



**AUTOMOTIVE GASOLINE  
AND SOME OXYGENATED  
GASOLINE ADDITIVES**

VOLUME 138

IARC MONOGRAPHS  
ON THE IDENTIFICATION  
OF CARCINOGENIC HAZARDS  
TO HUMANS



# **AUTOMOTIVE GASOLINE AND SOME OXYGENATED GASOLINE ADDITIVES**

**VOLUME 138**

This publication represents the views and expert  
opinions of an IARC Working Group on the  
Identification of Carcinogenic Hazards to Humans,  
which met in Lyon, France, 25 February to 4 March 2025

LYON, FRANCE - 2026

**IARC MONOGRAPHS  
ON THE IDENTIFICATION  
OF CARCINOGENIC HAZARDS  
TO HUMANS**

## IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic hazard of chemicals to humans, involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic hazards associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of cancer hazard to humans with the help of international working groups of experts in carcinogenesis and related fields; and to identify gaps in evidence. The lists of IARC evaluations are regularly updated and are available on the internet at <https://monographs.iarc.who.int/>.

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## NOTE TO THE READER

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Anyone who is aware of published data that may alter the evaluation of the carcinogenic hazard of an agent to humans is encouraged to make this information available to the *IARC Monographs* programme, International Agency for Research on Cancer, 25 avenue Tony Garnier, CS 90627, 69366 Lyon Cedex 07, or via email at [imo@iarc.who.int](mailto:imo@iarc.who.int), in order that the agent may be considered for re-evaluation by a future Working Group.

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<sup>3</sup> Dr Terron reported receiving personal consultancy fees from Accelera and Evotec for providing expert pathology advice on pharmaceutical toxicology studies. This was determined not to present a competing interest for the topics covered in the present meeting.

<sup>4</sup> Dr Hoel's travel costs to attend the meeting were paid by Exxon-Mobil, which is a multinational oil and gas corporation in the USA.



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<sup>6</sup> Dr Rushton is employed by LyondellBasell, which is a chemical company manufacturing methyl *tert*-butyl ether, ethyl *tert*-butyl ether, and *tert*-butyl alcohol gasoline ethers, and is Chair of the Health, Safety and Environment Committee at Sustainable Fuels (a sector group of Cefic, the European Chemical Association). These organizations paid his travel costs.

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# PREAMBLE

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The Preamble to the *IARC Monographs* describes the objective and scope of the programme, general principles and procedures, and scientific review and evaluations. The *IARC Monographs* embody principles of scientific rigour, impartial evaluation, transparency, and consistency. The Preamble should be consulted when reading a *Monograph* or a summary of a *Monograph's* evaluations. Separate Instructions for Authors describe the operational procedures for the preparation and publication of a volume of the *Monographs*.

## A. GENERAL PRINCIPLES AND PROCEDURES

### 1. Background

Soon after the International Agency for Research on Cancer (IARC) was established in 1965, it started to receive frequent requests for advice on the carcinogenicity of chemicals, including requests for lists of established and suspected human carcinogens. In 1970, an IARC Advisory Committee on Environmental Carcinogenesis recommended “that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.” The next year, the IARC Governing Council adopted a resolution that IARC should prepare “monographs on the evaluation of carcinogenic risk of chemicals to man”, which became the initial title of the series.

In succeeding years, the scope of the programme broadened as *Monographs* were developed for complex mixtures, occupational

exposures, physical agents, biological organisms, pharmaceuticals, and other exposures. In 1988, “of chemicals” was dropped from the title, and in 2019, “evaluation of carcinogenic risks” became “identification of carcinogenic hazards”, in line with the objective of the programme.

Identifying the causes of human cancer is the first step in cancer prevention. The identification of a cancer hazard may have broad and profound implications. National and international authorities and organizations can and do use information on causes of cancer in support of actions to reduce exposure to carcinogens in the workplace, in the environment, and elsewhere. Cancer prevention is needed as much today as it was when IARC was established, because the global burden of cancer is high and continues to increase as a result of population growth and ageing and upward trends in some exposures, especially in low- and middle-income countries (<https://publications.iarc.who.int/Non-Series-Publications/World-Cancer-Reports>).

IARC's process for developing *Monographs*, which has evolved over several decades, involves

the engagement of international, interdisciplinary Working Groups of expert scientists, the transparent synthesis of different streams of evidence (exposure characterization, cancer in humans, cancer in experimental animals, and mechanisms of carcinogenesis), and the integration of these streams of evidence into an overall evaluation and classification according to criteria developed and refined by IARC. Since the *Monographs* programme was established, the understanding of carcinogenesis has greatly deepened. Scientific advances are incorporated into the evaluation methodology. In particular, strong mechanistic evidence has had an increasing role in the overall evaluations since 1991.

The Preamble is primarily a statement of the general principles and procedures used in developing a *Monograph*, to promote transparency and consistency across *Monographs* evaluations. In addition, IARC provides Instructions for Authors (<https://monographs.iarc.who.int/preamble-instructions-for-authors/>), which specify more detailed working procedures. IARC routinely updates these Instructions for Authors to reflect advances in methods for cancer hazard identification and accumulated experience, including input from experts.

## 2. Objective and scope

The objective of the programme is to prepare, with the engagement of international, interdisciplinary Working Groups of experts, scientific reviews and evaluations of evidence on the carcinogenicity of a wide range of agents.

The *Monographs* assess the strength of the available evidence that an agent can cause cancer in humans, based on three streams of evidence: on cancer in humans (see Part B, Section 2), on cancer in experimental animals (see Part B, Section 3), and on mechanistic evidence (see Part B, Section 4). In addition, the exposure to each agent is characterized (see Part B, Section 1). In this Preamble, the term “agent” refers to any

chemical, physical, or biological entity or exposure circumstance (e.g. occupation as a painter) for which evidence on the carcinogenicity is evaluated.

A cancer *hazard* is an agent that is capable of causing cancer, whereas a cancer *risk* is an estimate of the probability that cancer will occur given some level of exposure to a cancer hazard. The *Monographs* assess the strength of evidence that an agent is a cancer hazard. The distinction between hazard and risk is fundamental. The *Monographs* identify cancer hazards even when risks appear to be low in some exposure scenarios. This is because the exposure may be widespread at low levels, and because exposure levels in many populations are not known or documented.

Although the *Monographs* programme has focused on hazard identification, some epidemiological studies used to identify a cancer hazard are also used to estimate an exposure–response relationship within the range of the available data. However, extrapolating exposure–response relationships beyond the available data (e.g. to lower exposures, or from experimental animals to humans) is outside the scope of *Monographs* Working Groups ([IARC, 2014](#)). In addition, the *Monographs* programme does not review quantitative risk characterizations developed by other health agencies.

The identification of a cancer hazard should trigger some action to protect public health, either directly as a result of the hazard identification or through the conduct of a risk assessment. Although such actions are outside the scope of the programme, the *Monographs* are used by national and international authorities and organizations to inform risk assessments, formulate decisions about preventive measures, motivate effective cancer control programmes, and choose among options for public health decisions. *Monographs* evaluations are only one part of the body of information on which decisions to control exposure to carcinogens may be based.

Options to prevent cancer vary from one situation to another and across geographical regions and take many factors into account, including different national priorities. Therefore, no recommendations are given in the *Monographs* with regard to regulation, legislation, or other policy approaches, which are the responsibility of individual governments or organizations. The *Monographs* programme also does not make research recommendations. However, it is important to note that *Monographs* contribute significantly to the science of carcinogenesis by synthesizing and integrating streams of evidence about carcinogenicity and pointing to critical gaps in knowledge.

### 3. Selection of agents for review

Since 1984, about every five years IARC convenes an international, interdisciplinary Advisory Group to recommend agents for review by the *Monographs* programme. IARC selects Advisory Group members who are knowledgeable about current research on carcinogens and public health priorities. Before an Advisory Group meets, IARC solicits nominations of agents from scientists and government agencies worldwide. Since 2003, IARC also invites nominations from the public. IARC charges each Advisory Group with reviewing nominations, evaluating exposure and hazard potential, and preparing a report that documents the Advisory Group's process for these activities and its rationale for the recommendations.

For each new volume of the *Monographs*, IARC selects the agents for review from those recommended by the most recent Advisory Group, considering the availability of pertinent research studies and current public health priorities. On occasion, IARC may select other agents if there is a need to rapidly evaluate an emerging carcinogenic hazard or an urgent need to re-evaluate a previous classification. All evaluations consider the full body of available evidence,

not just information published after a previous review.

A *Monograph* may review:

- (a) An agent not reviewed in a previous *Monograph*, if there is potential human exposure and there is evidence for assessing its carcinogenicity. A group of related agents (e.g. metal compounds) may be reviewed together if there is evidence for assessing carcinogenicity for one or more members of the group.
- (b) An agent reviewed in a previous *Monograph*, if there is new evidence of cancer in humans or in experimental animals, or mechanistic evidence to warrant re-evaluation of the classification. In the interests of efficiency, the literature searches may build on previous comprehensive searches.
- (c) An agent that has been established to be carcinogenic to humans and has been reviewed in a previous *Monograph*, if there is new evidence of cancer in humans that indicates new tumour sites where there might be a causal association. In the interests of efficiency, the review may focus on these new tumour sites.

### 4. The Working Group and other meeting participants

Five categories of participants can be present at *Monographs* meetings:

- (i) *Working Group* members are responsible for all scientific reviews and evaluations developed in the volume of the *Monographs*. The Working Group is interdisciplinary and comprises subgroups of experts in the fields of (a) exposure characterization, (b) cancer in humans, (c) cancer in experimental animals, and (d) mechanistic evidence. IARC selects Working Group members on the basis of expertise related to the subject matter and relevant methodologies, and absence

of conflicts of interest. Consideration is also given to diversity in scientific approaches and views, as well as demographic composition. Working Group members generally have published research related to the exposure or carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Since 2006, IARC also has encouraged public nominations through its Call for Experts. IARC's reliance on experts with knowledge of the subject matter and/or expertise in methodological assessment is confirmed by decades of experience documenting that there is value in specialized expertise and that the overwhelming majority of Working Group members are committed to the objective evaluation of scientific evidence and not to the narrow advancement of their own research results or a pre-determined outcome ([Wild and Coglianò, 2011](#)). Working Group members are expected to serve the public health mission of IARC, and should refrain from consulting and other activities for financial gain that are related to the agents under review, or the use of inside information from the meeting, until the full volume of the *Monographs* is published.

IARC identifies, from among Working Group members, individuals to serve as Meeting Chair and Subgroup Chairs. At the opening of the meeting, the Working Group is asked to endorse the selection of the Meeting Chair, with the opportunity to propose alternatives. The Meeting Chair and Subgroup Chairs take a leading role at all stages of the review process (see Part A, Section 7), promote open scientific discussions that involve all Working Group members in accordance with normal committee procedures, and ensure adherence to the Preamble.

(ii) *Invited Specialists* are experts who have critical knowledge and experience but who also have a conflict of interest that warrants

exclusion from developing or influencing the evaluations of carcinogenicity. Invited Specialists do not draft any section of the *Monograph* that pertains to the description or interpretation of cancer data, and they do not participate in the evaluations. These experts are invited in limited numbers when necessary to assist the Working Group by contributing their unique knowledge and experience to the discussions.

(iii) *Representatives of national and international health agencies* may attend because their agencies are interested in the subject of the meeting. They do not draft any section of the *Monograph* or participate in the evaluations.

(iv) *Observers* with relevant scientific credentials may be admitted in limited numbers. Attention is given to the balance of Observers from constituencies with differing perspectives. Observers are invited to observe the meeting and should not attempt to influence it, and they agree to respect the [Guidelines for Observers at IARC Monographs meetings](#). Observers do not draft any section of the *Monograph* or participate in the evaluations.

(v) The *IARC Secretariat* consists of scientists who are designated by IARC and who have relevant expertise. The IARC Secretariat coordinates and facilitates all aspects of the evaluation and ensures adherence to the Preamble throughout development of the scientific reviews and classifications (see Part A, Sections 5 and 6). The IARC Secretariat organizes and announces the meeting, identifies and recruits the Working Group members, and assesses the declared interests of all meeting participants. The IARC Secretariat supports the activities of the Working Group (see Part A, Section 7) by searching the literature and performing title and abstract screening, organizing conference calls to coordinate the development of pre-meeting



**Table 1 Roles of participants at IARC Monographs meetings**

| Category of participant            | Role                               |                            |                            |                            |
|------------------------------------|------------------------------------|----------------------------|----------------------------|----------------------------|
|                                    | Prepare text, tables, and analyses | Participate in discussions | Participate in evaluations | Eligible to serve as Chair |
| Working Group members              | ✓                                  | ✓                          | ✓                          | ✓                          |
| Invited Specialists                | ✓ <sup>a</sup>                     | ✓                          |                            |                            |
| Representatives of health agencies |                                    | ✓ <sup>b</sup>             |                            |                            |
| Observers                          |                                    | ✓ <sup>b</sup>             |                            |                            |
| IARC Secretariat                   | ✓ <sup>c</sup>                     | ✓                          | ✓ <sup>d</sup>             |                            |

<sup>a</sup> Only for the section on exposure characterization.

<sup>b</sup> Only at times designated by the Meeting Chair and Subgroup Chairs.

<sup>c</sup> When needed or requested by the Meeting Chair and Subgroup Chairs.

<sup>d</sup> Only for clarifying or interpreting the Preamble.

drafts and discuss cross-cutting issues, and reviewing drafts before and during the meeting. Members of the IARC Secretariat serve as meeting rapporteurs, assist the Meeting Chair and Subgroup Chairs in facilitating all discussions, and may draft text or tables when designated by the Meeting Chair and Subgroup Chairs. Their participation in the evaluations is restricted to the role of clarifying or interpreting the Preamble.

All participants are listed, with their principal affiliations, in the front matter of the published volume of the *Monographs*. Working Group members and Invited Specialists serve as individual scientists and not as representatives of any organization, government, or industry (Cogliano et al., 2004).

The roles of the meeting participants are summarized in Table 1.

## 5. Working procedures

A separate Working Group is responsible for developing each volume of the *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year before the meeting of a Working Group, a preliminary list of agents to be reviewed, together with a Call

for Data and a Call for Experts, is announced on the *Monographs* programme website (<https://monographs.iarc.who.int/>).

Before a meeting invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests form to report financial interests, employment and consulting (including remuneration for serving as an expert witness), individual and institutional research support, and non-financial interests such as public statements and positions related to the subject of the meeting. IARC assesses the declared interests to determine whether there is a conflict that warrants any limitation on participation (see Table 2).

Approximately two months before a *Monographs* meeting, IARC publishes the names and affiliations of all meeting participants together with a summary of declared interests, in the interests of transparency and to provide an opportunity for undeclared conflicts of interest to be brought to IARC's attention. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano et al., 2005).

The Working Group meets at IARC for approximately eight days to discuss and finalize the scientific review and to develop summaries

**Table 2 Public engagement during *Monographs* development**

| Approximate timeframe                        | Engagement                                                                                                                                                                                                                                                   |
|----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Every 5 years                                | IARC convenes an Advisory Group to recommend high-priority agents for future review                                                                                                                                                                          |
| ~1 year before a <i>Monographs</i> meeting   | IARC selects agents for review in a new volume of the <i>Monographs</i><br>IARC posts on its website:<br>Preliminary List of Agents to be reviewed<br>Call for Data and Call for Experts<br>Request for Observer Status<br>WHO Declaration of Interests form |
| ~8 months before a <i>Monographs</i> meeting | Call for Experts closes                                                                                                                                                                                                                                      |
| ~4 months before a <i>Monographs</i> meeting | Request for Observer Status closes                                                                                                                                                                                                                           |
| ~2 months before a <i>Monographs</i> meeting | IARC posts the names of all meeting participants together with a summary of declared interests, and a statement discouraging contact of the Working Group by interested parties                                                                              |
| ~1 month before a <i>Monographs</i> meeting  | Call for Data closes                                                                                                                                                                                                                                         |
| ~2–4 weeks after a <i>Monographs</i> meeting | IARC publishes a summary of evaluations and key supporting evidence                                                                                                                                                                                          |
| ~9 months after a <i>Monographs</i> meeting  | IARC Secretariat publishes the verified and edited master copy of plenary drafts as a <i>Monographs</i> volume                                                                                                                                               |

and evaluations. At the opening of the meeting, all participants update their Declaration of Interests forms, which are then reviewed by IARC. Declared interests related to the subject of the meeting are disclosed to the meeting participants during the meeting and in the published volume ([Cogliano et al., 2004](#)). The objectives of the meeting are peer review and consensus. During the first part of the meeting, subgroup sessions (covering exposure characterization, cancer in humans, cancer in experimental animals, and mechanistic evidence) review the pre-meeting drafts, develop a joint subgroup draft, and draft subgroup summaries. During the last part of the meeting, the Working Group meets in plenary session to review the subgroup drafts and summaries and to develop the consensus evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections. After the meeting, the master copy is verified by the IARC Secretariat and is then edited and prepared for publication. The aim is to publish the volume within approximately nine months of the Working Group meeting. A summary of the

evaluations and key supporting evidence is prepared for publication in a scientific journal or is made available on the *Monographs* programme website soon after the meeting.

In the interests of transparency, IARC engages with the public throughout the process, as summarized in [Table 2](#).

## 6. Overview of the scientific review and evaluation process

The Working Group considers all pertinent epidemiological studies, cancer bioassays in experimental animals, and mechanistic evidence, as well as pertinent information on exposure in humans. In general, for cancer in humans, cancer in experimental animals, and mechanistic evidence, only studies that have been published or accepted for publication in the openly available scientific literature are reviewed. Under some circumstances, materials that are publicly available and whose content is final may be reviewed if there is sufficient information to permit an evaluation of the quality of the methods and results of the studies (see Step 1,



below). Such materials may include reports and databases publicly available from government agencies, as well as doctoral theses. The reliance on published and publicly available studies promotes transparency and protects against citation of premature information.

The principles of systematic review are applied to the identification, screening, synthesis, and evaluation of the evidence related to cancer in humans, cancer in experimental animals, and mechanistic evidence (as described in Part B, Sections 2–4 and as detailed in the Instructions for Authors). Each *Monograph* specifies or references information on the conduct of the literature searches, including search terms and inclusion/exclusion criteria that were used for each stream of evidence.

In brief, the steps of the review process are as follows:

*Step 1. Comprehensive and transparent identification of the relevant information:* The IARC Secretariat identifies relevant studies through initial comprehensive searches of literature contained in authoritative biomedical databases (e.g. PubMed, PubChem) and through a Call for Data. These literature searches, designed in consultation with a librarian and other technical experts, address whether the agent causes cancer in humans, causes cancer in experimental systems, and/or exhibits key characteristics of established human carcinogens (in humans or in experimental systems). The Working Group provides input and advice to IARC to refine the search strategies, and identifies literature through other searches (e.g. from reference lists of past *Monographs*, retrieved articles, and other authoritative reviews).

For certain types of agents (e.g. regulated pesticides and pharmaceuticals), IARC also provides an opportunity to relevant regulatory authorities, and regulated parties through such authorities, to make pertinent

unpublished studies publicly available by the date specified in the Call for Data. Consideration of such studies by the Working Group is dependent on the public availability of sufficient information to permit an independent evaluation of (a) whether there has been selective reporting (e.g. on outcomes, or from a larger set of conducted studies); (b) study quality (e.g. design, methodology, and reporting of results), and (c) study results.

*Step 2. Screening, selection, and organization of the studies:* The IARC Secretariat screens the retrieved literature for inclusion based on title and abstract review, according to pre-defined exclusion criteria. For instance, studies may be excluded if they were not about the agent (or a metabolite of the agent), or if they reported no original data on epidemiological or toxicological end-points (e.g. review articles). The Working Group reviews the title and abstract screening done by IARC, and performs full-text review. Any reasons for exclusion are recorded, and included studies are organized according to factors pertinent to the considerations described in Part B, Sections 2–4 (e.g. design, species, and endpoint). Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results.

*Step 3. Evaluation of study quality:* The Working Group evaluates the quality of the included studies based on the considerations (e.g. design, methodology, and reporting of results) described in Part B, Sections 2–4. Based on these considerations, the Working Group may accord greater weight to some of the included studies. Interpretation of the results and the strengths and limitations of a study are clearly outlined in square brackets at the end of study descriptions (see Part B).

*Step 4: Report characteristics of included studies, including assessment of study*

*quality*: Pertinent characteristics and results of included studies are reviewed and succinctly described, as detailed in Part B, Sections 1–4. Tabulation of data may facilitate this reporting. This step may be iterative with Step 3.

*Step 5: Synthesis and evaluation of strength of evidence*: The Working Group summarizes the overall strengths and limitations of the evidence from the individual streams of evidence (cancer in humans, cancer in experimental animals, and mechanistic evidence; see Part B, Section 5). The Working Group then evaluates the strength of evidence from each stream of evidence by using the transparent methods and defined descriptive terms given in Part B, Sections 6a–c. The Working Group then develops, and describes the rationale for, the consensus classification of carcinogenicity that integrates the conclusions about the strength of evidence from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic evidence (see Part B, Section 6d).

## 7. Responsibilities of the Working Group

The Working Group is responsible for identifying and evaluating the relevant studies and developing the scientific reviews and evaluations for a volume of the *Monographs*. The IARC Secretariat supports these activities of the Working Group (see Part A, Section 4). Briefly, the Working Group's tasks in developing the evaluation are, in sequence:

(i) Before the meeting, the Working Group ascertains that all appropriate studies have been identified and selected, and assesses the methods and quality of each individual study, as outlined above (see Part A, Section 6). The Working Group members

prepare pre-meeting working drafts that present accurate tabular or textual summaries of informative studies by extracting key elements of the study design and results, and highlighting notable strengths and limitations. They participate in conference calls organized by IARC to coordinate the development of working drafts and to discuss cross-cutting issues. Pre-meeting reviews of all working drafts are generally performed by two or more subgroup members who did not participate in study identification, data extraction, or study review for the draft. Each study summary is written or reviewed by someone who is not associated with the study.

(ii) At the meeting, within subgroups, the Working Group members critically review, discuss, and revise the pre-meeting drafts and adopt the revised versions as consensus subgroup drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary. A proposed classification of the strength of the evidence reviewed in the subgroup using the *IARC Monographs* criteria (see Part B, Sections 6a–c) is then developed from the consensus subgroup drafts of the evidence summaries (see Part B, Section 5).

(iii) During the plenary session, each subgroup presents its drafts for scientific review and discussion to the other Working Group members, who did not participate in study identification, data extraction, or study review for the drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary. After review, discussion, and revisions as needed, the subgroup drafts are adopted as a consensus Working Group product. The summaries and classifications of the strength of the evidence, developed in the subgroup in line with the *IARC Monographs* criteria

(see Part B, Sections 6a–c), are considered, revised as needed, and adopted by the full Working Group. The Meeting Chair proposes an overall evaluation using the guidance provided in Part B, Section 6d.

The Working Group strives to achieve consensus evaluations. Consensus reflects broad agreement among the Working Group, but not necessarily unanimity. The Meeting Chair may poll the Working Group to determine the diversity of scientific opinion on issues where consensus is not apparent.

Only the final product of the plenary session represents the views and expert opinions of the Working Group. The entire *Monographs* volume is the joint product of the Working Group and represents an extensive and thorough peer review of the body of evidence (individual studies, synthesis, and evaluation) by an interdisciplinary expert group. Initial working papers and subsequent revisions are not released, because they would give an incomplete and possibly misleading impression of the consensus developed by the Working Group over a full week of deliberation.

## B. SCIENTIFIC REVIEW AND EVALUATION

This part of the Preamble discusses the types of evidence that are considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations. In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and any key issues encountered during the meeting.

### 1. Exposure characterization

This section identifies the agent and describes its occurrence, main uses, and production locations and volumes, where relevant. It also

summarizes the prevalence, concentrations in relevant studies, and relevant routes of exposure in humans worldwide. Methods of exposure measurement and analysis are described, and methods of exposure assessment used in key epidemiological studies reviewed by the Working Group are described and evaluated.

Over the course of the *Monographs* programme, concepts of exposure and dose have evolved substantially with deepening understanding of the interactions of agents and biological systems. The concept of exposure has broadened and become more holistic, extending beyond chemical, physical, and biological agents to stressors as construed generally, including psychosocial stressors ([National Research Council, 2012](#); [National Academies of Sciences, Engineering, and Medicine, 2017](#)). Overall, this broader conceptualization supports greater integration between exposure characterization and other sections of the *Monographs*. Concepts of absorption, distribution, metabolism, and excretion are considered in the first subsection of mechanistic evidence (see Part B, Section 4a), whereas validated biomarkers of internal exposure or metabolites that are routinely used for exposure assessment are reported on in this section (see Part B, Section 1b).

#### (a) Identification of the agent

The agent being evaluated is unambiguously identified. Details will vary depending on the type of agent but will generally include physical and chemical properties relevant to the agent's identification, occurrence, and biological activity. If the material that has been tested in experimental animals or in vitro systems is different from that to which humans are exposed, these differences are noted.

For chemical agents, the Chemical Abstracts Service Registry Number is provided, as well as the latest primary name and other names in common use, including important trade names,

along with available information on the composition of common mixtures or products containing the agent, and potentially toxic and/or carcinogenic impurities. Physical properties relevant to understanding the potential for human exposure and measures of exposure used in studies in humans are summarized. These might include physical state, volatility, aqueous and fat solubility, and half-life in the environment and/or in human tissues.

For biological agents, taxonomy and structure are described. Mode of replication, life-cycle, target cells, persistence, latency, and host responses, including morbidity and mortality through pathologies other than cancer, are also presented.

For foreign bodies, fibres and particles, composition, size range, relative dimensions, and accumulation, persistence, and clearance in target organs are summarized. Physical agents that are forms of radiation are described in terms of frequency spectrum and energy transmission.

Exposures may result from, or be influenced by, a diverse range of social and environmental factors, including components of diet, sleep, and physical activity patterns. In these instances, this section will include a description of the agent, its variability across human populations, and its composition or characteristics relevant to understanding its potential carcinogenic hazard to humans and to evaluating exposure assessments in epidemiological studies.

#### *(b) Detection and analysis*

Key methods of detection and quantification of the agent are presented, with an emphasis on those used most widely in surveillance, regulation, and epidemiological studies. Measurement methods for sample matrices that are deemed important sources of human exposure (e.g. air, drinking-water, food, residential dust) and for validated exposure biomarkers (e.g. the agent or its metabolites in human blood, urine, or

saliva) are described. Information on detection and quantification limits is provided when it is available and is useful for interpreting studies in humans and in experimental animals. This is not an exhaustive treatise but is meant to help readers understand the strengths and limitations of the available exposure data and of the epidemiological studies that rely on these measurements.

#### *(c) Production and use*

Historical and geographical patterns and trends in production and use are included when they are available, to help readers understand the contexts in which exposures may occur, both within key epidemiological studies reviewed by the Working Group and in human populations generally. Industries that produce, use, or dispose of the agent are described, including their global distribution, when available. National or international listing as a high-production-volume chemical or similar classification may be included. Production processes with significant potential for occupational exposure or environmental pollution are indicated. Trends in global production volumes, technologies, and other data relevant to understanding exposure potential are summarized. Minor or historical uses with significant exposure potential or with particular relevance to key epidemiological studies are included. Particular effort may be directed towards finding data on production in low- and middle-income countries, where rapid economic development may lead to higher exposures than those in high-income countries.

#### *(d) Exposure*

A concise overview of quantitative information on sources, prevalence, and levels of exposure in humans is provided. Representative data from research studies, government reports and websites, online databases, and other citable, publicly available sources are tabulated. Data

from low- and middle-income countries are sought and included to the extent feasible; information gaps for key regions are noted. Naturally occurring sources of exposure, if any, are noted. Primary exposure routes (e.g. inhalation, ingestion, skin uptake) and other considerations relevant to understanding the potential for cancer hazard from exposure to the agent are reported.

For occupational settings, information on exposure prevalence and levels (e.g. in air or human tissues) is reported by industry, occupation, region, and other characteristics (e.g. process, task) where feasible. Information on historical exposure trends, protection measures to limit exposure, and potential co-exposures to other carcinogenic agents in workplaces is provided when available.

For non-occupational settings, the occurrence of the agent is described with environmental monitoring or surveillance data. Information on exposure prevalence and levels (e.g. concentrations in human tissues) as well as exposure from and/or concentrations in food and beverages, consumer products, consumption practices, and personal microenvironments is reported by region and other relevant characteristics. Particular importance is placed on describing exposures in life stages or in states of disease or nutrition that may involve greater exposure or susceptibility.

Current exposures are of primary interest; however, information on historical exposure trends is provided when available. Historical exposures may be relevant for interpreting epidemiological studies, and when agents are persistent or have long-term effects. Information gaps for important time periods are noted. Exposure data that are not deemed to have high relevance to human exposure are generally not considered.

#### (e) *Regulations and guidelines*

Regulations or guidelines that have been established for the agent (e.g. occupational exposure limits, maximum permitted levels in foods and water, pesticide registrations) are described in brief to provide context about government efforts to limit exposure; these may be tabulated if they are informative for the interpretation of existing or historical exposure levels. Information on applicable populations, specific agents concerned, basis for regulation (e.g. human health risk, environmental considerations), and timing of implementation may be noted. National and international bans on production, use, and trade are also indicated.

This section aims to include major or illustrative regulations and may not be comprehensive, because of the complexity and range of regulatory processes worldwide. An absence of information on regulatory status should not be taken to imply that a given country or region lacks exposure to, or regulations on exposure to, the agent.

#### (f) *Critical review of exposure assessment in key epidemiological studies*

Epidemiological studies evaluate cancer hazard by comparing outcomes across differently exposed groups. Therefore, the type and quality of the exposure assessment methods used are key considerations when interpreting study findings for hazard identification. This section summarizes and critically reviews the exposure assessment methods used in the individual epidemiological studies that contribute data relevant to the *Monographs* evaluation.

Although there is no standard set of criteria for evaluating the quality of exposure assessment methods across all possible agents, some concepts are universally relevant. Regardless of the agent, all exposures have two principal dimensions: intensity (sometimes defined as concentration or dose) and time. Time considerations include



duration (time from first to last exposure), pattern or frequency (whether continuous or intermittent), and windows of susceptibility. This section considers how each of the key epidemiological studies characterizes these dimensions. Interpretation of exposure information may also be informed by consideration of mechanistic evidence (e.g. as described in Part B, Section 4a), including the processes of absorption, distribution, metabolism, and excretion.

Exposure intensity and time in epidemiological studies can be characterized by using environmental or biological monitoring data, records from workplaces or other sources, expert assessments, modelled exposures, job-exposure matrices, and subject or proxy reports via questionnaires or interviews. Investigators use these data sources and methods individually or in combination to assign levels or values of an exposure metric (which may be quantitative, semi-quantitative, or qualitative) to members of the population under study.

In collaboration with the Working Group members reviewing human studies (of cancer and of mechanisms), key epidemiological studies are identified. For each selected study, the exposure assessment approach, along with its strengths and limitations, is summarized using text and tables. Working Group members identify concerns about exposure assessment methods and their impacts on overall quality for each study reviewed (see Part B, Sections 2d and 4d). In situations where the information provided in the study is inadequate to properly consider the exposure assessment, this is indicated. When adequate information is available, the likely direction of bias due to error in exposure measurement, including misclassification (overestimated effects, underestimated effects, or unknown) is discussed.

## 2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part B, Section 2b) that include cancer as an outcome. These studies encompass certain types of biomarker studies, for example, studies with biomarkers as exposure metrics (see Part B, Section 2) or those evaluating histological or tumour subtypes and molecular signatures in tumours consistent with a given exposure ([Alexandrov et al., 2016](#)). Studies that evaluate early biological effect biomarkers are reviewed in Part B, Section 4.

### (a) *Types of study considered*

Several types of epidemiological studies contribute to the assessment of carcinogenicity in humans; they typically include cohort studies (including variants such as case-cohort and nested case-control studies), case-control studies, ecological studies, and intervention studies. Rarely, results from randomized trials may be available. Exceptionally, case reports and case series of cancer in humans may also be reviewed. In addition to these designs, innovations in epidemiology allow for many other variants that may be considered in any given *Monographs* evaluation.

Cohort and case-control studies typically have the capacity to relate individual exposures under study to the occurrence of cancer in individuals, and provide an estimate of effect (such as relative risk) as the main measure of association. Well-conducted cohort and case-control studies provide most of the evidence of cancer in humans evaluated by Working Groups. Intervention studies are much less common, but when available can provide strong evidence for making causal inferences.

In ecological studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary



measure of the exposure in the population under study. In ecological studies, data on individual exposure and outcome are not available, which renders this type of study more prone to confounding and exposure misclassification. In some circumstances, however, ecological studies may be informative, especially when the unit of exposure is most accurately measured at the population level (see, for example, the *Monograph* on arsenic in drinking-water; [IARC, 2004](#)).

Exceptionally, case reports and case series may provide compelling evidence about the carcinogenicity of an agent. In fact, many of the early discoveries of occupational cancer hazards came about because of observations by workers and their clinicians, who noted a high frequency of cancer in workers who share a common occupation or exposure. Such observations may be the starting point for more structured investigations, but in exceptional circumstances, when the risk is high enough, the case series may in itself provide compelling evidence. This would be especially warranted in situations where the exposure circumstance is fairly unusual, as it was in the example of plants containing aristolochic acid ([IARC, 2012a](#)).

The uncertainties that surround the interpretation of case reports, case series, and ecological studies typically make them inadequate, except in rare instances as described above, to form the sole basis for inferring a causal relationship. However, when considered together with cohort and case-control studies, these types of study may support the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, pre-neoplastic lesions, malignant precursors, and other end-points are also reviewed when they relate to the agents reviewed. On occasion they can strengthen inferences drawn from studies of cancer itself. For example, benign brain tumours may share common risk factors with those that are malignant, and benign neoplasms (or those of uncertain behaviour) may be

part of the causal path to malignancies (e.g. myelodysplastic syndromes, which may progress to acute myeloid leukaemia).

#### *(b) Identification of eligible studies of cancer in humans*

Relevant studies of cancer in humans are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Eligible studies include all studies in humans of exposure to the agent of interest with cancer as an outcome. Multiple publications on the same study population are identified so that the number of independent studies is accurately represented. Multiple publications may result, for example, from successive follow-ups of a single cohort, from analyses focused on different aspects of an exposure-disease association, or from inclusion of overlapping populations. Usually in such situations, only the most recent, most comprehensive, or most informative report is reviewed in detail.

#### *(c) Assessment of study quality and informativeness*

Epidemiological studies are potentially susceptible to several different sources of error, summarized briefly below. Qualities of individual studies that address these issues are also described below.

Study quality is assessed as part of the structured expert review process undertaken by the Working Group. A key aspect of quality assessment is consideration of the possible roles of chance and bias in the interpretation of epidemiological studies. Chance, which is also called random variation, can produce misleading study results. This variability in study results is strongly influenced by the sample size: smaller studies are more likely than larger studies to have effect estimates that are imprecise. Confidence intervals

around a study's point estimate of effect are used routinely to indicate the range of values of the estimate that could easily be produced by chance alone.

Bias is the effect of factors in study design or conduct that lead an association to erroneously appear stronger or weaker than the association that really exists between the agent and the disease. Biases that require consideration are varied but are usually categorized as selection bias, information bias (e.g. error in measurement of exposure and diseases), and confounding (or confounding bias) ([Rothman et al., 2008](#)). Selection bias in an epidemiological study occurs when inclusion of participants from the eligible population or their follow-up in the study is influenced by their exposure or their outcome (usually disease occurrence). Under these conditions, the measure of association found in the study will not accurately reflect the association that would otherwise have been found in the eligible population ([Hernán et al., 2004](#)). Information bias results from inaccuracy in exposure or outcome measurement. Both can cause an association between hypothesized cause and effect to appear stronger or weaker than it really is. Confounding is a mixing of extraneous effects with the effects of interest ([Rothman et al., 2008](#)). An association between the purported causal factor and another factor that is associated with an increase or decrease in incidence of disease can lead to a spurious association or absence of a real association of the presumed causal factor with the disease. When either of these occurs, confounding is present.

In assessing study quality, the Working Group consistently considers the following aspects:

- **Study description:** Clarity in describing the study design and its implementation, and the completeness of reporting of all other key information about the study and its results.
- **Study population:** Whether the study population was appropriate for evaluating the association between the agent and cancer. Whether the study was designed and carried out to minimize selection bias. Cancer cases in the study population must have been identified in a way that was independent of the exposure of interest, and exposure assessed in a way that was not related to disease (outcome) status. In these respects, completeness of recruitment into the study from the population of interest and completeness of follow-up for the outcome are essential measures.
- **Outcome measurement:** The appropriateness of the cancer outcome measure (e.g. mortality vs incidence) for the agent and cancer type under consideration, outcome ascertainment methodology, and the extent to which outcome misclassification may have led to bias in the measure(s) of association.
- **Exposure measurement:** The adequacy of the methods used to assess exposure to the agent, and the likelihood (and direction) of bias in the measure(s) of association due to error in exposure measurement, including misclassification (as described in Part B, Section 1f).
- **Assessment of potential confounding:** To what extent the authors took into account in the study design and analysis other variables (including co-exposures, as described in Part B, Section 1d) that can influence the risk of disease and may have been related to the exposure of interest. Important sources of potential confounding by such variables should have been addressed either in the design of the study, such as by matching or restriction, or in the analysis, by statistical adjustment. In some instances, where direct information on confounders is unavailable, use of indirect methods to evaluate the potential impact of confounding on exposure–disease associations is appropriate (e.g. [Axelson and Steenland, 1988](#); [Richardson et al., 2014](#)).

- **Other potential sources of bias:** Each epidemiological study is unique in its study population, its design, its data collection, and, consequently, its potential biases. All possible sources of bias are considered for their possible impact on the results. The possibility of reporting bias (i.e. selective reporting of some results and the suppression of others) should be explored.
- **Statistical methodology:** Adequacy of the statistical methods used and their ability to obtain unbiased estimates of exposure–outcome associations, confidence intervals, and test statistics for the significance of measures of association. Appropriateness of methods used to investigate confounding, including adjusting for matching when necessary and avoiding treatment of probable mediating variables as confounders. Detailed analyses of cancer risks in relation to summary measures of exposure such as cumulative exposure, or temporal variables such as age at first exposure or time since first exposure, are reviewed and summarized when available.

For the sake of economy and simplicity, in this Preamble the list of possible sources of error is referred to with the phrase “chance, bias, and confounding”, but it should be recognized that this phrase encompasses a comprehensive set of concerns pertaining to study quality.

These sources of error do not constitute and should not be used as a formal checklist of indicators of study quality. The judgement of experienced experts is critical in determining how much weight to assign to different issues in considering how all of these potential sources of error should be integrated and how to rate the potential for error related to each of these considerations.

The informativeness of a study is its ability to show a true association, if there is one, between the agent and cancer, and the lack of an association, if no association exists. Key determinants of

informativeness include: having a study population of sufficient size to obtain precise estimates of effect; sufficient elapsed time from exposure to measurement of outcome for an effect, if present, to be observable; presence of an adequate exposure contrast (intensity, frequency, and/or duration); biologically relevant definitions of exposure; and relevant and well-defined time windows for exposure and outcome.

#### (d) *Meta-analyses and pooled analyses*

Independent epidemiological studies of the same agent may lead to inconsistent results that are difficult to interpret or reconcile. Combined analyses of data from multiple studies may be conducted as a means to address this ambiguity. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis), and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) ([Greenland and O’Rourke, 2008](#)).

The strengths of combined analyses are increased precision because of increased sample size and, in the case of pooled analyses, the opportunity to better control for potential confounders and to explore in more detail interactions and modifying effects that may explain heterogeneity among studies. A disadvantage of combined analyses is the possible lack of comparability of data from various studies, because of differences in population characteristics, subject recruitment, procedures of data collection, methods of measurement, and effects of unmeasured covariates that may differ among studies. These differences in study methods and quality can influence results of either meta-analyses or pooled analyses. If published meta-analyses are to be considered by the Working Group, their adequacy needs to be carefully evaluated, including the methods used to identify eligible studies

and the accuracy of data extracted from the individual studies.

The Working Group may conduct ad hoc meta-analyses during the course of a *Monographs* meeting, when there are sufficient studies of an exposure–outcome association to contribute to the Working Group’s assessment of the association. The results of such unpublished original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies, or de novo analyses.

Irrespective of the source of data for the meta-analyses and pooled analyses, the following key considerations apply: the same criteria for data quality must be applied as for individual studies; sources of heterogeneity among studies must be carefully considered; and the possibility of publication bias should be explored.

#### (e) *Considerations in assessing the body of epidemiological evidence*

The ability of the body of epidemiological evidence to inform the Working Group about the carcinogenicity of the agent is related to both the quantity and the quality of the evidence. There is no formulaic answer to the question of how many studies of cancer in humans are needed from which to draw inferences about causality, although more than a single study in a single population will almost always be needed. The number will depend on the considerations relating to evidence described below.

After the quality of individual epidemiological studies of cancer has been assessed and the informativeness of the various studies on the association between the agent and cancer has been evaluated, a judgement is made about the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several aspects of the body of evidence (e.g. [Hill, 1965](#);

[Rothman et al., 2008](#); [Vandenbroucke et al., 2016](#)).

A strong association (e.g. a large relative risk) is more likely to indicate causality than is a weak association, because it is more difficult for confounding to falsely create a strong association. However, it is recognized that estimates of effect of small magnitude do not imply lack of causality and may have impact on public health if the disease or exposure is common. Estimates of effect of small magnitude could also contribute useful information to the assessment of causality if level of risk is commensurate with level of exposure when compared with risk estimates from populations with higher exposure (e.g. as seen in residential radon studies compared with studies of radon from uranium mining).

Associations that are consistently observed in several studies of the same design, or in studies that use different epidemiological approaches, or under different circumstances of exposure are more likely to indicate a causal relationship than are isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (e.g. differences in study informativeness because of latency, exposure levels, or assessment methods). Results of studies that are judged to be of high quality and informativeness are given more weight than those of studies judged to be methodologically less sound or less informative.

Temporality of the association is an essential consideration: that is, the exposure must precede the outcome.

An observation that cancer risk increases with increasing exposure is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship, and there are several reasons why the shape of the exposure–response association may be non-monotonic (e.g. [Stayner et al., 2003](#)). The demonstration of a decline in risk after cessation of or reduction in exposure



in individuals or in whole populations also supports a causal interpretation of the findings.

Confidence in a causal interpretation of the evidence from studies of cancer in humans is enhanced if it is coherent with physiological and biological knowledge, including information about exposure to the target organ, latency and timing of the exposure, and characteristics of tumour subtypes.

The Working Group considers whether there are subpopulations with increased susceptibility to cancer from the agent. For example, molecular epidemiology studies that identify associations between genetic polymorphisms and inter-individual differences in cancer susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. Such studies may be particularly informative if polymorphisms are found to be modifiers of the exposure–response association, because evaluation of polymorphisms may increase the ability to detect an effect in susceptible subpopulations.

When, in the process of evaluating the studies of cancer in humans, the Working Group identifies several high-quality, informative epidemiological studies that clearly show either no positive association or an inverse association between an exposure and a specific type of cancer, a judgement may be made that, in the aggregate, they suggest evidence of lack of carcinogenicity for that cancer type. Such a judgement requires, first, that the studies strictly meet the standards of design and analysis described above. Specifically, the possibility that bias, confounding, or misclassification of exposure or outcome could explain the observed results should be considered and ruled out with reasonable confidence. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of relative effect of unity (or below unity) for any observed level of exposure, (b) when considered together, provide a combined estimate of relative risk that is at or below unity, and (c) have a narrow confidence interval. Moreover, neither any

individual well-designed and well-conducted study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It must be noted that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the exposure levels reported and the timing and route of exposure studied, to the intervals between first exposure and disease onset observed in these studies, and to the general population(s) studied (i.e. there may be susceptible subpopulations or life stages). Experience from studies of cancer in humans indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; therefore, latency periods substantially shorter than about 30 years cannot provide evidence of lack of carcinogenicity. Furthermore, there may be critical windows of exposure, for example, as with diethylstilboestrol and clear cell adenocarcinoma of the cervix and vagina ([IARC, 2012a](#)).

### 3. Studies of cancer in experimental animals

Most human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species. For some agents, carcinogenicity in experimental animals was demonstrated before epidemiological studies identified their carcinogenicity in humans. Although this observation cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, such as strong evidence that a given agent causes cancer in

experimental animals through a species-specific mechanism that does not operate in humans (see Part B, Sections 4 and 6; [Capen et al., 1999](#); [IARC, 2003](#)), these agents are considered to pose a potential carcinogenic hazard to humans. The inference of potential carcinogenic hazard to humans does not imply tumour site concordance across species ([Baan et al., 2019](#)).

#### (a) *Types of studies considered*

Relevant studies of cancer in experimental animals are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (or possibly metabolites or derivatives of the agent) (see Part A, Section 7) after a thorough evaluation of the study features (see Part B, Section 3b). Those studies that are judged to be irrelevant to the evaluation or judged to be inadequate (e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. [OECD, 2018](#)).

In addition to conventional long-term bioassays, alternative studies (e.g. in genetically engineered mouse models) may be considered in assessing carcinogenicity in experimental animals, also after a critical evaluation of the study features. For studies of certain exposures, such as viruses that typically only infect humans, use of such specialized experimental animal models may be particularly important; models include genetically engineered mice with targeted expression of viral genes to tissues from which human cancers arise, as well as humanized mice implanted with the human cells usually infected by the virus.

Other types of studies can provide supportive evidence. These include: experiments in which the agent was administered in the presence of

factors that modify carcinogenic effects (e.g. initiation–promotion studies); studies in which the end-point was not cancer but a defined precancerous lesion; and studies of cancer in non-laboratory animals (e.g. companion animals) exposed to the agent.

#### (b) *Study evaluation*

Considerations of importance in the interpretation and evaluation of a particular study include: (i) whether the agent was clearly characterized, including the nature and extent of impurities and contaminants and the stability of the agent, and, in the case of mixtures, whether the sample characterization was adequately reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration and frequency of treatment, duration of observation, and route of exposure were appropriate; (iv) whether appropriate experimental animal species and strains were evaluated; (v) whether there were adequate numbers of animals per group; (vi) whether animals were allocated randomly to groups; (vii) whether the body weight, food and water consumption, and survival of treated animals were affected by any factors other than the test agent; (viii) whether the histopathology review was adequate; and (ix) whether the data were reported and analysed adequately.

#### (c) *Outcomes and statistical analyses*

An assessment of findings of carcinogenicity in experimental animals involves consideration of (i) study features such as route, doses, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age, and duration of follow-up; (ii) the spectrum of neoplastic response, from pre-neoplastic lesions and benign tumours to malignant neoplasms; (iii) the incidence, latency, severity, and multiplicity of neoplasms and pre-neoplastic



lesions; (iv) the consistency of the results for a specific target organ or organs across studies of similar design; and (v) the possible role of modifying factors (e.g. diet, infection, stress).

Key factors for statistical analysis include: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type or lesion, and (iii) duration of survival.

Benign tumours may be combined with malignant tumours in the assessment of tumour incidence when (a) they occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) they appear to represent a stage in the progression to malignancy ([Huff et al., 1989](#)). The occurrence of lesions presumed to be pre-neoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed.

Evidence of an increased incidence of neoplasms with increasing level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms. The form of the dose–response relationship can vary widely, including non-linearity, depending on the particular agent under study and the target organ. The dose–response relationship can also be affected by differences in survival among the treatment groups.

The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose ([Peto et al., 1980](#); [Gart et al., 1986](#); [Portier and Bailer, 1989](#); [Bieler and Williams, 1993](#)). The choice of the most appropriate statistical method requires consideration of whether there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life and a survival-adjusted analysis would be warranted. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing

animals among the effective number of animals (alive at the time that the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel–Haenszel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the poly-*k* test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other, more complicated statistical procedures may be needed ([Sherman et al., 1994](#); [Dunson et al., 2003](#)).

The concurrent control group is generally the most appropriate comparison group for statistical analysis; however, for uncommon tumours, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, sex, and strain, as well as other factors, such as basal diet and general laboratory environment, which may affect tumour response rates in control animals ([Haseman et al., 1984](#); [Fung et al., 1996](#); [Greim et al., 2003](#)). It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls.

Meta-analyses and pooled analyses may be appropriate when the experimental protocols are sufficiently similar.

## 4. Mechanistic evidence

Mechanistic data may provide evidence of carcinogenicity and may also help in assessing the relevance and importance of findings of cancer in experimental animals and in humans ([Guyton et al., 2009](#); [Parkkinen et al., 2018](#)) (see Part B, Section 6). Mechanistic studies have gained in prominence, increasing in their volume, diversity, and relevance to cancer hazard evaluation, whereas studies pertinent to other streams of evidence evaluated in the *Monographs* (i.e. studies of cancer in humans and lifetime cancer bioassays in rodents) may only be available for a fraction of agents to which humans are currently exposed ([Guyton et al., 2009, 2018](#)). Mechanistic studies and data are identified, screened, and evaluated for quality and importance to the evaluation by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below.

The Working Group's synthesis reflects the extent of available evidence, summarizing groups of included studies with an emphasis on characterizing consistencies or differences in results within and across experimental designs. Greater emphasis is given to informative mechanistic evidence from human-related studies than to that from other experimental test systems, and gaps are identified. Tabulation of data may facilitate this review. The specific topics addressed in the evidence synthesis are described below.

### (a) Absorption, distribution, metabolism, and excretion

Studies of absorption, distribution, metabolism, and excretion in mammalian species are addressed in a summary fashion; exposure characterization is addressed in Part B, Section 1. The Working Group describes the metabolic fate of the agent in mammalian species, noting the metabolites that have been identified and their chemical reactivity. A metabolic schema

may indicate the relevant metabolic pathways and products and whether supporting evidence is from studies in humans and/or studies in experimental animals. Evidence on other adverse effects that indirectly confirm absorption, distribution, and/or metabolism at tumour sites is briefly summarized when direct evidence is sparse.

### (b) Evidence relevant to key characteristics of carcinogens

A review of Group 1 human carcinogens classified up to and including *IARC Monographs* Volume 100 revealed several issues relevant to improving the evaluation of mechanistic evidence for cancer hazard identification ([Smith et al., 2016](#)). First, it was noted that human carcinogens often share one or more characteristics that are related to the multiple mechanisms by which agents cause cancer. Second, different human carcinogens may exhibit a different spectrum of these key characteristics and operate through distinct mechanisms. Third, for many carcinogens evaluated before Volume 100, few data were available on some mechanisms of recognized importance in carcinogenesis, such as epigenetic alterations ([Herceg et al., 2013](#)). Fourth, there was no widely accepted method to search systematically for relevant mechanistic evidence, resulting in a lack of uniformity in the scope of mechanistic topics addressed across *IARC Monographs* evaluations.

To address these challenges, the key characteristics of human carcinogens were introduced to facilitate systematic consideration of mechanistic evidence in *IARC Monographs* evaluations ([Smith et al., 2016](#); [Guyton et al., 2018](#)). The key characteristics described by [Smith et al. \(2016\)](#) (see [Table 3](#)), such as “is genotoxic”, “is immunosuppressive”, or “modulates receptor-mediated effects”, are based on empirical observations of the chemical and biological properties associated with the human carcinogens identified by

**Table 3 The key characteristics of carcinogens**

| Ten key characteristics of carcinogens |                                                                       |
|----------------------------------------|-----------------------------------------------------------------------|
| 1.                                     | Is electrophilic or can be metabolically activated to an electrophile |
| 2.                                     | Is genotoxic                                                          |
| 3.                                     | Alters DNA repair or causes genomic instability                       |
| 4.                                     | Induces epigenetic alterations                                        |
| 5.                                     | Induces oxidative stress                                              |
| 6.                                     | Induces chronic inflammation                                          |
| 7.                                     | Is immunosuppressive                                                  |
| 8.                                     | Modulates receptor-mediated effects                                   |
| 9.                                     | Causes immortalization                                                |
| 10.                                    | Alters cell proliferation, cell death, or nutrient supply             |

From [Smith et al. \(2016\)](#).

the *IARC Monographs* programme up to and including Volume 100. The list of key characteristics and associated end-points may evolve, based on the experience of their application and as new human carcinogens are identified. Key characteristics are distinct from the “hallmarks of cancer”, which relate to the properties of cancer cells ([Hanahan and Weinberg, 2000, 2011](#)). Key characteristics are also distinct from hypothesized mechanistic pathways, which describe a sequence of biological events postulated to occur during carcinogenesis. As such, the evaluation approach based on key characteristics, outlined below, “avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence” ([National Academies of Sciences, Engineering, and Medicine, 2017](#)).

Studies in exposed humans and in human primary cells or tissues that incorporate end-points relevant to key characteristics of carcinogens are emphasized when available. For each key characteristic with adequate evidence for evaluation, studies are grouped according to whether they involve (a) humans or human primary cells or tissues or (b) experimental systems; further organization (as appropriate) is by end-point (e.g. DNA damage), duration, species, sex, strain, and target organ as well as strength of

study design. Studies investigating susceptibility related to key characteristics of carcinogens (e.g. of genetic polymorphisms, or in genetically engineered animals) can be highlighted and may provide additional support for conclusions on the strength of evidence. Findings relevant to a specific tumour type may be noted.

### (c) Other relevant evidence

Other informative evidence may be described when it is judged by the Working Group to be relevant to an evaluation of carcinogenicity and to be of sufficient importance to affect the overall evaluation. Quantitative structure–activity information, such as on specific chemical and/or biological features or activities (e.g. electrophilicity, molecular docking with receptors), may be informative. In addition, evidence that falls outside of the recognized key characteristics of carcinogens, reflecting emerging knowledge or important novel scientific developments on carcinogen mechanisms, may also be included. Available evidence relevant to criteria provided in authoritative publications (e.g. [Capen et al., 1999](#); [IARC, 2003](#)) on thyroid, kidney, urinary bladder, or other tumours in experimental animals induced by mechanisms that do not operate in humans is also described.

(d) *Study quality and importance to the evaluation*

Based on formal considerations of the quality of the studies (e.g. design, methodology, and reporting of results), the Working Group may give greater weight to some included studies.

For observational and other studies in humans, the quality of study design, exposure assessment, and assay accuracy and precision are considered, in collaboration with the Working Group members reviewing exposure characterization and studies of cancer in humans, as are other important factors, including those described above for evaluation of epidemiological evidence ([García-Closas et al., 2006, 2011](#); [Vermeulen et al., 2018](#)) (Part B, Sections 1 and 2).

In general, in experimental systems, studies of repeated doses and of chronic exposures are accorded greater importance than are studies of a single dose or time-point. Consideration is also given to factors such as the suitability of the dosing range, the extent of concurrent toxicity observed, and the completeness of reporting of the study (e.g. the source and purity of the agent, the analytical methods, and the results). Route of exposure is generally considered to be a less important factor in the evaluation of experimental studies, recognizing that the exposures and target tissues may vary across experimental models and in exposed human populations. Non-mammalian studies can be synthetically summarized when they are considered to be supportive of evidence in humans or higher organisms.

In vitro test systems can provide mechanistic insights, but important considerations include the limitations of the test system (e.g. in metabolic capabilities) as well as the suitability of a particular test article (i.e. because of physical and chemical characteristics) ([Hopkins et al., 2004](#)). For studies on some end-points, such as for traditional studies of mutations in bacteria and in mammalian cells, formal guidelines, including

those from the Organisation for Economic Co-operation and Development, may be informative in conducting the quality review ([OECD, 1997, 2016a, b](#)). However, existing guidelines will not generally cover all relevant assays, even for genotoxicity. Possible considerations when evaluating the quality of in vitro studies encompass the methodology and design (e.g. the end-point and test method, the number of replicate samples, the suitability of the concentration range, the inclusion of positive and negative controls, and the assessment of cytotoxicity) as well as reporting (e.g. of the source and purity of the agent, and of the analytical methods and results). High-content and high-throughput in vitro data can serve as an additional or supportive source of mechanistic evidence ([Chiu et al., 2018](#); [Guyton et al., 2018](#)), although large-scale screening programmes measuring a variety of end-points were designed to evaluate large chemical libraries in order to prioritize chemicals for additional toxicity testing rather than to identify the hazard of a specific chemical or chemical group.

The synthesis is focused on the evidence that is most informative for the overall evaluation. In this regard, it is of note that some human carcinogens exhibit a single or primary key characteristic, evidence of which has been influential in their cancer hazard classifications. For instance, ethylene oxide is genotoxic ([IARC, 1994](#)), 2,3,7,8-tetrachlorodibenzo-*para*-dioxin modulates receptor-mediated effects ([IARC, 1997](#)), and etoposide alters DNA repair ([IARC, 2012a](#)). Similarly, oncogenic viruses cause immortalization, and certain drugs are, by design, immunosuppressive ([IARC, 2012a, b](#)). Because non-carcinogens can also induce oxidative stress, this key characteristic should be interpreted with caution unless it is found in combination with other key characteristics ([Guyton et al., 2018](#)). Evidence for a group of key characteristics can strengthen mechanistic conclusions (e.g. “induces oxidative stress” together with “is electrophilic or can be metabolically activated to an

electrophile”, “induces chronic inflammation”, and “is immunosuppressive”); see, for example, 1-bromopropane ([IARC, 2018](#)).

## 5. Summary of data reported

### (a) *Exposure characterization*

Exposure data are summarized to identify the agent and describe its production, use, and occurrence. Information on exposure prevalence and intensity in different settings, including geographical patterns and time trends, may be included. Exposure assessment methods used in key epidemiological studies reviewed by the Working Group are described and evaluated.

### (b) *Cancer in humans*

Results of epidemiological studies pertinent to an evaluation of carcinogenicity in humans are summarized. The overall strengths and limitations of the epidemiological evidence base are highlighted to indicate how the evaluation was reached. The target organ(s) or tissue(s) in which a positive association between the agent and cancer was observed are identified. Exposure–response and other quantitative data may be summarized when available. When the available epidemiological studies pertain to a mixed exposure, process, occupation, or industry, the Working Group seeks to identify the specific agent considered to be most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data permit.

### (c) *Cancer in experimental animals*

Results pertinent to an evaluation of carcinogenicity in experimental animals are summarized to indicate how the evaluation was reached. For each animal species, study design, and route of administration, there is a statement about whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms

or pre-neoplastic lesions was observed, and the tumour sites are indicated. Special conditions resulting in tumours, such as prenatal exposure or single-dose experiments, are mentioned. Negative findings, inverse relationships, dose–response patterns, and other quantitative data are also summarized.

### (d) *Mechanistic evidence*

Results pertinent to an evaluation of the mechanistic evidence on carcinogenicity are summarized to indicate how the evaluation was reached. The summary encompasses the informative studies on absorption, distribution, metabolism, and excretion; on the key characteristics with adequate evidence for evaluation; and on any other aspects of sufficient importance to affect the overall evaluation, including on whether the agent belongs to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans, and on criteria with respect to tumours in experimental animals induced by mechanisms that do not operate in humans. For each topic addressed, the main supporting findings are highlighted from exposed humans, human cells or tissues, experimental animals, or in vitro systems. When mechanistic studies are available in exposed humans, the tumour type or target tissue studied may be specified. Gaps in the evidence are indicated (i.e. if no studies were available in exposed humans, in in vivo systems, etc.). Consistency or differences of effects across different experimental systems are emphasized.



## 6. Evaluation and rationale

Consensus evaluations of the strength of the evidence of cancer in humans, the evidence of cancer in experimental animals, and the mechanistic evidence are made using transparent criteria and defined descriptive terms. The Working Group then develops a consensus overall evaluation of the strength of the evidence of carcinogenicity for each agent under review.

An evaluation of the strength of the evidence is limited to the agents under review. When multiple agents being evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single and unified evaluation of the strength of the evidence.

The framework for these evaluations, described below, may not encompass all factors relevant to a particular evaluation of carcinogenicity. After considering all relevant scientific findings, the Working Group may exceptionally assign the agent to a different category than a strict application of the framework would indicate, while providing a clear rationale for the overall evaluation.

When there are substantial differences of scientific interpretation among the Working Group members, the overall evaluation will be based on the consensus of the Working Group. A summary of the alternative interpretations may be provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

The categories of the classification refer to the strength of the evidence that an exposure is carcinogenic and not to the risk of cancer from particular exposures. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used as descriptors of different strengths of evidence of carcinogenicity in humans; *probably carcinogenic* signifies a greater strength of evidence than *possibly carcinogenic*.

### (a) Carcinogenicity in humans

Based on the principles outlined in Part B, Section 2, the evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

**Sufficient evidence of carcinogenicity:** A causal association between exposure to the agent and human cancer has been established. That is, a positive association has been observed in the body of evidence on exposure to the agent and cancer in studies in which chance, bias, and confounding were ruled out with reasonable confidence.

**Limited evidence of carcinogenicity:** A causal interpretation of the positive association observed in the body of evidence on exposure to the agent and cancer is credible, but chance, bias, or confounding could not be ruled out with reasonable confidence.

**Inadequate evidence regarding carcinogenicity:** The available studies are of insufficient quality, consistency, or statistical precision to permit a conclusion to be drawn about the presence or the absence of a causal association between exposure and cancer, or no data on cancer in humans are available. Common findings that lead to a determination of inadequate evidence of carcinogenicity include: (a) there are no data available in humans; (b) there are data available in humans, but they are of poor quality or informativeness; and (c) there are studies of sufficient quality available in humans, but their results are inconsistent or otherwise inconclusive.

**Evidence suggesting lack of carcinogenicity:** There are several high-quality studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and the studied cancers at any observed level of exposure. The results from these studies



alone or combined should have narrow confidence intervals with an upper limit below or close to the null value (e.g. a relative risk of unity). Bias and confounding were ruled out with reasonable confidence, and the studies were considered informative. A conclusion of *evidence suggesting lack of carcinogenicity* is limited to the cancer sites, populations and life stages, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

When there is *sufficient evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a causal interpretation has been established. When there is *limited evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a positive association between exposure to the agent and the cancer(s) was observed in humans. When there is *evidence suggesting lack of carcinogenicity*, a separate sentence identifies the target organ(s) or tissue(s) where evidence of lack of carcinogenicity was observed in humans. Identification of a specific target organ or tissue as having *sufficient evidence* or *limited evidence* or *evidence suggesting lack of carcinogenicity* does not preclude the possibility that the agent may cause cancer at other sites.

#### (b) *Carcinogenicity in experimental animals*

The evidence relevant to carcinogenicity from studies in experimental animals is classified into one of the following categories:

***Sufficient evidence of carcinogenicity:*** A causal relationship has been established between exposure to the agent and cancer in experimental animals based on an increased incidence of malignant neoplasms

or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories and/or under different protocols. An increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices (GLP), can also provide *sufficient evidence*.

Exceptionally, a single study in one species and sex may be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour, or age at onset, or when there are marked findings of tumours at multiple sites.

***Limited evidence of carcinogenicity:*** The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, for example, (a) the evidence of carcinogenicity is restricted to a single experiment and does not meet the criteria for *sufficient evidence*; (b) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; (c) the agent increases tumour multiplicity or decreases tumour latency but does not increase tumour incidence; (d) the evidence of carcinogenicity is restricted to initiation–promotion studies; (e) the evidence of carcinogenicity is restricted to observational studies in non-laboratory animals (e.g. companion animals); or (f) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the available studies.

***Inadequate evidence regarding carcinogenicity:*** The studies cannot be interpreted as showing either the presence or the absence of a carcinogenic effect because of major

qualitative or quantitative limitations, or no data are available on cancer in experimental animals.

**Evidence suggesting lack of carcinogenicity:** Well-conducted studies (e.g. conducted under GLP) involving both sexes of at least two species are available showing that, within the limits of the tests used, the agent was not carcinogenic. The conclusion of *evidence suggesting lack of carcinogenicity* is limited to the species, tumour sites, age at exposure, and conditions and levels of exposure covered by the available studies.

### (c) *Mechanistic evidence*

Based on the principles outlined in Part B, Section 4, the mechanistic evidence is classified into one of the following categories:

**Strong mechanistic evidence:** Results in several different experimental systems are consistent, and the overall mechanistic database is coherent. Further support can be provided by studies that demonstrate experimentally that the suppression of key mechanistic processes leads to the suppression of tumour development. Typically, a substantial number of studies on a range of relevant end-points are available in one or more mammalian species. Quantitative structure–activity considerations, in vitro tests in non-human mammalian cells, and experiments in non-mammalian species may provide corroborating evidence but typically do not in themselves provide strong evidence. However, consistent findings across a number of different test systems in different species may provide strong evidence.

Of note, “strong” relates not to potency but to strength of evidence. The classification applies to three distinct topics:

(a) Strong evidence that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans. The considerations can go beyond quantitative structure–activity relationships to incorporate similarities in biological activity relevant to common key characteristics across dissimilar chemicals (e.g. based on molecular docking, –omics data).

(b) Strong evidence that the agent exhibits key characteristics of carcinogens. In this case, three descriptors are possible:

1. The strong evidence is in exposed humans. Findings relevant to a specific tumour type may be informative in this determination.
2. The strong evidence is in human primary cells or tissues. Specifically, the strong findings are from biological specimens obtained from humans (e.g. ex vivo exposure), from human primary cells, and/or, in some cases, from other humanized systems (e.g. a human receptor or enzyme).
3. The strong evidence is in experimental systems. This may include one or a few studies in human primary cells and tissues.

(c) Strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Certain results in experimental animals (see Part B, Section 6b) would be discounted, according to relevant criteria and considerations in authoritative publications (e.g. [Capen et al., 1999](#); [IARC, 2003](#)). Typically, this classification would not apply when there is strong mechanistic evidence that the agent exhibits key characteristics of carcinogens.

**Limited mechanistic evidence:** The evidence is suggestive, but, for example, (a) the studies cover a narrow range of experiments, relevant end-points, and/or species; (b) there are unexplained inconsistencies in the studies of similar design; and/or (c) there is unexplained incoherence across studies of different end-points or in different experimental systems.

**Inadequate mechanistic evidence:** Common findings that lead to a determination of inadequate mechanistic evidence include: (a) few or no data are available; (b) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the studies; (c) the available results are negative.

#### (d) Overall evaluation

Finally, the bodies of evidence included within each stream of evidence are considered as a whole, in order to reach an overall evaluation of the carcinogenicity of the agent to humans. The three streams of evidence are integrated and the agent is classified into one of the following categories (see [Table 4](#)), indicating that the Working Group has established that:

#### **The agent is carcinogenic to humans (Group 1)**

This category applies whenever there is *sufficient evidence of carcinogenicity* in humans.

In addition, this category may apply when there is both *strong evidence in exposed humans that the agent exhibits key characteristics of carcinogens* and *sufficient evidence of carcinogenicity* in experimental animals.

#### **The agent is probably carcinogenic to humans (Group 2A)**

This category generally applies when the Working Group has made at least *two of the following* evaluations, *including at least one* that

involves either exposed humans or human cells or tissues:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,
- *Strong evidence that the agent exhibits key characteristics of carcinogens.*

If there is *inadequate evidence regarding carcinogenicity* in humans, there should be *strong evidence in human cells or tissues that the agent exhibits key characteristics of carcinogens*. If there is *limited evidence of carcinogenicity in humans*, then the second individual evaluation may be from experimental systems (i.e. *sufficient evidence of carcinogenicity* in experimental animals or *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens*).

Additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2A.

Separately, this category generally applies if there is *strong evidence that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A*.

#### **The agent is possibly carcinogenic to humans (Group 2B)**

This category generally applies when only one of the following evaluations has been made by the Working Group:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,

**Table 4 Integration of streams of evidence in reaching overall classifications (the evidence in *bold italic* represents the basis of the overall evaluation)**

| Stream of evidence                                |                                                      |                                                                                                 | Classification based on strength of evidence                          |
|---------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|
| Evidence of cancer in humans <sup>a</sup>         | Evidence of cancer in experimental animals           | Mechanistic evidence                                                                            |                                                                       |
| <b><i>Sufficient</i></b><br>Limited or Inadequate | Not necessary<br><b><i>Sufficient</i></b>            | Not necessary<br><b><i>Strong (b)(1) (exposed humans)</i></b>                                   | <b>Carcinogenic to humans (Group 1)</b>                               |
| <b><i>Limited</i></b><br>Inadequate               | <b><i>Sufficient</i></b><br><b><i>Sufficient</i></b> | Strong (b)(2–3), Limited, or Inadequate<br><b><i>Strong (b)(2) (human cells or tissues)</i></b> | <b>Probably carcinogenic to humans (Group 2A)</b>                     |
| <b><i>Limited</i></b><br>Limited or Inadequate    | Less than Sufficient<br>Not necessary                | <b><i>Strong (b)(1–3)</i></b><br><b><i>Strong (a) (mechanistic class)</i></b>                   | <b>Possibly carcinogenic to humans (Group 2B)</b>                     |
| <b><i>Limited</i></b><br>Inadequate               | Less than Sufficient<br><b><i>Sufficient</i></b>     | Limited or Inadequate<br>Strong (b)(3), Limited, or Inadequate                                  |                                                                       |
| Inadequate                                        | Less than Sufficient                                 | <b><i>Strong (b)(1–3)</i></b>                                                                   |                                                                       |
| <b><i>Limited</i></b>                             | <b><i>Sufficient</i></b>                             | <b><i>Strong (c) (does not operate in humans)<sup>b</sup></i></b>                               |                                                                       |
| Inadequate                                        | <b><i>Sufficient</i></b>                             | <b><i>Strong (c) (does not operate in humans)<sup>b</sup></i></b>                               | <b>Not classifiable as to its carcinogenicity to humans (Group 3)</b> |
| All other situations not listed above             |                                                      |                                                                                                 |                                                                       |

<sup>a</sup> Human cancer(s) with highest evaluation.

<sup>b</sup> The *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* must specifically be for the tumour sites supporting the classification of *sufficient evidence in experimental animals*.

- *Strong evidence that the agent exhibits key characteristics of carcinogens.*

Because this category can be based on evidence from studies in experimental animals alone, there is **no** requirement that the strong mechanistic evidence be in exposed humans or in human cells or tissues. This category may be based on *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens*.

As with Group 2A, additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2B.

### ***The agent is not classifiable as to its carcinogenicity to humans (Group 3)***

Agents that do not fall into any other group are generally placed in this category.

This includes the case when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites in experimental animals, the remaining tumour sites do not support an evaluation of *sufficient evidence in experimental animals*, and other categories are not supported by data from studies in humans and mechanistic studies.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that the agent is of unknown carcinogenic potential and that there are significant gaps in research.

If the evidence suggests that the agent exhibits no carcinogenic activity, either through *evidence suggesting lack of carcinogenicity* in both humans and experimental animals, or through *evidence suggesting lack of carcinogenicity* in

experimental animals complemented by strong negative mechanistic evidence in assays relevant to human cancer, then the Working Group may add a sentence to the evaluation to characterize the agent as well-studied and without evidence of carcinogenic activity.

### (e) *Rationale*

The reasoning that the Working Group used to reach its evaluation is summarized so that the basis for the evaluation offered is transparent. This section integrates the major findings from studies of cancer in humans, cancer in experimental animals, and mechanistic evidence. It includes concise statements of the principal line(s) of argument that emerged in the deliberations of the Working Group, the conclusions of the Working Group on the strength of the evidence for each stream of evidence, an indication of the body of evidence that was pivotal to these conclusions, and an explanation of the reasoning of the Working Group in making its evaluation.

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## GENERAL REMARKS

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This one-hundred-thirty-eighth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of automotive gasoline and five oxygenated gasoline additives.

Automotive gasoline was previously evaluated by the *IARC Monographs* programme in 1988, when it was classified as *possibly carcinogenic to humans* (Group 2B) (Volume 45; [IARC, 1989](#)). The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that automotive gasoline be re-evaluated with high priority ([IARC, 2019a](#)), because new evidence had become available since the previous evaluation, including studies of cancer in humans, studies of cancer in experimental animals, and mechanistic evidence related to the key characteristics of carcinogens.

The Advisory Group also assigned high priority to the evaluation of the oxygenated additives included in the present volume. Methyl *tert*-butyl ether (MTBE) was previously evaluated as *not classifiable as to its carcinogenicity to humans* (Group 3) (Volume 73; [IARC, 1999a](#)). Ethyl *tert*-butyl ether (ETBE), *tert*-butyl alcohol (TBA), diisopropyl ether (DIPE), and *tert*-amyl methyl ether (TAME) have not been previously evaluated by the *IARC Monographs* programme.

A summary of the findings of the present volume appears in *The Lancet Oncology* ([Turner et al., 2025](#)).

### Challenges in the evaluation of automotive gasoline

The composition of automotive gasoline has changed over time and is currently strictly regulated. The Working Group evaluated studies covering a broad time period and several different countries, and they attributed the findings to the complex mixture of gasoline.

Gasoline is a complex mixture of volatile hydrocarbons derived from petroleum, including alkanes, alkenes, and aromatics (primarily C5–C10), blended with various additives. Lead-based compounds, once widely used as anti-knock agents in gasoline worldwide, have now been phased out and are prohibited globally. After the removal of lead-based compounds during the period between the 1980s and 2000s, oxygenated additives such as ethanol, MTBE, ETBE, and others were introduced to improve fuel performance, although maximum concentrations used have varied by region.

The studies of cancer in humans that were evaluated in the present volume encompassed periods when both leaded and unleaded gasoline were in use, whereas nearly all studies in experimental animals were conducted with unleaded gasoline. Most mechanistic evidence in exposed humans derived from studies conducted after 2000 that involved primarily unleaded gasoline exposure.

There is a high probability of lead exposure in earlier studies; however, the spectrum of cancer types associated with *sufficient* or *limited* evidence in humans does not generally align with that for cancer types linked to lead exposure, with the exception of stomach cancer, for which *limited* evidence exists for inorganic lead. Consequently, the Working Group did not attribute the observed findings for automotive gasoline to exposure to leaded gasoline or any specific gasoline formulation.

### Etiological relevance of the other components of gasoline for studies of cancer in humans and mechanistic studies in humans

Benzene has been previously evaluated by the IARC *Monographs* programme as *carcinogenic to humans* (Group 1) ([IARC, 2018](#)). The maximum allowed concentration of benzene has been decreased over the years and is now restricted to below 1% in most countries. The Working Group noted that the pattern of cancers with *sufficient* or *limited* evidence in humans exposed to automotive gasoline generally differs from that associated with benzene exposure, with the exception of acute myeloid leukaemia (AML), non-Hodgkin lymphoma (including chronic lymphocytic leukaemia), and multiple myeloma in adults. In addition, several mechanistic studies in exposed humans showed effects in service station attendants who were exposed to relatively low levels of benzene. Thus, the Working Group noted that the findings cannot be attributed to benzene exposure alone.

The Working Group concluded that there was *sufficient* evidence for a causal association between automotive gasoline and bladder cancer in humans. Several aromatic amines are known or suspected bladder carcinogens. For example, benzidine, 2-naphthylamine, and *ortho*-toluidine are classified as *carcinogenic to humans*

(Group 1), with *sufficient* evidence for bladder cancer in humans, and 4-chloro-*ortho*-toluidine is classified as *probably carcinogenic to humans* (Group 2A), with *limited* evidence for bladder cancer ([IARC, 2026](#)). As noted in Section 1 of the monograph on automotive gasoline, certain aromatic amines (e.g. aniline and *n*-methylaniline) have been added to gasoline to increase the octane number over the decades.

Evidence is emerging regarding the bladder carcinogenicity of the major solvent components of gasoline. In the re-evaluation of the carcinogenicity of benzene by the IARC *Monographs* programme in 2017 ([IARC, 2018](#)), the evidence for bladder cancer in humans was considered to be *inadequate*, but the available studies were small, few in number, and had little quantitative exposure information. A large, high-quality study in the USA recently found a strong association between quantitative metrics of benzene, toluene, and xylene (BTX) exposure and the incidence of bladder cancer ([Xie et al., 2024](#)). This study team also reported evidence that participants carrying known bladder cancer susceptibility genetic loci were at even higher risk of bladder cancer, providing a mechanistic linkage between exposure to BTX and bladder cancer ([Tadesse et al., 2025](#)).

The Working Group noted that exposure to both gasoline vapours and engine exhausts may occur in some occupational settings. Available measurements indicated that concentrations of gasoline vapours were higher in service stations than in other urban ambient environments. Measurements of pollutants from diesel and gasoline engine exhaust, however, were generally similar in service stations and in urban ambient air. These findings were interpreted as indicating higher occupational exposure to gasoline vapours, but similar exposure to engine exhausts, among service station attendants compared with the general population.



Diesel exhaust and gasoline exhaust have been evaluated previously by the *IARC Monographs* programme in 2012 as Group 1 and Group 2B, with *sufficient* evidence that diesel exhaust causes cancer of the lung (Volume 105; [IARC, 2013](#)). As lung cancer was not consistently more frequent in studies of individuals exposed to automotive gasoline, the concerns that confounding by diesel exhaust was responsible for the observed increases in bladder cancer and adult AML were allayed.

### Evaluation of myelodysplastic syndrome in humans

The Working Group concluded that the evidence was *limited* for myelodysplastic syndrome (MDS) in humans. This is the first evaluation of this type of cancer within the *IARC Monographs* programme. MDS, currently referred to as “myelodysplastic neoplasm” ([WHO Classification of Tumours Editorial Board, 2024](#)), is a group of haematological neoplasms characterized by clonal expansion of myeloid stem cells with cytopenia, morphological dysplasia, and progressive clinical ineffective haematopoiesis ([WHO Classification of Tumours Editorial Board, 2024](#)). In nearly 30% of cases, MDS could progress to AML; however, risk of progression to AML is highly variable between patients. The incidence of MDS increases with age, with a median age at diagnosis of 77 years ([WHO Classification of Tumours Editorial Board, 2024](#); [Bewersdorf et al., 2026](#)). In its evaluation of automotive gasoline, the Working Group concluded that there was *sufficient* evidence for AML and *limited* evidence for MDS. The *sufficient* evaluation for AML was strengthened by the evidence from studies estimating the association between gasoline and MDS, which was consistent with a credible positive association. However, results were generally weaker for MDS than for AML; therefore, the Working Group could not exclude

chance, bias, and confounding, and concluded that the evidence was *limited* for MDS.

### Mechanistic evaluation of kidney tumours in male rats

The Working Group noted that the development of renal tumours in male rats exposed to automotive gasoline or MTBE has been reported to be mediated by a male rat-specific mechanism involving increased accumulation of  $\alpha_{2u}$ -globulin. Several studies on  $\alpha_{2u}$ -globulin nephropathy in male rats and its role in renal tumorigenesis were assessed by the Working Group (see the monographs on automotive gasoline and MTBE, Section 4.3). An IARC Scientific Publication discusses seven criteria that need to be met to conclude that renal tubule tumours arising in male rats are caused only by a mechanism that is specific to male rats. The Working Group concluded that not all the seven criteria were met ([IARC, 1999b](#)). The Working Group noted that the first criterion that needs to be considered is that an agent causing renal tumours should give negative results in a battery of genotoxicity tests, thereby supporting a non-genotoxic mechanism of tumour formation. There was consistent and coherent evidence that automotive gasoline is genotoxic in exposed humans, and there was also suggestive evidence that MTBE is genotoxic in experimental systems. In addition, in light of the most recent scientific literature and the present evaluation based upon the latest revision of the *IARC Monographs* Preamble ([IARC, 2019b](#)), it was noted that these criteria would have benefited from an update, as they no longer accounted for evidence related to all 10 key characteristics of carcinogens. Therefore, the Working Group concluded that the available mechanistic evidence did not support attributing the kidney carcinogenicity of automotive gasoline or MTBE in male rats solely to an  $\alpha_{2u}$ -globulin-mediated male rat-specific mechanism.

The evidence of carcinogenicity in experimental animals for automotive gasoline and MTBE was considered *sufficient*, and this determination would have been reached even if the findings of renal adenomas and carcinomas observed in male rats had been excluded. This evaluation was based also on compelling evidence of statistically significant increases in the incidence of tumours at multiple additional sites, besides kidney, in mice and rats exposed to automotive gasoline or MTBE and, for MTBE, on the occurrence of the rare tumour type, brain astrocytoma.

## Gaps in research for human cancer and mechanistic evidence

Research gaps were noted by the Working Group. Notably, a study on cancer incidence within households affected by a gasoline spill in Pennsylvania, USA, provided evidence complementary to studies in service station attendants or people with occupational exposure to gasoline. However, only one study of cancer incidence after a gasoline spill was available to the Working Group. Further studies of cancer incidence within other areas affected by gasoline spills may provide additional information to complement the available studies on occupational cancer.

For childhood acute lymphoblastic leukaemia, studies of cancer incidence in relation to residential proximity to gas stations were informative. Additional studies using linkage between data on geographical residence and cancer incidence may provide useful information in relation to cancer types related to automotive gasoline exposure.

In the evaluation of automotive gasoline, informative studies on human cancer were primarily conducted in high-income countries and were generally performed earlier than were the mechanistic studies. In contrast, mechanistic studies in exposed humans have been conducted

across more diverse geographical settings, including many low- and middle-income countries (LMICs), highlighting a gap in contemporary epidemiological evidence of cancer from these regions. However, the available studies on cancer in humans were still considered to be relevant in the context of LMICs, as they were conducted when vapour control technologies were not widely implemented.

The Working Group noted that, whereas certain exposure settings (such as those involving service station attendants) have been relatively well characterized in the literature, important gaps remain in the assessment of both historical and current gasoline exposures among workers and in the general population. Four examples are listed here. Firstly, vapour recovery systems are critical for reducing emissions of volatile organic compounds from fuel stations; although widely implemented across countries, there is still limited available information on their deployment and effectiveness, particularly in LMICs, which hinders accurate exposure assessment in these populations. Secondly, exposure of the general population outside service station areas is not well documented, despite its likely occurrence. Vehicles can emit gasoline vapours through evaporative losses, especially during prolonged parking. The resulting gasoline vapour concentrations in enclosed environments such as garages remain insufficiently characterized. Thirdly, additional exposure scenarios, including the use of automotive gasoline in small engines (e.g. lawnmowers) or as a solvent, are not well described. There is also a gap in documentation on the nature of additives used in such settings, where regulations on gasoline composition are less stringent. Lastly, benzene is commonly used as a marker of gasoline exposure in occupational settings; however, its levels may not be specific to gasoline exposure, because it is also a component of ambient air pollution and engine exhaust. Further data are needed to improve the interpretation of measurements of benzene and volatile

organic compounds in occupations with mixed exposures, such as mechanics, tanker drivers, and fuel distribution workers. Gasoline distribution workers, especially before the implementation of the use of closed systems, have been among those workers with the highest gasoline exposures. However, compared with service station attendants, there were few available mechanistic studies related to this occupation.

## Scope of the systematic review

Standardized searches of the PubMed database (NCBI, 2024) were conducted for automotive gasoline and oxygenated additives for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). The literature trees for automotive gasoline and oxygenated additives, including the full set of search terms for each agent name and each outcome type, are available online.<sup>a</sup>

As described in the Preamble to the *IARC Monographs* (last revised in 2019; IARC, 2019b), the Working Group reviews publicly available scientific data, such as peer-reviewed papers in the scientific literature, and may also review unpublished reports, if made available in their final form by governmental agencies and if they contain enough detail for critical review. A public Call for Data was opened on the IARC website 1 year ahead of the meeting for Volume 138. Eligible studies were only those published or accepted for publication in the openly available scientific literature by the time of the Working Group meeting.

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<sup>a</sup> The literature trees for the monographs in the present volume are available at: <https://hawcproject.iarc.who.int/assessment/748/> (automotive gasoline) and <https://hawcproject.iarc.who.int/assessment/747/> (oxygenated gasoline additives).

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# AUTOMOTIVE GASOLINE

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## 1. Exposure Characterization

### 1.1 Identification of the agent

#### 1.1.1 Nomenclature, synonyms, and trade names

##### *Automotive gasoline*

Automotive gasoline is a generic term used to describe a commercial product that is a complex mixture of volatile hydrocarbons derived from petroleum and blended with additives. It is primarily used in internal combustion engines, mainly passenger cars and other types of vehicles. The composition of gasoline varies according to the crude oil used, the refinery processes available, and the product specifications. The main hydrocarbons present in gasoline are alkanes, alkenes (olefins), isoalkanes, cycloalkanes, cycloalkenes, and aromatics, including benzene ([IARC, 1989](#); [Kaplan et al., 1997](#); [Hegedüs et al., 2024](#)). Additives and blending agents are added to the hydrocarbon mixture derived from petroleum distillation to improve the performance and stability of the gasoline (see Section 1.2; [Lane, 1980](#); [IARC, 1989](#)). These compounds include anti-knock agents such as ethanol or other oxygenated additives, antioxidants, metal deactivators, anti-rust agents, anti-icing agents, upper-cylinder lubricants, detergents, and dyes ([Lane, 1980](#); [IARC, 1989](#); [Stolark, 2016](#)). At the

end of the production process, the finished gasoline typically contains more than 150 separate compounds, although as many as 1000 compounds have been estimated to be present in some blends ([Domask, 1984](#); [Mehlman, 1990](#)). [The Working Group noted that other types of fuel, such as aviation gasoline, marine gasoline, and diesel fuel, were not included in the definition of the agent. Alternative fuels containing higher proportions of ethanol (commonly above 10–15%) that are required for specific engines were not evaluated in the present monograph. In addition, individual components of gasoline, such as benzene, were not evaluated separately. Five oxygenated gasoline additives were evaluated in the other monographs in the present volume. The Working Group also noted that engine combustion emissions and the resulting air pollution are not included in the definition of the agent.]

*Chem. Abstr. Serv. Reg. No.:* 86290-81-5 ([NCBI, 2025](#))

[The Working Group noted that Chemical Abstracts Service (CAS) Registration No. 8006-61-9 defines natural gasoline, which is produced from natural gas and is not used in modern internal combustion engines. This was not evaluated in the present monograph.]

*EC/List No.:* 289-220-8 ([ECHA, 2023](#))



*Synonyms and registered trade names:* petrol [the Working Group noted that “petrol” is used in some English-speaking countries and is considered to be equivalent to automotive gasoline in this context]; benzine; automotive fuel; automotive gasoline (more extensive list available in: [NIOSH, 2019](#); [ECHA, 2023, 2025a](#)).

### 1.1.2 Chemical and physical properties

[The chemical composition of gasoline is highly variable, because a product with automotive fuel characteristics can be formulated in a number of ways, and therefore its properties vary.]

*Description:* gasoline is a volatile, flammable, clear, colourless to amber-coloured liquid with a petroleum-like odour ([NCBI, 2025](#))

*Boiling-point:* 32–210 °C [the Working Group noted that the size of this range is the result of gasoline being composed of multiple compounds with various boiling-points] ([NCBI, 2025](#))

*Melting point:* –90.5 to –95.4 °C ([NCBI, 2025](#))

*Flashpoint:* –46 to –38 °C ([NCBI, 2025](#))

*Autoignition temperature:* 280 °C ([NCBI, 2025](#))

*Density:* 0.7–0.8 g/cm<sup>3</sup> ([NCBI, 2025](#))

*Solubility:* insoluble in water; soluble in ethanol, ether, and chloroform ([NCBI, 2025](#))

*Vapour pressure:* 41–78 kPa (see [Table 1.1](#)).

### 1.1.3 Chemical composition

The chemical composition of gasoline is highly variable, because a product with specific automotive fuel properties can be formulated in different ways. Therefore, its composition is determined on the basis of aspects such as the required performance, legal specifications, refining facilities available in a specific region, crude source,

and petroleum product specifications ([Concawe, 1987](#); [IARC, 1989](#)). [The Working Group noted that few data were publicly available on the composition and production volumes of gasoline and its additives. Because independently verifiable data were lacking, data published by Concawe, the science division of a trade organization, are presented.] The hydrocarbon components are predominantly in the range of C5–C10 with an overall range of C4–C12. The legal requirements for the properties and composition of gasoline are presented in [Table 1.1](#). Whereas the maximal permissible contents of some components, such as the total aromatic content and the benzene and lead contents, are similar internationally, other requirements, such as the maximum amount of ethanol or oxygenates permitted, differ between countries.

[The Working Group noted that the composition of gasoline varies widely and depends on many factors, including the season. In the absence of comprehensive data on the historic gasoline composition in different countries, several studies are discussed that only displayed snapshots of gasoline composition at specific points in time or in specific geographical locations.]

For example, the typical composition of gasoline in the 1980s was 25–40% isoalkanes, 20–50% total aromatics (including 0.5–2.5% benzene), 4–8% alkanes, 3–7% cycloalkanes, 2–5% alkenes, and 1–4% cycloalkenes (all percentage volume) ([IARC, 1989](#)). The composition rapidly changed in the 1990s in California, USA, when the contents of both aromatics and alkenes were reduced ([Harley and Kean, 2004](#)). More recently, gasoline has principally consisted of 55–77% saturated hydrocarbons, with 9–36% aromatics, some unsaturated hydrocarbons, and <10% ethanol; the benzene content has been reduced ([Chin and Batterman, 2012](#)). In the USA, gasoline contained an average of approximately 1.5% benzene between 1970 and 1990, but this may have reached 5% by volume ([Infante et al., 1990](#)). Historically, gasoline in most European

**Table 1.1 Regulatory requirements for automotive gasoline in selected countries**

| Parameters                                                     | Units | EU Directive<br>2009/30/EC |       | Brazil<br>ANP 807 |       | China<br>GB17930 |       | Australia<br>F2019L00455 |       | Japan                      |         | Thailand         |          |
|----------------------------------------------------------------|-------|----------------------------|-------|-------------------|-------|------------------|-------|--------------------------|-------|----------------------------|---------|------------------|----------|
|                                                                |       | Min.                       | Max.  | Min.              | Max.  | Min.             | Max.  | Min.                     | Max.  | Min.                       | Max.    | Min.             | Max.     |
| Research octane number (RON)                                   | NA    | 95                         |       | 91                |       | 95               |       | 95                       |       | Regular, 89<br>Premium, 96 |         | 91               | 95       |
| Motor octane number                                            | NA    | 85                         |       | NR                |       | 90               |       | 95                       |       |                            |         | 80               |          |
| Vapour pressure, summer period                                 | kPa   | NR                         | 60    |                   | 62    |                  | 65    |                          | 65    | 41                         | 78      |                  | 62       |
| <i>Distillation:</i>                                           |       |                            |       |                   |       |                  |       |                          |       |                            |         |                  |          |
| Percentage evaporated                                          | % v/v | 46% at<br>100 °C           |       | 50% at<br>120 °C  |       | 50% at<br>110 °C |       |                          |       |                            |         | 50% at<br>70 °C  |          |
|                                                                |       | 75% at<br>150 °C           |       | 90% at<br>190 °C  |       | 90% at<br>190 °C |       |                          |       |                            |         | 90% at<br>170 °C |          |
| <i>Hydrocarbon analysis:</i>                                   |       |                            |       |                   |       |                  |       |                          |       |                            |         |                  |          |
| Olefins                                                        | % v/v |                            | 18.0  |                   | 25    |                  | 15    |                          | 18    |                            |         |                  | 18       |
| Aromatics                                                      | % v/v |                            | 35.0  |                   | 35    |                  | 35    |                          | 45    |                            |         |                  | 35       |
| Benzene                                                        | % v/v |                            | 1     |                   | 1     |                  | 0.8   |                          | 1     |                            | 1       |                  | 1.0      |
| Oxygen content                                                 | % m/m |                            | 3.7   |                   |       |                  | 2.7   |                          | 2.7   |                            | 1.3     |                  |          |
| <i>Oxygenates:</i>                                             |       |                            |       |                   |       |                  |       |                          |       |                            |         |                  |          |
| Methanol                                                       | % v/v |                            | 3.0   | 18                | 25    |                  | 0.3   |                          | 10    |                            | ND      |                  | 9        |
| Ethanol<br>(stabilizing agents may<br>be necessary)            | % v/v |                            | 10.0  |                   |       |                  |       |                          | 0.5   |                            | 3       |                  |          |
| Iso-propyl alcohol                                             | % v/v |                            | 12.0  |                   |       |                  |       |                          | 1     |                            |         |                  |          |
| <i>tert</i> -Butyl alcohol                                     | % v/v |                            | 15.0  |                   |       |                  |       |                          |       |                            |         |                  |          |
| Iso-butyl alcohol                                              | % v/v |                            | 15.0  |                   |       |                  |       |                          |       |                            |         |                  |          |
| Ethers containing five or<br>more carbon atoms per<br>molecule | % v/v |                            | 22.0  |                   |       |                  |       |                          |       |                            |         |                  |          |
| Other oxygenates                                               | % v/v |                            | 15.0  |                   |       |                  |       |                          |       |                            | MTBE, 7 | MTBE, 5.5        | MTBE, 11 |
| <i>Other:</i>                                                  |       |                            |       |                   |       |                  |       |                          |       |                            |         |                  |          |
| Sulfur content                                                 | mg/kg | NR                         | 10    |                   | 50    |                  | 10    |                          | 50    |                            | 10      |                  | 50       |
| Lead content                                                   | g/L   | NR                         | 0.005 |                   | 0.005 |                  | 0.005 |                          | 0.005 |                            | ND      |                  | 0.005    |

EU, European Union; max., maximum; min., minimum; MTBE, methyl *tert*-butyl ether; % m/m, percentage by mass (wt%); NA, not applicable; ND, not detected; NR, not reported; % v/v, percentage by volume.

From [European Union \(2009\)](#); [ICCT \(2011\)](#); [Ministry of Energy \(2019\)](#); [Hegedüs et al. \(2024\)](#); [Ministry of Justice Japan \(2025\)](#).

countries contained more benzene than did varieties in the USA, a trend that persisted through 1994 ([Deschamps, 1995](#)), but benzene content is now limited to 1% ([Table 1.1](#)). In the United Kingdom (UK), gasoline was reported to contain on average 2% benzene in 1993 ([TBV Science, 1993](#)). According to data available from the United States Environmental Protection Agency (US EPA), the average content of total aromatics and of benzene in gasoline in the United States of America (USA) decreased from 25% to 19% and from 1% to 0.6%, respectively, between 2000 and 2015 ([Fig. 1.1](#); [US EPA, 2025a](#)). Methyl *tert*-butyl ether (MTBE) was present in gasoline in the USA at an average of approximately 3% until 2005, after which its use was phased out. At the same time, the average content of ethanol increased from 1% in 2000 to 7% in 2010.

[Chin and Batterman \(2012\)](#) investigated the fuel and vapour compositions of current and commercially available fuels, including gasoline (< 10% ethanol content). [The Working Group noted that the 29 measured compounds accounted for only a portion (15% by weight) of gasoline, and therefore the rankings represented only the measured compounds.] Of the 29 target volatile organic compounds (VOCs), the five compounds with the highest concentrations measured were toluene (15 g/L in gasoline; classified by IARC in Group 3, *not classifiable as to its carcinogenicity to humans*), *n*-heptane (13 g/L), 1,2,4-trimethylbenzene (11 g/L), cyclohexane (10 g/L), and *p*- and *m*-xylene (9 g/L; IARC Group 3), accounting for 51% of the total VOC concentration. Other compounds of interest that were measured and are commonly used as exposure indicators for gasoline were benzene (6 g/L; classified by IARC in Group 1, *carcinogenic to humans*) and ethylbenzene (3 g/L; classified by IARC in Group 2B, *possibly carcinogenic to humans*).

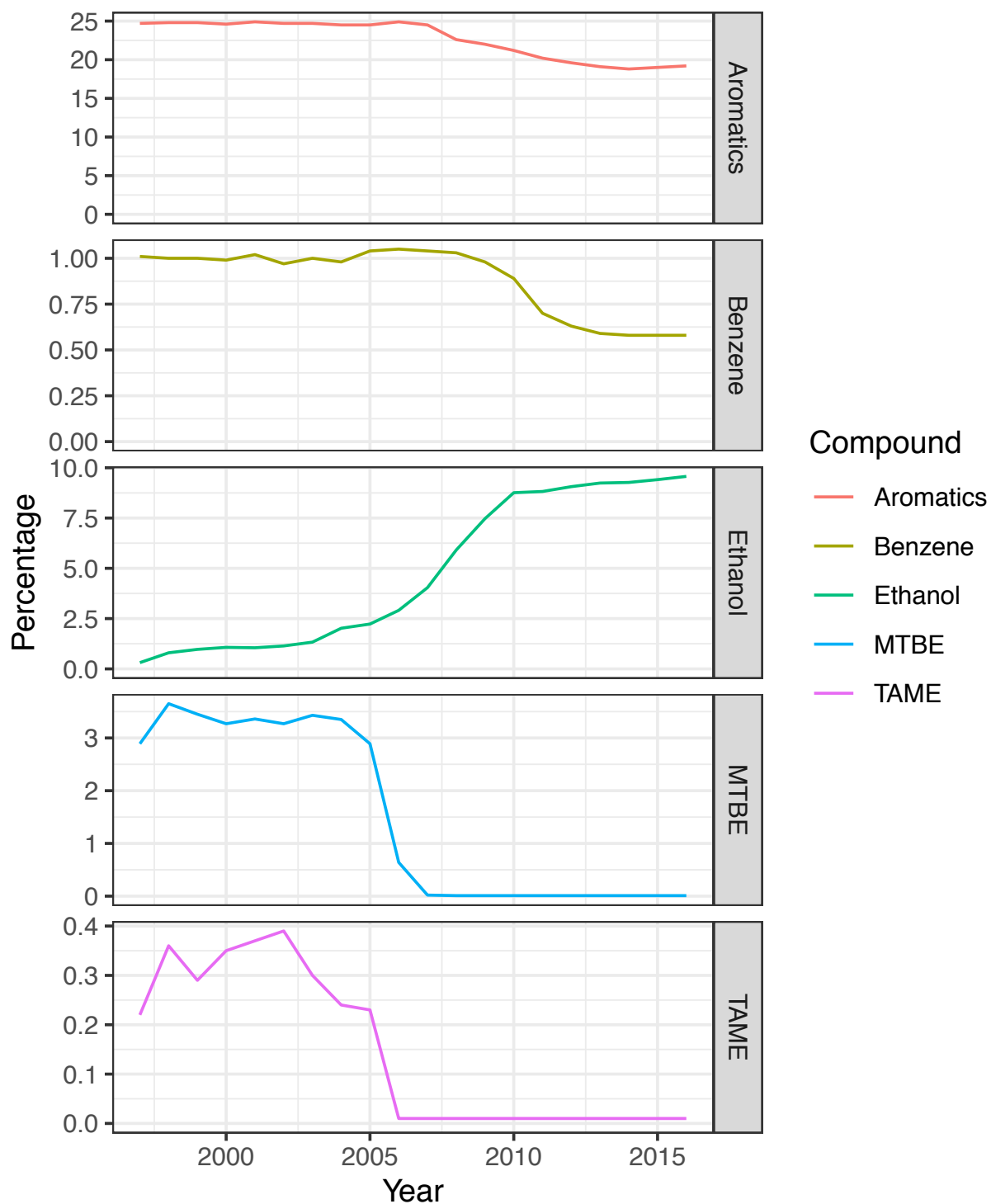
### 1.1.4 Additives and octane number

#### (a) Engine knocking and octane number

In addition to petroleum distillates, gasoline contains various blending components that help with the desired performance characteristics (see also Section 1.2 on gasoline production). In an internal combustion engine, gasoline is vapourized, mixed with air, and compressed before ignition ([Concawe, 2019](#)). However, under high pressure, the fuel/air mixture can spontaneously ignite before the spark plug fires, causing engine knocking. Knocking reduces combustion efficiency and engine performance and longevity.

To indicate a fuel's resistance to knocking, an octane number is assigned ([Badia et al., 2021](#); [US EIA, 2025](#)). The research octane number (RON) is a key measurement for evaluating knock resistance. It is determined by comparing the ignition properties of a gasoline sample to those of a reference mixture containing iso-octane and *n*-heptane ([Sobarsah et al., 2021](#)). Iso-octane, which is highly resistant to knocking, has been assigned an octane number of 100, whereas *n*-heptane, which is very prone to knocking, has been assigned an octane number of 0 ([Concawe, 2019](#)). Thus, the higher the octane number, the greater the fuel's ability to resist premature ignition. A high octane number makes a fuel suitable for high-performance engines that operate with higher levels of compression. [Table 1.2](#) lists the octane ratings for common types of gasoline.

Additives that increase the octane rating have taken many forms over the years ([Badia et al., 2021](#)). Historically, lead alkyls were used to meet the octane requirements, but virtually all the gasoline available worldwide was unleaded by 2021 ([US EPA, 1985](#); [Concawe, 1997](#); [Lacerda et al., 2023](#)). Lead was initially replaced by increasing the proportion of aromatics, but these compounds were then largely replaced by various oxygenated additives ([Stolark, 2016](#)).

**Fig. 1.1 Average content of specific components in gasoline in the USA from 1997 to 2017**

MTBE, methyl *tert*-butyl ether; TAME, *tert*-amyl methyl ether.  
Note the different scales on the y axes of the panels.  
Created by the Working Group with data from [US EPA \(2025a\)](#).

**Table 1.2 Octane rating of common types of gasoline**

| Gasoline type          | Octane rating                                     | Common use                                                                             |
|------------------------|---------------------------------------------------|----------------------------------------------------------------------------------------|
| Regular gasoline       | Generally 87 (in the USA) or 91 (in Europe)       | Ideal for most passenger vehicles with standard engines                                |
| Premium gasoline       | Generally 91–93 (in the USA) or 95–98 (in Europe) | Recommended for vehicles with high-compression engines, such as sports and luxury cars |
| Super premium gasoline | > 93 (in the USA) or > 98 (in Europe)             | Used in some high-performance and luxury vehicles                                      |

USA, United States of America.  
Data from [INSPENET \(2024\)](#).

*(b) Oxygenated gasoline additives*

The oxygenates that replaced lead and increased the octane number are mainly ethers and alcohols such as MTBE, ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), diisopropyl ether (DIPE), *tert*-butyl alcohol (TBA), and ethanol ([IARC, 1989](#); [Concawe, 2012](#); [Shi et al., 2024](#)). These oxygenates can also enhance fuel combustion and thereby reduce exhaust emissions ([Abdellatief et al., 2021](#); [US EIA, 2025](#)). The cancer hazard evaluations for several of these oxygenates can be found in dedicated monographs in the present volume.

MTBE was introduced in the USA in the 1980s but has since been replaced with ethanol (see [Fig. 1.1](#)). In Europe, both oxygenated ethers, such as MTBE and more recently ETBE, and ethanol are used as additives to increase the RON ([Concawe, 2012](#)). [The Working Group noted that most automotive gasoline nowadays contains some oxygenated additives. However, fuels such as gasohol, containing high proportions of additives such as ethanol, which are required for specific engines, are not included in the definition of the agent.]

In 2018, Concawe [the science division of an oil industry trade organization] updated a report first published in 2012, compiling data on the ether oxygenates present in gasoline available in Europe ([Concawe, 2012](#)). [Table 1.3](#) presents data on the ether oxygenates measured in gasoline obtained from the Société Générale

de Surveillance database from winter 1999–2000 to summer 2009 in 27 European countries. These data were obtained by the analysis of 1239 samples in which the MTBE, ETBE, and TBA contents were measured, and 650 samples in which the TAME and DIPE contents were measured ([Concawe, 2018](#)). The mean Europe-wide concentrations were 5.39, 0.91, 0.29, < 0.01, and 0.03 mass per mass percentage (m/m%) (or wt%) for MTBE, ETBE, TAME, DIPE, and TBA, respectively.

The same report also provided the ethanol content of gasoline, which increased between the surveys performed in 2006–2009 (0.76 m/m%), 2010 (3.42 m/m%), and 2015 (4.60 m/m%) (wt%) in four European countries (France, Italy, Spain, and the UK) ([Concawe, 2018](#)).

Modern commercial gasoline is also classified by the maximum content of biofuel, such as ethanol, in gasoline ([European Commission, 2018](#)). E5 includes a maximum of 5% biofuel, E10 contains a maximum of 10%, etc. This information is crucial, because older engines (made before 2000) might not be compatible with high biofuel contents ([Automobile Association, 2019](#)). [The Working Group noted that fuels with high ethanol contents, known as “gasohol”, such as E85, were not included in the definition of the agent (see Section 1.1.1).]



**Table 1.3 Concentration of ether oxygenates in gasoline from winter 1999–2000 to summer 2009 in 27 countries in Europe**

| Concentration                                                                 | Ether oxygenates                   |                                        |                                         |                                       |                                            |
|-------------------------------------------------------------------------------|------------------------------------|----------------------------------------|-----------------------------------------|---------------------------------------|--------------------------------------------|
|                                                                               | MTBE                               | ETBE                                   | TAME                                    | DIPE                                  | TBA                                        |
| Mean concentration in all European gasoline samples during 1999–2009 (m/m%)   | 5.39                               | 0.91                                   | 0.29                                    | < 0.01                                | 0.03                                       |
| Median concentration in all European gasoline samples during 1999–2009 (m/m%) | 4.25                               | < 0.01                                 | < 0.01                                  | < 0.01                                | 0.01                                       |
| Maximum concentration in individual sampling rounds (m/m%), period, country   | 20.43<br>Summer<br>2001<br>Romania | 15.50<br>Winter<br>2008/2009<br>France | 11.22<br>Winter<br>2007/2008<br>Finland | 0.88<br>Winter<br>2002/2003<br>Greece | 1.03<br>Winter<br>2000/2001<br>Switzerland |

DIPE, diisopropyl ether; ETBE, ethyl *tert*-butyl ether; m/m%, percentage by mass; MTBE, methyl *tert*-butyl ether; TAME, *tert*-amyl methyl ether; TBA, *tert*-butyl alcohol.

Data from [Concawe \(2012, 2018\)](#).

### (c) Aromatic amines

Since the 1920s, aromatic amines have also been added to gasoline to increase the octane number. Some examples of these amines are aniline (IARC Group 2A), *n*-methylaniline, and 2,4-dimethyl-aniline ([Brown et al., 1955](#); [Mamytov et al., 2012](#); [Badia et al., 2021](#)). These aromatic amines have been added to various types of gasoline in various volumes (%). For instance, aniline has been added at 1–2% volume (RON, 85.6) and 2,4-dimethyl-aniline and *n*-methylaniline have been added at 1–3% volume (RON, 85.6) ([Badia et al., 2021](#)). *n*-Methylaniline is the most relevant, because it causes a substantial increase in RON, but is associated with the least gum formation (formation of a deposit in the fuel tank), which is one of the main drawbacks of adding aromatic amines ([Danilov, 2001](#)). Higher concentrations are permitted by most regulatory agencies if a detergent and a fuel combustion modifier are also added to the gasoline to prevent gum formation. China banned the use of *n*-methylaniline as a gasoline additive in 2013 ([ACFA, 2016](#)), but in the European Union (EU) it can still be used for this purpose. The most relevant use of the substance in the EU is as

a gasoline additive, with an aggregated registered use of this substance of 100–1000 tonnes per year (reported as an estimated tonnage on the European Chemicals Agency public chemicals database, ECHA CHEM) ([Bureau for Chemical Substances, 2024](#)).

### (d) Manganese compounds

Methylcyclopentadienyl manganese tricarbonyl (MMT), an organic derivative of manganese, was used initially as an anti-knocking agent, replacing lead, in many regions of the world. Researchers have noted a decrease in its use in recent years, and manganese is now prohibited in the fuel specifications of most countries, especially high-income countries ([Badia et al., 2021](#)).

As of 2004, the legal limit for manganese in gasoline in the USA was 8.3 mg/L ([Blumberg and Walsh, 2004](#)), and the addition of MMT to reformulated gasoline was not allowed.

MMT has been added to unleaded gasoline in Canada since 1976, and it was used in 90% of gasoline as a replacement for lead when its use was discontinued in 1991 ([Zayed et al., 2003](#); [Blumberg and Walsh, 2004](#)). The mean MMT concentration in Canadian gasoline equated

to a manganese level of 6.3–10.8 mg/L, which was significantly below the limit of 18 mg/L ([Environment Canada, 2003](#)). The Manganese-based Fuel Additives Act now bans adding these to unleaded gasoline in Canada ([Government of Canada, 2025](#)).

A survey conducted in Latvia showed manganese levels of up to 55 mg/L in gasoline ([Schindler, 2004](#)). The specifications for gasoline intended for export from the Kingdom of the Netherlands to low- and middle-income countries (LMICs) outside the EU from 1 April 2023 permitted a maximum manganese concentration of 2 mg/L in gasoline. In Europe, the manganese content of gasoline has been limited to a maximum of 6 mg/L since 2009, and the upper limit was reduced to 2 mg/L in 2014. The addition of manganese to gasoline is prohibited in Europe ([HETI, 2022](#)).

In a survey in China during 2000–2003, the maximum concentration of manganese measured in gasoline was 30 mg/L, but >30% of gasoline contained no manganese, approximately 50% contained manganese at <5 mg/L, and approximately 10% contained manganese at 5–10 mg/L ([Schindler, 2004](#)).

As of 2023, manganese was still common in gasoline in several African countries, south Asia, and the Russian Federation. The highest concentrations were detected in Senegal (65 mg/kg), Gabon (59 mg/kg), and Mauritania (49 mg/kg) ([Fuelsandlubes, 2023](#)).

#### (e) Other additives

Other additives with various functions can also be included in gasoline, such as detergents (e.g. aminohydroxyamide), anti-rust agents (e.g. fatty acid amines and sulfonates), antioxidants (e.g. aminophenols), dyes (e.g. alkyl derivatives of azobenzene-4-azo-2-naphthol), anti-icing agents (e.g. glycols), scavengers (e.g. 1,2-dibromoethane, also known as ethylene dibromide, which was historically added to leaded gasoline to prevent the build-up of lead oxide deposits in

engines), and many others, depending on the fuel requirements and regulations in place in a specific region or country (for more details, see Section 1.5) ([IARC, 1989](#); [Hatzinger et al., 2018](#); [Badia et al., 2021](#)). [The Working Group noted that the first reference was fairly dated, and it was not clear if these additives mentioned above were still in use.]

#### 1.1.5 Composition of gasoline vapour

The composition of gasoline vapour significantly differs from that of the bulk liquid, owing to the differing vapour pressures of the individual components. For example, aromatic hydrocarbons such as toluene, trimethylbenzene, and xylenes make up a significant proportion of liquid gasoline, but their proportions in the vapour phase are far lower, because aromatics have lower vapour pressures than the lower-molecular-weight components ([Concawe, 1987](#)). In a study performed by Concawe, the vapours from several European gasoline mixtures were found to contain on average 80% C3–C5 hydrocarbons by volume, compared with 26% by volume for the equivalent liquid phases. For example, *n*-pentane was present in liquid gasoline at 9.7% and in gasoline vapour at 23.3%. For the aromatics, the corresponding figures were <5% by volume in the vapour and 45% by volume in the liquid ([Concawe, 1987](#)). However, several factors may influence the composition of gasoline vapour. A study in the USA aimed to measure the concentration of benzene in the vapours associated with two different brands of gasoline ([McMullen et al., 2014](#)). It was shown that benzene concentrations (0–0.2 ppm) were affected by the atmospheric conditions. The atmospheric conditions that varied the most between the two sampling days were the relative humidity and the dew point; when these were high, the benzene concentrations were highest ([McMullen et al., 2014](#)).

### 1.1.6 Impurities

Other substances that are recognized as impurities may also be present in gasoline.

A common impurity in gasoline is sulfur. It is present in crude oil but can be removed during the refining process. This impurity can lead to higher emissions of nitrogen and sulfur oxides ( $\text{NO}_x$  and  $\text{SO}_x$ ) and higher fuel consumption and, at worst, can permanently damage engines ([Blumberg et al., 2003](#); [Schifter et al., 2003](#); [OICA, 2025](#)). In a study in which data on the sulfur content of gasoline from 16 European countries were assessed, mean values ranged between [0 and 700] mg/kg gasoline ([Concawe, 1998](#)). The limits for the sulfur content of gasoline can be found in [Table 1.1](#).

The adulteration of automotive gasoline is common in some LMICs. Typical adulterants include kerosene, ethanol, thinner, turpentine, and benzene ([Muralikrishna et al., 2006](#); [Osueke and Ofondu, 2011](#); [Kulathunga and Mahanama, 2013](#); [Fadairo et al., 2020](#); [Gonçalves et al., 2022](#)).

## 1.2 Production and use

### 1.2.1 Production

#### (a) Production process

Gasoline is produced in petroleum refineries that convert (refine) crude oil into petroleum products, which are used in fuels for transportation (such as gasoline), heating, paving roads, and generating electricity, and as feedstocks for the production of other chemicals ([US EIA, 2023](#)).

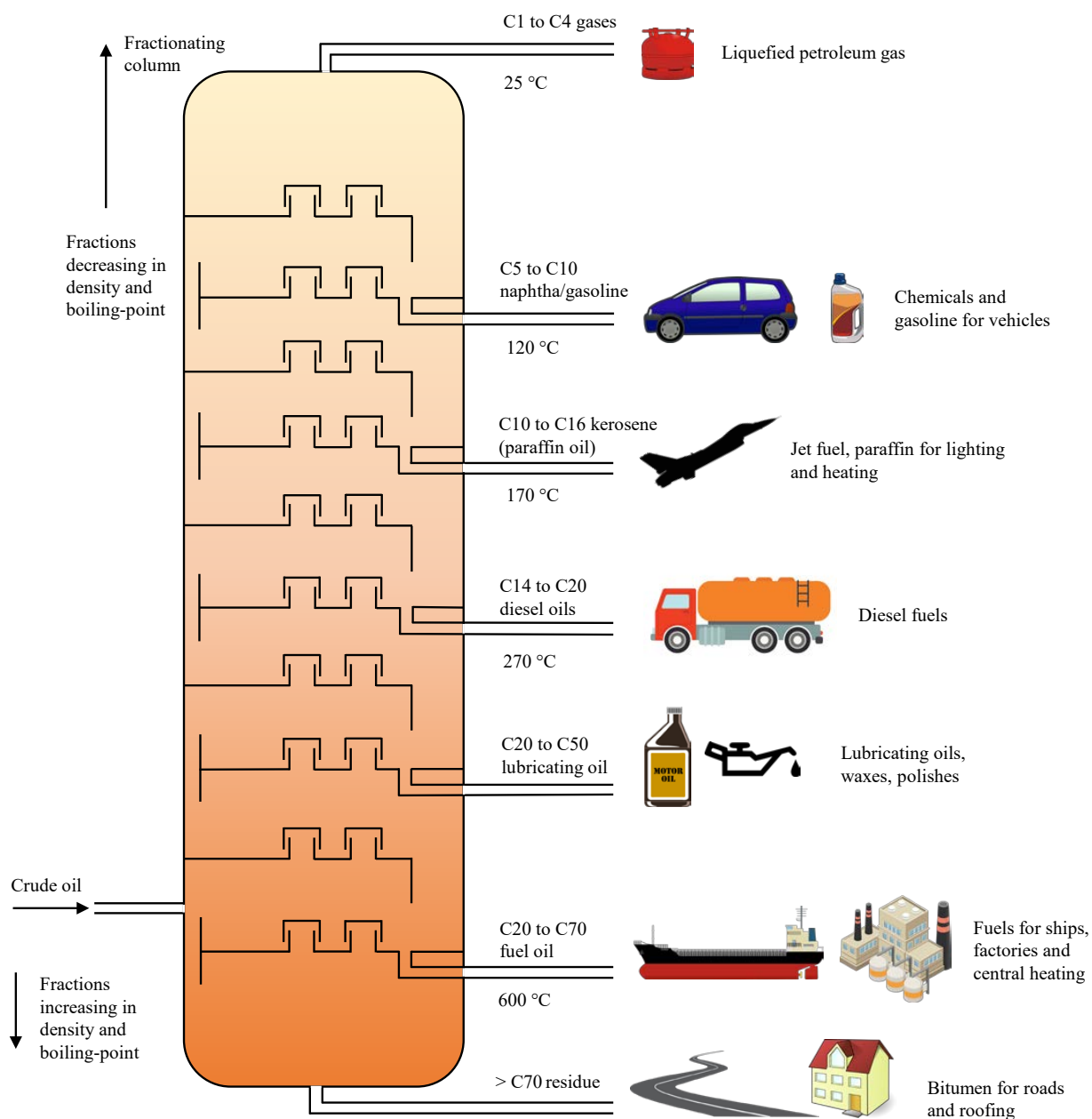
Initially, “straight-run” gasoline was produced by the simple distillation of crude oil, without the use of chemical conversion processes ([Lane, 1980](#); [Sax and Lewis, 1987](#)). However, after 1900, motor vehicles began to appear in growing numbers, and gasoline began to have a marketable value as a refinery product. By around 1912, the distillation of crude oil alone had become insufficient

to meet the rapidly growing demand for gasoline. At this time, gasoline-range hydrocarbons were also recovered from “wet” natural gas. However, only a limited amount of natural gasoline could be included in the final gasoline product because of the former’s high volatility and relatively low anti-knock quality ([Lane, 1980](#)). Since then, petroleum refineries have developed several processes to increase the production of gasoline ([ATSDR, 1995](#)).

Automotive gasoline is produced primarily by blending component streams from petroleum refinery processing units. Petroleum refining is a complex industrial process that consists of three production phases: separation, conversion, and treatment. First, inside the distillation units, the liquids and vapours separate into petroleum components, referred to as fractions, according to their boiling-points. Heavy fractions settle at the bottom, whereas light fractions rise to the top. Gasoline is a component of the lightest fractions and liquefied refinery gases. It is vapourized during the distillation process and then rises to the top of the distillation tower, where it is condensed to the liquid form ([US EIA, 2023](#)). [Fig. 1.2](#) describes a crude oil distillation unit and its products.

At this point in the process, fractions from the distillation units are transformed into streams (intermediate components), which eventually become finished products. The heavy, lower-value distillation fractions can be processed further to yield lighter, higher-value products, such as gasoline. The most widely used conversion method is referred to as “cracking”, because it uses heat, pressure, catalysts, and sometimes hydrogen to “crack” heavy hydrocarbon molecules to form lighter ones. More complex refineries use several types of “crackers”, including fluid catalytic cracking units and hydrocracking/hydrocracker units ([US EIA, 2023](#)).

The final step in the production of gasoline involves the careful combination of a variety of streams obtained from the processing units.

**Fig. 1.2 Schematic of a crude oil distillation unit and its products**

The various distillation fractions obtained by refining petroleum are used in numerous products, including automotive gasoline. Adapted from [US EIA \(2023\)](#) and [Oil and gas info \(2025\)](#).

The octane levels, vapour pressure ratings, and other considerations determine the gasoline blend produced ([US EIA, 2023](#)). The blending of various stocks is a high-volume operation. Gasoline components, including alkylates and other high-octane components, are blended with octane-improving additives (such as oxygenated additives), carburettor detergents, anti-rust agents, anti-icing agents, and other additives, as described in Section 1.1 ([IARC, 1989](#)).

#### (b) *Production volumes*

The USA is by far the largest gasoline producer worldwide, producing over 360 million tonnes per year in recent years, followed by China, the Russian Federation, Japan, and Canada, in decreasing order of production volume ([United Nations Data, 2025](#); see [Fig. 1.3](#)). Another source stated that India was ranked fifth worldwide in gasoline production ([Global Economy, 2024](#)). [The Working Group noted that the overall rankings were similar, but not identical, in the different sources.] There was a sharp dip in gasoline production in 2020 [that was likely to be a result of the COVID-19 pandemic], followed by a recovery.

In Europe, under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, companies must register substances that they import or manufacture in the European Economic Area (EEA) at  $\geq 1$  tonne/year. With respect to gasoline (CAS No. 86290-81-5), there were 195 registrants in the EEA in 2025, with an estimated tonnage band of 10 000 000–100 000 000 tonnes ([ECHA, 2025a](#)). With respect to natural gasoline (CAS No. 8006-61-9), there were only five registrants in the EEA, with an estimated tonnage band of 100 000–1 000 000 tonnes ([ECHA, 2025b](#)).

#### 1.2.2 *Uses*

The primary use of automotive gasoline is as a fuel for spark ignition in internal combustion engines, but a small quantity is also used for aviation purposes ([Concawe, 1997](#)). The known misuses of gasoline include use as a solvent, as a cleaning agent, and for sniffing (solvent abuse) ([Concawe, 1997](#); [Barillo et al., 1998](#)).

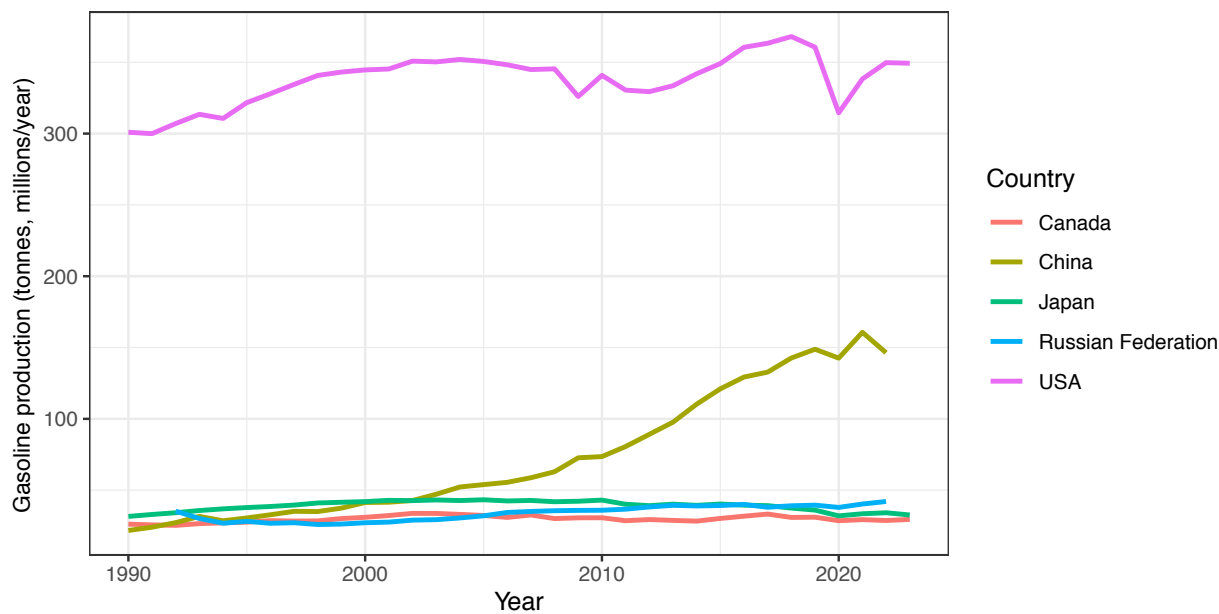
The USA is the largest consumer of gasoline worldwide, and consumption follows a trend similar to that for production. There was a clear upward trend from the mid-1990s to the mid-2000s, followed by a plateau or a slight decline in recent years ([US Department of Energy, 2024](#)) ([Fig. 1.4](#)). China, the Russian Federation, and Japan have shown rising consumption, closely matching the local increases in production. Canada's consumption of gasoline has remained relatively stable when compared with that of other countries. Another source stated that India ranked fifth in gasoline consumption ([Global Economy, 2024](#)). [The Working Group noted that the overall rankings were similar, but not exactly the same, according to the various sources.]

Using different metrics to describe gasoline consumption around the world, for 2022, based on Global Economy data, the mean gasoline consumption across 187 countries was 21.3 million litres/day, and this had increased during the preceding years. This included the consumption of conventional gasoline; all types of oxygenated gasoline, including gasohol ([Energy Business Services, 2024](#)), and reformulated gasoline, but excluded the consumption of aviation gasoline ([Global Economy, 2024](#)).

Given the 1.4 billion litres of gasoline dispensed every day in the USA, it was expected that there would be approximately 37 million fill-ups per day (based on a 38 L fill-up) at the 118 678 convenience stores selling fuel in the USA, where the main form of gasoline sold is automotive gasoline ([NACS, 2024](#)).

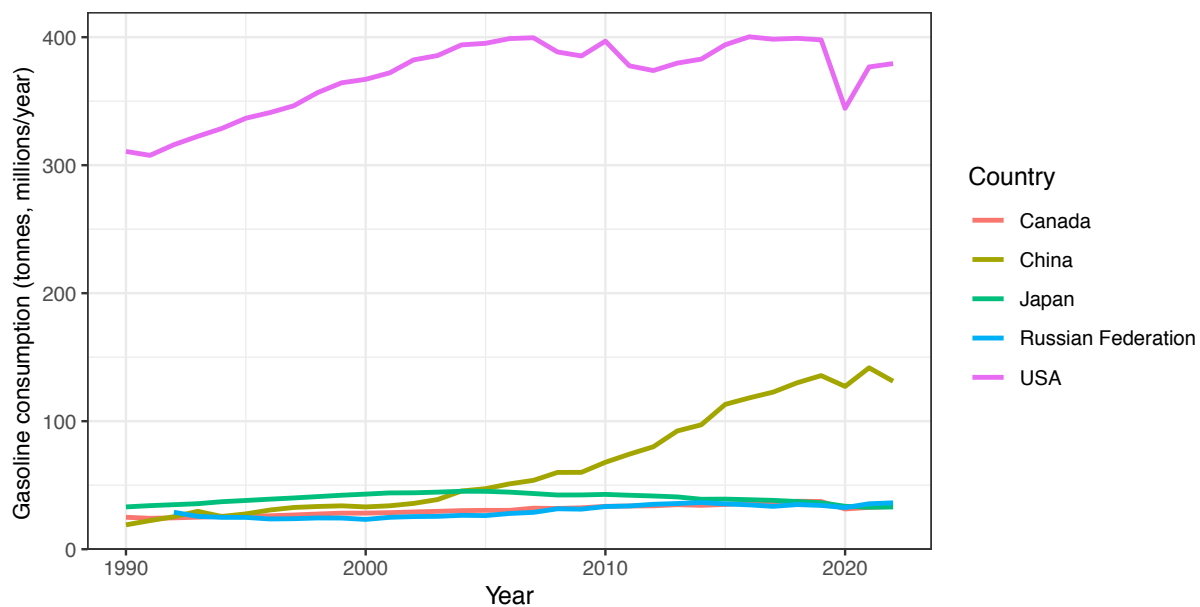


**Fig. 1.3 Trends in the production of automotive gasoline in the top five producer countries (1992–2022)**



From [United Nations Data \(2025\)](#).

**Fig. 1.4 Trends in the consumption of automotive gasoline in the top five consumer countries (1992–2022)**



From [United Nations Data \(2025\)](#).

In the EU, the majority of gasoline sales in 2020 comprised fuels with a RON of 95 (79.3% of the total gasoline sales), 14.3% of sales were  $95 < \text{RON} < 98$ , and 6.4% were  $\text{RON} \geq 98$ . There was also a very low proportion of RON 91-grade sales (0.01%) ([European Commission, 2022](#)).

### 1.3 Analytical methods

Gasoline is a complex mixture of hydrocarbons and other additives (see Section 1.1 for details on gasoline composition). Summarized in this section are a selection of methods dedicated to the analysis of gasoline components that are commonly used as indicators of contamination and exposure to gasoline, including hydrocarbons; benzene, toluene, ethylbenzene, and xylenes (BTEX); and VOCs. Oxygenated additives such as MTBE have also been used to monitor gasoline levels in the environment, and the corresponding methods are described in Section 1.3 of the monograph on MTBE in the present volume. [The Working Group noted that the measurement of the concentrations of these gasoline components as a proxy for gasoline is adequate only in settings in which gasoline is expected to be present.] An overview of some examples of commonly accepted methods for the analysis of various matrices is given in [Table 1.4](#).

#### 1.3.1 Air

[The Working Group noted that this section describes methods for the measurement of VOC concentrations in gasoline vapour. Some of these vapours also occur in engine exhaust.] Benzene is the indicator that is commonly used to evaluate the presence and level of gasoline in ambient air, and there are several methods available to measure it. These methods involve the collection of samples using sorbent tubes, canisters, or passive samplers and subsequent analysis by gas chromatography, with detection using methods such as mass spectrometry (MS) and flame

ionization detection. In a review of 151 studies in which the level of benzene in ambient air was analysed, approximately 60% involved sampling using sorbent tubes ([Sekar et al., 2019](#)).

Other techniques that can be used for sampling and analysis include real-time continuous monitoring systems, portable instruments for direct reading, and other suitable methods, which are chosen according to the source of the emission and the information needed ([Sekar et al., 2019](#); [Kumari et al., 2024](#)). The European Standard EN 14662-3:2015 describes a standard method for the measurement of benzene in ambient air using automated pumped sampling and an in situ gas chromatograph ([European Standards, 2015](#)).

Methods to determine VOCs in ambient air have been published by the US EPA. Method TO-17 involves sample collection in a stainless-steel sorbent tube ([US EPA, 1999a](#)). This method permits the measurement of several VOCs, including benzene, in samples of ambient air by gas chromatography-mass spectrometry (GC-MS). The limits of detection (LODs) are below the 0.5 ppb level [ $\leq 1.62 \mu\text{g}/\text{m}^3$ ] for benzene. US EPA method TO-15 ([US EPA, 1999b](#)) has an LOD of 0.29 ppm [ $0.94 \mu\text{g}/\text{m}^3$ ]; therefore, it is suitable for the detection of analytes present as pollutants in ambient air.

One of the most commonly used methods of assessing the concentrations of BTEX and other aromatics in occupational studies is the National Institute for Occupational Safety and Health (NIOSH) method 1501 ([NIOSH, 2003](#)). This method involves the use of a solid sorbent tube coupled to an active pump for the sampling of analytes, and gas chromatography-flame ionization detection (GC-FID) to analyse the samples ([NIOSH, 2003](#)). The LOD for benzene using this method was  $0.5 \mu\text{g}/\text{sample}$  with a sampling volume of 5–30 L air. Another validated method used to study occupational exposure to BTEX is the NIOSH method 3900 ([NIOSH, 2018a](#)). This method involves the use of a fused silica-lined

**Table 1.4 Analytical methods for the measurement of gasoline in air, water, and soil samples**

| Sample matrix                        | Agent                                       | Sample preparation                                                                                                                              | Instrument (LOD)                                                                                                                               | Comments                                      | Reference                         |
|--------------------------------------|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|-----------------------------------|
| <i>Water samples</i>                 |                                             |                                                                                                                                                 |                                                                                                                                                |                                               |                                   |
| Drinking-water                       | Purgeable organic compounds, including BTEX | 5 mL water extracted using purge-and-trap                                                                                                       | GC-MS (benzene LOD 0.017 µg/L)                                                                                                                 | US EPA method 524.3                           | <a href="#">US EPA (2009)</a>     |
| <i>Air samples</i>                   |                                             |                                                                                                                                                 |                                                                                                                                                |                                               |                                   |
| Air                                  | Alcohols                                    | Sampling using an activated charcoal sorbent tube at ≤ 0.01–0.2 L/min and desorption with CS <sub>2</sub>                                       | GC-FID (LOD, 0.01 mg/sample for an air volume of 1–10 L)                                                                                       | NIOSH method 1400 issue 2                     | <a href="#">NIOSH (1994)</a>      |
| Air                                  | BTEX and other aromatics                    | Sampling using an activated charcoal sorbent tube at ≤ 0.2 L/min and desorption with C <sub>s</sub>                                             | GC-FID (benzene LOD, 0.5 µg/sample) for an air sample volume of 5–30 L                                                                         | NIOSH method 1501                             | <a href="#">NIOSH (2003)</a>      |
| Air                                  | VOCs, including benzene                     | Air sampled in stainless steel sorbent tubes (example volume 6 L)                                                                               | Analytes are trapped and concentrated, then thermally desorbed. Analysis by GC-MS (benzene LOD 0.29 ppb [0.94 µg/m <sup>3</sup> ] in SIM mode) | US EPA Compendium Method TO-17                | <a href="#">US EPA (1999a, b)</a> |
| Air                                  | VOCs C1–C10 in mixed exposure environments  | Sampling using fused silica-lined stainless steel canisters of volume 400 mL to 6 L at 0.06–50 mL/min                                           | GC-MS (benzene LOD, 0.2 ppb [0.65 µg/m <sup>3</sup> ])                                                                                         | NIOSH method 3900; modified from US EPA TO-15 | <a href="#">NIOSH (2018a)</a>     |
| Air                                  | VOCs from fugitive and area sources         | Passive sampling using sorbent tubes (various adsorbents possible), then thermal desorption (the method can be used also after active sampling) | TD/GC-MS; other detection methods, such as FID, can also be used (benzene LOD, 0.05 ppb [0.16 µg/m <sup>3</sup> ])                             | US EPA 325B, based on Compendium Method TO-17 | <a href="#">US EPA (2019)</a>     |
| <i>Soil and other solid matrices</i> |                                             |                                                                                                                                                 |                                                                                                                                                |                                               |                                   |
| Soil                                 | VOCs                                        | Various preparation techniques, including purge-and-trap, with TD or vacuum system distillation                                                 | GC-MS (LOD varies)                                                                                                                             | US EPA 8260C                                  | <a href="#">US EPA (2006)</a>     |
| Soil                                 | VOCs, BTEX, naphthalene                     | Purge-and-trap with TD                                                                                                                          | GC-FID (LOQ for VOCs, 0.1 mg/kg)<br>GC-MS (< 0.1 mg/kg)                                                                                        | ISO 15009                                     | <a href="#">ISO (2016)</a>        |

BTEX, benzene, toluene, ethylbenzene, and xylene; FID, flame ionization detection; GC, gas chromatography; ISO, International Organization for Standardization; LOD, limit of detection; min, minute(s); MS, mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; ppb, parts per billion; SIM, selected ion monitoring; TD, thermal desorption; US EPA, United States Environmental Protection Agency; VOC, volatile organic compound.

stainless-steel canister as a sampler and GC-MS to analyse the chemicals of interest. Both methods (GC-FID and GC-MS) can be used for the analysis of ambient air samples, with some adaptations to the sampling procedures ([Chaiklieng, 2021](#)).

There are also methods that use passive samplers, also termed “diffusive” samplers, which rely on the passive diffusion of air pollutants into a sorbent medium without the mechanical convection of air. These are commonly used for ambient air measurements and are sometimes used for occupational exposure assessments ([Zabiegała et al., 2010](#); [Pienaar et al., 2015](#); [Cruz et al., 2017](#)). US EPA method 325B ([US EPA, 2019](#)) is among these and is useful for the assessment of VOCs from fugitive emissions. Passive tubes with various adsorbing materials can be used to trap the analytes, which can then be analysed, after thermal desorption, using GC-MS or GC-FID. The performance of this method varies according to the sorbent and detector used. Under optimized conditions (the analysis of indoor air), benzene and other VOCs can be analysed with a high level of sensitivity, with LODs in the range of 0.002–0.298 mg/m<sup>3</sup> ([Baysal et al., 2021](#)).

Direct-reading equipment, including photoionization detector devices, has also been used to measure the concentrations of BTEX ([Kamani et al., 2023](#)). Although they have some limitations with respect to specificity and sensitivity, these direct-reading instruments are also valuable tools for the assessment of worker exposure and the sources of emissions ([Coffey and Pearce, 2010](#); [OSHA, 2025](#)).

### 1.3.2 Water

Gasoline does not dissolve readily in water. However, some of the chemicals that make up gasoline can dissolve easily in water ([ATSDR, 2025](#)).

Liquid–liquid extraction is commonly used for water sample extraction ([Yue et al., 2021](#)). US EPA method 524.3 for the analysis of purgeable organic compounds, including BTEX, in water involves the extraction of organic compounds from 5 mL of water and their analysis using GC-MS. The LOD for benzene using this method was 0.017 µg/L ([US EPA, 2009](#)).

### 1.3.3 Soil

Soil samples are collected in airtight, solvent-resistant containers (e.g. glass jars) and stored at ≤4 °C to prevent losses owing to volatilization. Extraction is performed using solvents such as methanol, hexane, or dichloromethane, and methods that include agitation, sonication, Soxhlet extraction, or purge-and-trap techniques. For volatile hydrocarbons, headspace sampling or solid-phase microextraction (SPME) can also be used to isolate and concentrate analytes before their analysis.

US EPA method 8260C is suitable for the measurement of VOC concentrations in solid matrices, including soil ([US EPA, 2006](#)). Various preparation techniques were suggested in this publication, including purge-and-trap with thermal desorption and vacuum system distillation. GC-MS was the technique used for the analysis, and the LOD depended on the preparation technique used ([US EPA, 2006](#)).

In China, method HJ 1020-2019 is used to determine the concentrations of C6–C9 petroleum hydrocarbons in soil using purge-and-trap and GC-FID, and this is highly sensitive for VOCs. In addition, HJ 605-2011 can be used for the analysis of BTEX ([Jia et al., 2023](#)).

For highly volatile gasoline fractions, headspace analysis with purge-and-trap GC can be used for direct vapour-phase quantification. This has a limit of quantification (LOQ) of 0.1 mg/kg using FID. Lower LOQs can be achieved using MS with selected ion detection (ISO 15009; [ISO, 2016](#); from 0.001 to 0.010 mg/kg for benzene;

[Environment Agency, 2009](#)). SPME coupled to GC-MS is another technique used for the analysis of BTEX, and it has an LOQ of 1 mg/m<sup>3</sup> for soil vapour ([Wang et al., 2023](#)) and an LOD of 5 µg/kg for soil ([Orazbayeva et al., 2017](#)). These methodologies permit an accurate assessment of the gasoline contamination of soil ([Pinedo et al., 2013](#); [ISO, 2016](#)).

### 1.3.4 Food

Sample preparation methods such as distillation and extraction, followed by GC-FID or GC-MS, have been used for the determination of hydrocarbons in food ([Concawe, 2000](#)). Sample concentration is typically performed by head-space sampling or purge-and-trap methods, the latter being more sensitive ([Concawe, 2000](#)).

Developments in dynamic headspace-GC-quadrupole MS have permitted the optimization of information obtained from the analysis of the volatile fraction of the muscle and fat tissue of animals destined for human consumption ([Ratel and Engel, 2009](#)). However, natural matrices such as animal tissues or fluids are complex. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOF-MS) increases the separation space and improves chromatographic resolution, such that the constituents of a sample can be separated in a single analysis ([Ratel and Engel, 2009](#)).

### 1.3.5 Human biomarkers

Because gasoline is a mixture of chemicals (see Section 1.1 for details on the composition of gasoline), the assessment of gasoline exposure in biological specimens commonly involves a focus on specific components that are chosen for their abundance and/or toxicological relevance. Those that have been or are currently being used include lead, BTEX, and some oxygenated components, including MTBE, ETBE, and TAME.

Some commonly used methods are presented in [Table 1.5](#). [The Working Group noted that these exposure biomarkers are only adequate in settings in which exposure to gasoline is the main source of these compounds.]

All these chemicals or their metabolites have been assessed in biological fluid samples collected during studies investigating occupationally exposed individuals and/or the general population. [The Working Group noted that in occupational settings, direct exposure to gasoline occurs mainly during its production, use, distribution, or transportation; therefore, the associations between gasoline exposure and the relevant biomarkers are quite reliable. In studies involving the general population, co-exposure to gasoline vapour, engine exhaust fumes, first-hand or secondhand tobacco smoke, or other substances makes it more difficult to interpret biological monitoring data and to identify the role played by each source in the total internal dose.]

Blood lead concentrations have been assessed in occupationally exposed individuals ([Olowoyo et al., 2024](#)) and in the general population exposed to leaded gasoline (reviewed in papers by [Angrand et al., 2022](#); [Lacerda et al., 2023](#)). Lead in the blood is the preferred biomarker of lead exposure; it can be assessed using graphite furnace atomic absorption spectroscopy, which has an LOD of 5 µg/100 g blood ([NIOSH, 1994](#)), or using inductively coupled plasma MS, which has a lower LOD of 0.07 µg/100 mL ([Jones et al., 2017](#)) (see [Table 1.5](#)).

The most commonly investigated chemicals are BTEX. For benzene, various biomarkers have been proposed and reported, such as S-phenylmercapturic acid (SPMA), *trans,trans*-muconic acid (*t,t*-MA), and benzene in the blood and urine. Of these, the most suitable for the assessment of low exposure levels and for general population studies are urinary SPMA and urinary benzene. [The Working Group noted that because benzene exposure



**Table 1.5 Summary of biomarkers for the assessment of exposure to selected chemicals in gasoline**

| Chemical in gasoline | Biomarker                                                      | Assay        | LOD or LOQ          | Reference                                |
|----------------------|----------------------------------------------------------------|--------------|---------------------|------------------------------------------|
| Lead                 | Lead in the blood                                              | AAS          | LOD, 5 µg/100 g     | <a href="#">NIOSH (1994)</a>             |
|                      |                                                                | ICP-MS       | LOD, 0.07 µg/100 mL | <a href="#">Jones et al. (2017)</a>      |
| Benzene              | Benzene in urine<br>SPMA in urine                              | SPME-GC-MS   | LOD, 25 ng/L        | <a href="#">Fustinoni et al. (1999)</a>  |
|                      |                                                                | LC-MS/MS     | LOQ, 0.01 µg/L      | <a href="#">Frigerio et al. (2019)</a>   |
|                      |                                                                |              | LOQ, 0.15 µg/L      | <a href="#">Schettgen et al. (2021)</a>  |
|                      |                                                                | SPE-LC-MS/MS | LOD, 0.2 µg/L       | <a href="#">NIOSH (2014)</a>             |
| Toluene              | Toluene in urine<br><i>o</i> -Cresol in urine<br>SBMA in urine | SPME-GC-MS   | LOD, 34 ng/L        | <a href="#">Fustinoni et al. (1999)</a>  |
|                      |                                                                | SPME-GC-MS   | LOD, 6 µg/L         | <a href="#">Fustinoni et al. (2005b)</a> |
|                      |                                                                | LC-MS/MS     | LOQ, 0.02 µg/L      | <a href="#">Frigerio et al. (2019)</a>   |
|                      |                                                                |              | LOQ, 1.5 µg/L       | <a href="#">Schettgen et al. (2021)</a>  |
| Ethylbenzene         | Ethylbenzene in urine<br>Phenylglyoxylic acid in urine         | SPE-LC-MS/MS | LOD, 0.2 µg/L       | <a href="#">NIOSH (2014)</a>             |
|                      |                                                                | SPME-GC-MS   | LOD, 12 ng/L        | <a href="#">Fustinoni et al. (1999)</a>  |
|                      |                                                                | HPLC-UV      | LOQ, 4–11 mg/L      | <a href="#">Chromsystems (2024)</a>      |
|                      |                                                                |              |                     |                                          |
| Xylenes              | Xylenes in urine<br>Methylhippuric acid in urine               | SPME-GC-MS   | LOD, 15–23 ng/L     | <a href="#">Fustinoni et al. (1999)</a>  |
|                      |                                                                | HPLC-UV      | LOQ, 4–11 mg/L      | <a href="#">Chromsystems (2024)</a>      |
| MTBE                 | MTBE in urine                                                  | SPME-GC-MS   | LOD, 6 ng/L         | <a href="#">Scibetta et al. (2007)</a>   |
| ETBE                 | ETBE in urine                                                  | SPME-GC-MS   | LOD, 6 ng/L         | <a href="#">Scibetta et al. (2007)</a>   |
| TAME                 | TAME in urine                                                  | SPME-GC-MS   | LOD, 6 ng/L         | <a href="#">Scibetta et al. (2007)</a>   |

AAS, atomic absorption spectroscopy; ETBE, ethyl *tert*-butyl ether; GC-MS, gas chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; ICP-MS, inductively coupled plasma-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MTBE, methyl *tert*-butyl ether; SBMA, S-benzylmercapturic acid; SPE, solid-phase extraction; SPMA, S-phenylmercapturic acid; SPME, solid-phase microextraction; TAME, *tert*-amyl methyl ether; UV, ultraviolet (spectroscopy).

can be derived from both gasoline and smoking, these markers represent measures of exposure from both sources. In smokers, tobacco smoke contributes to benzene biomarker levels.]

SPMA is a minor but specific metabolite that accounts for <1% of the absorbed dose ([Rappaport et al., 2009](#); [Tranfo et al., 2018](#)). It is assayed using solid-phase extraction, followed by liquid chromatography coupled to tandem mass spectrometry (LS-MS/MS), which has an LOD of 0.2 µg/L ([NIOSH, 2014](#), method 8326). Other simple assays based on isotope dilution, online enrichment, and LC-MS/MS analysis are also possible and have LOQs below the microgram per litre range (e.g. [Frigerio et al., 2019](#); [Schettgen et al., 2021](#)).

Another metabolite of benzene is *t,t*-MA, which accounts for approximately 4% of the absorbed dose. The principal limitation of its use is poor specificity, because *t,t*-MA is also produced by the metabolism of the preservatives sorbic acid or sorbates, which may be present in food and beverages ([Ruppert et al., 1997](#); [Weaver et al., 2000](#)). The use of *t,t*-MA is recommended when the level of benzene exposure is > 0.2 ppm [or 0.6 mg/m<sup>3</sup>] ([Kim et al., 2006](#)). [The Working Group noted that this concentration was much higher than that documented after gasoline evaporation. For this reason, *t,t*-MA is not recommended for use as a biomarker of exposure to benzene in workers who have been personally exposed to gasoline at levels lower than approximately 0.6 mg/m<sup>3</sup>, for example, in service stations with efficient vapour recovery

systems, when there has been no skin exposure.] The concentration of *t,t*-MA is usually measured by high-performance liquid chromatography (HPLC) with ultraviolet detection ([Lee et al., 2005](#)), and standardized assays are present on the market.

For toluene, the possible biomarkers are urinary toluene, *o*-cresol, and *S*-benzylmercapturic acid (SBMA). Hippuric acid, a major urinary metabolite of toluene used in biomonitoring campaigns in the past, is now not regarded as suitable, owing to its low specificity ([Foo et al., 1991](#)). It may be derived from the catabolism of proteins and from the metabolism of benzoic acid, which is present in some fruits and is used as a food preservative. Indeed, international agencies, such as the American Conference of Governmental Industrial Hygienists and the German Research Foundation, which regularly publish the details of biological markers for use in occupational exposure assessments, no longer recommend the use of hippuric acid to assess benzene exposure ([ACGIH, 2023](#); [DFG MAK, 2024](#)).

*o*-Cresol concentrations can be measured using SPME coupled to GC-MS, which has an LOD in the low microgram per litre range, and SBMA concentrations can be measured simultaneously by LC-MS/MS, using the method reported above for SPMA (NIOSH method 8326; [NIOSH, 2014](#); [Frigerio et al., 2019](#); [Schettgen et al., 2021](#)).

For ethylbenzene, the proposed biomarkers are urinary ethylbenzene and phenylglyoxylic acid, the latter also being a metabolite of styrene. The phenylglyoxylic acid concentration can be determined using a simple commercial kit and an HPLC-UV method that has an LOQ in the low milligrams per litre range ([Chromsystems, 2024](#)).

For xylenes, the biomarkers are urinary xylenes and methylhippuric acid. Methylhippuric acid concentrations can be measured using the same commercial kit used to measure phenyl-

glyoxylic acid concentrations ([Chromsystems, 2024](#)).

Although measuring the concentrations of urinary metabolites of BTEX requires specific methods, the measurement of non-metabolized urinary BTEX concentrations offers a good opportunity to assess all these chemicals in a single assay based on headspace GC-MS, using SPME for the sampling of analytes in the headspace. The LOQs for the published methods are in the order of  $\leq 10$  ng/L ([Fustinoni et al., 2010](#); [Campo et al., 2016](#)).

For several BTEX biomarkers, including urinary SPME and BTEX, laboratories can test the accuracy of their assays by participating in the German external quality assessment scheme for biological materials ([G-EQUAS, 2024](#)).

To assess exposure to MTBE, ETBE, and/or TAME, blood and urinary ether concentrations can be measured. The performance of assays for these unmetabolized chemicals in the blood or urine for exposure assessment was similar. Therefore, urine sampling is preferable to blood sampling, because it is not invasive. MTBE, ETBE, and TAME concentrations can be measured in urine using SPME-GC-MS in the presence of deuterated MTBE (MTBE-d<sub>12</sub>) as an internal standard ([Scibetta et al., 2007](#)). The associated LOD was 6 ng/L for each analyte. The high sensitivity of this assay makes it useful for measuring ether concentrations in both occupationally and environmentally exposed individuals.

[In summary, the Working Group noted that the biological monitoring of several VOCs present in gasoline can be achieved by measuring the concentrations of unmetabolized chemicals in the urine. The use of the corresponding urinary metabolites is also possible, taking care to choose the markers with the highest specificities, such as SPMA for benzene, and *o*-cresol and/or SBMA for toluene, and considering the smoking habits of the individuals when interpreting the biomonitoring data.]

## 1.4 Occurrence and exposure

### 1.4.1 Environmental occurrence

#### (a) Air

Gasoline vapour is an air pollutant that is derived from the evaporation of gasoline. The evaporation rate and the chemical nature of the vapour depend on the composition of the gasoline, the vapour pressure, and the temperature. Gasoline vapour contains numerous VOCs, which are mostly hydrocarbons, and include alkanes, alkenes, aromatics, alcohols, and ethers (information on gasoline composition can be found in Section 1.1.5). For additional data on some of the oxygenated additives to gasoline that can be found in air, see the monographs on MTBE, ETBE, TBA, DIPE, and TAME in the present volume.

The monitoring of air pollution caused by gasoline evaporation is performed by measuring VOC concentrations, particularly those of non-methane volatile organic compounds (NMVOCs), including benzene and ethanol. [The Working Group noted that gasoline does not contain methane, and therefore, in the context of gasoline vapour, the terms VOC and NMVOC are equivalent in practice. Here, the term NMVOC is used in some instances, where the regulations and studies cited employ the term.] However, the detection of VOC and NMVOC in the air in the general environment cannot be directly linked to gasoline evaporation, because several other sources of the same chemicals exist, including vehicle exhausts, industrial activities, agriculture, domestic heating, and wildfires ([European Environment Agency, 2019](#)). [The Working Group noted that the presence of VOCs in the air is more specifically attributable to gasoline evaporation at service stations and at other locations where gasoline is dispensed, loaded, unloaded, used, or produced.]

Several initiatives have been adopted worldwide to reduce vehicular emissions, and these have led to decreases in exhaust emissions. However, gasoline evaporation from vehicles (vehicular evaporative emissions) that occur while they are parked and used still represents a major source of atmospheric VOCs. This evaporative emission occurs through four routes: permeation, hot-soak losses, canister breakthrough emission, and gasoline leaks from fuel systems ([Yamada, 2013](#); [Hata et al., 2020](#)). Permeation denotes evaporative emissions resulting from the penetration of parts of fuel systems, such as fuel hoses and pipes. Hot-soak loss is a type of evaporative emission resulting from the penetration of hot fuel system components within the hour after parking. A canister filled with an absorbent (usually charcoal) is used in cars to prevent evaporation from the gasoline tank into the environment. However, this absorbent eventually becomes saturated. While vehicles are parked, canister breakthrough emissions occur through evaporation from the empty volume of their gasoline tanks after saturation of the absorbent. This phenomenon is referred to as “breakthrough emission”. Evaporative emissions associated with fuel leaks are emitted from fuel caps, compromised vent lines with poor connections, or poor fittings ([Hata et al., 2020](#)). Evaporative emissions are regulated in most automotive markets around the world, and evaporative emission control systems are installed in all new vehicles, but the efficiency of these systems varies. A comparative evaluation showed that the installation of onboard refuelling vapour recovery (ORVR) systems in vehicles in the USA led to much more efficient reductions in evaporative emissions than did the canisters used in vehicles meeting the Euro 4 standard ([Liu et al., 2015](#)). It was shown that a car in Europe emitted 602–1147 g/year of VOCs through diurnal (daily) parking, hot soak, and permeation, and that a car in Japan emitted 526 g/year of VOCs during diurnal parking ([Yamada, 2013](#)). In addition,

each car in South Africa emitted 7647 g/year of VOCs ([Van der Westhuisen et al., 2004](#); [Liu et al., 2015](#)), although the ambient temperature significantly affected this quantity. However, these estimates are subject to change, owing to the adoption of ever stricter standards and technological improvements.

[The Working Group noted that many data regarding air pollution with gasoline-related chemicals were available worldwide. However, it was challenging to perform a proper source apportionment (the practice of obtaining information about pollution sources and the amounts they contribute to air pollution levels), especially because the share of air pollution that can be ascribed to gasoline changes over time and differs between countries. A few studies that yielded data on VOCs in the air and the share apportioned to automotive evaporative emissions are reported here.]

A study performed in Toronto, Canada, using data from the National Air Pollution Surveillance Program, showed that between 2000 and 2009 pollution related to traffic and transportation (in general assessed through the measurement of C4–C8 aliphatic alkane and alkene and aromatic hydrocarbon concentrations) dominated, with evaporative emissions contributing more than exhaust emissions to the urban VOC pollution ([Porada and Szyszkowicz, 2016](#)).

In the EU, the emission of NMVOCs was dominated by the “industrial processes and product use” sector (43%), followed by agriculture (24%), and residential heating and solvent use. The broad category of road transport, including both tailpipe emissions and gasoline evaporation from vehicles, accounted for 8% of the total ([European Environment Agency, 2019, 2023](#)).

In other parts of the world, the situation has been different, particularly in Asia, where the number of vehicles has significantly increased during recent decades. A VOC emission inventory showed that the storage and distribution

of fuel contributed approximately 3–4% to the total amount of VOCs (TVOC) emitted in China ([Wei et al., 2008](#)), and fuel evaporation made the largest contribution to this. Because diesel fuel has a lower vapour pressure, the contribution of diesel evaporation was assumed to be negligible compared with that of gasoline, and therefore in this context, diesel vapour only contributed a negligible part to the TVOC. The contribution of the storage and distribution of fuel was expected to decrease as a result of several governmental initiatives in China, such as the introduction of strict VOC emission limit standards ([Wei et al., 2011](#)).

In a more recent study conducted in Zhengzhou, China, in 2019, 29.3% of the TVOC emissions was estimated to be derived from mobile sources, including both gasoline evaporation and the emission of fumes derived from incompletely combusted fuel from tailpipes ([Zhang et al., 2023](#)).

Higher percentage contributions of gasoline evaporation to air pollution have been shown in other studies. A source apportionment analysis of urban air pollutants in Taipei, Taiwan, China, which considered nine sources of VOCs and particulate matter of  $\leq 2.5$   $\mu\text{m}$  in diameter ( $\text{PM}_{2.5}$ ) in urban air, showed that gasoline evaporation contributed 15% to the total ([Liao et al., 2017](#)). A similar finding was made in another study performed in Harbin, China, in which the contribution of gasoline evaporation to TVOC air pollution was 12.4% ([Xuan et al., 2021](#)).

In addition to VOCs, another substance that can pollute the air and that was previously present in gasoline is lead (Pb). Although leaded gasoline has been banned all over the world (in the last country in 2021) ([Lacerda et al., 2023](#)), airborne particles in large cities still contain lead, as a consequence of the remobilization of lead that was historically deposited in soils ([Resongles et al., 2021](#)). In a site with heavy traffic in central London, UK, the concentration of lead in airborne particulate matter of  $\leq 10$   $\mu\text{m}$  in



diameter ( $PM_{10}$ ) ranged from 3.9 to 19.4  $ng/m^3$ , with the contribution of lead from leaded gasoline being estimated to range from 32% to 43% ([Resongles et al., 2021](#)).

(i) *Air monitoring near service stations*

At service stations, the sources of gasoline vapour include vehicle tank vapour releases when refuelling, leaks from storage tanks, accidental spills from the nozzles of gasoline dispensers, fugitive vapour emissions from leaky pipes and fittings, and leaky hoses ([Patton et al., 2021](#)).

To reduce gasoline evaporation at service stations, stage I and stage II vapour recovery systems have been installed in many countries. Stage I gasoline vapour recovery systems capture vapour expelled from underground storage tanks at service stations when they are being refilled by tanker trucks, and stage II systems capture gasoline vapour that would otherwise be vented during individual vehicle refuelling at service stations. These systems were adopted in the USA in 1992 and have been gradually implemented in many countries and regions around the world, such as in the EU (Directive 94/63/EC; [European Commission, 2009](#)) and China ([DEE, 2020](#)). Recently, cars in the USA have been equipped with ORVR systems, which return gasoline vapour displaced during refuelling to the vehicle fuel system. When vehicles equipped with ORVR systems came to dominate the market, and the efficiency of this system was proven to be > 95% ([International Council on Clean Transportation, 2011](#)), the US EPA waived the requirements for states to install stage II gasoline vapour recovery systems at gasoline dispensing facilities ([Office of the Federal Register, 2012](#)).

The adoption of the aforementioned recovery systems dramatically reduced evaporative emissions. In the EU, after the introduction of stage II systems, the proportion of anthropogenic VOC emissions in the air that was attributable to refuelling was approximately 0.7% ([European Commission, 2017](#)). Similarly, in China, reduc-

tions of >80% in the concentration of VOCs in refuelling vapours were reported after the introduction of these vapour recovery systems ([Zhang et al., 2013](#)). Moreover, strict regulations were introduced in China in 2020 that stipulated that non-methane hydrocarbon concentrations on the boundaries of relevant facilities, such as service stations, had to be < 4.0  $mg/m^3$  ([National Standards of the People's Republic of China, 2020](#); GB 20952-2020). [The Working Group noted that the term “non-methane hydrocarbon” used in the publication was likely to be the same as NMVOC.]

The authors of several studies have measured the concentrations of gasoline components in the air around service stations. These components included a number of VOCs, such as BTEX and MTBE, and some of the studies included modelling of the chemical dispersion around the source. Selected studies are summarized in Table S1.6 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>). The presence of recovery systems for gasoline vapour in service stations is highly relevant for the local concentrations of various air pollutants, and this information is reported when available. Moreover, after the year 2000, the amount of benzene in gasoline was regulated in the EU and the USA, and therefore the concentrations of benzene in the vapour decreased accordingly. For example, in Toulouse, France, the mean benzene concentration in the spring-to-summer period decreased significantly from a mean of 2.0 (range, 1.1–3.8)  $\mu g/m^3$  in 1999, before the regulations were introduced, to 1.1 (range, 0.7–2.0)  $\mu g/m^3$  in 2001, after the reformulated gasoline was introduced ([Simon et al., 2004](#)).

To verify the efficacy of the stage II recovery system, a 2-week monitoring study was performed in a modern service station located on a motorway in the rural location of Rambouillet, France, in 1999 ([González-Flesca et al., 2002](#)). During the first week, the vapour recovery



system of the station was operating, and it was disconnected during the second week. The mean benzene air concentration ranged from 2.6 to 3.5  $\mu\text{g}/\text{m}^3$  with the recovery system on and from 3.6 to 8.1  $\mu\text{g}/\text{m}^3$  with the recovery system off. It was concluded that stage II vapour recovery reduces the levels of benzene, toluene, and xylene (BTX), and especially benzene, near the fuel pumps and that the emissions released from a service station with stage I and II systems operating into its surroundings appear to be lower than those typically present in urban areas with traffic.

Outdoor concentrations of benzene and MTBE were measured in the vicinities of 25 houses in a residential area located within 50 m of a service station and another 25 houses located > 100 m from a service station in Taegu, the third most populous city in the Republic of Korea, during the summer and winter of 2020. There was no mention of the presence of any vapour recovery system in these service stations. Benzene and MTBE levels were higher in the houses that were closer to the stations, with mean concentrations of [5.5 and 10.3  $\mu\text{g}/\text{m}^3$ ] in these, versus [3.9 and 7.3  $\mu\text{g}/\text{m}^3$ ] in the more distant houses (Jo and Oh, 2001).

In another study performed in Yuanlin City, Taiwan, China, Lin et al. (2005) measured the levels of MTBE, benzene, and toluene in ambient air at four different positions in one service station: (i) at the boundaries of the traffic inlet and (ii) outlet areas, (iii) in the proximity of the three pumps dispensing gasoline for motorcycles, and (iv) at two pumps dispensing gasoline for cars. The service station was not equipped with any vapour recovery systems. The mean concentration of benzene close to the pumps was [127–169  $\mu\text{g}/\text{m}^3$ ], higher than in the inlet and outlet areas, where it was [41–33  $\mu\text{g}/\text{m}^3$ ]. The mean concentrations of toluene were between [172 and 1035  $\mu\text{g}/\text{m}^3$ ] and the mean concentrations of MTBE were between [51 and 699  $\mu\text{g}/\text{m}^3$ ] (Lin et al., 2005).

In a study performed in Taoyuan, China, Wu et al. (2006) measured the concentrations of 54 VOCs in four service stations, two of which were equipped with vapour recovery systems. The three sampling points were at the entry and exit of the service station and above the storage tank. The concentrations of VOCs were approximately seven times higher, and those of MTBE approximately 20 times higher, in the stations that did not have recovery systems than in those that did (Wu et al., 2006).

de Oliveira et al. (2007) measured the concentrations of several VOCs in a service station located in Rio de Janeiro, Brazil, in 2004. Sampling was performed at two locations, beside the fuel dispenser and in an area with a similar level of traffic that was 200 m from the station. The mean benzene concentration in the service station was 144.5  $\mu\text{g}/\text{m}^3$ , which was an increase of approximately 10-fold over that in the more distant location. In addition, the concentrations of toluene, ethylbenzene, and xylene in the service station were approximately three times as high as the control values. The station had no recovery systems (de Oliveira et al., 2007).

Morales Terrés et al. (2010) evaluated the effect of a stage I recovery system fitted in a service station on the exposure of residents living in the surrounding area in the city of Murcia, Spain, in May and June 2008. During two sampling periods, air samples were collected at 15 locations in the service station and its surroundings (up to a distance of 100 m), and the *n*-hexane, benzene, and cyclohexane concentrations were measured. For each of these pollutants, an isoconcentration curve was drawn for the locations around the station. The concentration of benzene in the station ranged from 4.6 to 37.3  $\mu\text{g}/\text{m}^3$ , and that in the surroundings ranged from 1.16 to 8.6  $\mu\text{g}/\text{m}^3$ . On the basis of the ratio of the concentrations of aliphatic and aromatic hydrocarbon pollutants in the air in the vicinity of the service stations, the researchers concluded that the effect of the service station differed in the various directions

analysed, but the compounds were never detectable at distances of  $> 75$  m ([Morales Terrés et al., 2010](#)). [The authors did not report the LOD, but the Working Group assumed on the basis of the data reported in the paper that it was  $< 1 \mu\text{g}/\text{m}^3$ .]

A dispersion model for gasoline vapour was developed by [Kountouriotis et al. \(2014\)](#) using VOC data published in Concawe report 09-02 ([Concawe, 2002](#)). The measurements of VOC levels were performed before the year 2000 in service stations that were not equipped with vapour recovery systems and dispensed gasoline with a benzene content of up to 5%. The predicted VOC concentrations ranged from a few parts per billion (ppb) to 6000 ppm, and the dispersion curves showed that the wind plays a major role in the direction and degree of dispersion of gasoline vapour ([Kountouriotis et al., 2014](#)). [The Working Group noted that the data used for these models were collected before the era of recovery system use, and therefore the conclusions were not applicable to the recent situation in Europe. However, these data were useful for the interpretation of data from older studies and from studies performed in settings in which vapour recovery systems had not been installed.]

[Barros et al. \(2019\)](#) measured BTEX levels in the surroundings of service stations in the urban environment of the medium-sized city of Porto, Portugal. No information regarding the presence or absence of recovery systems was reported. Outdoor sampling locations were chosen on the basis of their distance from a service station: exposed areas were defined as being within 80 m of a service station, and the control areas were approximately 300 m away. These areas were matched with respect to traffic, level of urbanization, and orientation. Forty-eight samples were collected over 3 weeks in August 2017. Higher BTEX concentrations were found in the exposed areas than in the control areas (the median benzene concentrations were  $1.8$  and  $1.5 \mu\text{g}/\text{m}^3$ , respectively).

[Hilpert et al. \(2019\)](#) performed a study in one service station in the Midwest of the USA during 2014/2015 and another on the northwest coast in 2009. These stations were equipped with stage I and stage II recovery systems. Sampling was performed for 18–20 days in the vent pipe of the gasoline tank, and the benzene concentration was estimated from the vapour flow. The authors found that the 1-hour acute reference exposure level for benzene for the general population ( $26 \mu\text{g}/\text{m}^3$ ) was exceeded only within 50 m of the centre of one of the service stations, and for the other, this concentration was only exceeded on 2 days, up to 160 m from its centre. The authors concluded that the distance recommended by the California Air Resources Board between service stations and schools, day care centres, and other sensitive locations of 91 m was not enough to ensure that the local benzene concentration was  $< 26 \mu\text{g}/\text{m}^3$  ([CalEPA/CARB, 2005](#)). In a more recent study, the gasoline vapour dispersion from a large, simulated service station equipped with a stage I recovery system in Maryland, USA, was modelled ([Hilpert, 2023](#)). The model predicted that, for a service station that dispensed [34 million litres] of gasoline per year using California dispensing technology, the concentrations of benzene did not exceed the guideline value of  $26 \mu\text{g}/\text{m}^3$  at a distance of 25 m.

[Zeng et al. \(2022\)](#) studied a service station in Guangzhou, China, which was equipped with stage I and stage II recovery systems. Two sampling sites were chosen, one above the fuel dispenser and the other on the edge of the station, 30 m apart. Thirty-three samples were collected for the measurement of 88 VOCs, including 58 non-methane hydrocarbons. The mean total concentration of non-methane hydrocarbons was  $3.2 \pm 0.6 \text{ mg}/\text{m}^3$ , and 5 of 21 samples collected at the fuel dispenser contained concentrations that exceeded the recently implemented national guideline limit of  $4.0 \text{ mg}/\text{m}^3$ .

Health Canada estimated the evaporative emission from service stations, focusing on the dispersion of benzene. Changes in the benzene concentration with increasing distance from the service station were modelled. It was estimated that a median-sized service station emitted benzene at a rate of 1.0 mg/second. The concentration of benzene in the air decreased gradually with increasing distance from the station and equalled the background level (the level of benzene in the air of the city) at approximately [50–220 m] ([Health Canada, 2023](#)).

[The Working Group noted that in service stations equipped with vapour recovery systems and when cars are equipped with ORVR units, the concentration of benzene in air was typically in the range of a few to tens of micrograms per cubic metre, with these concentrations decreasing with increasing distance from the pumps. The concentrations of benzene and any other VOCs were up to hundreds of micrograms per cubic metre in the absence of vapour recovery systems and in older studies. The Working Group noted that limited data were available on the implementation of vapour recovery systems in service stations and cars worldwide. For some examples of the regulations, see Section 1.5. The few studies that investigated the influence of a service station on the levels of VOCs in the air in the surrounding residential areas suggested that these may be high within 100–220 m of the service station, but rarely beyond this distance.]

(ii) *Air monitoring inside and around cars*

VOCs in and around cars originate from several sources, including gasoline evaporative emissions ([Hata et al., 2020](#)), car interior furnishing materials ([Yoshida et al., 2006](#)), the infiltration of engine exhausts ([Besis et al., 2023](#)), and other exterior environmental sources associated with various activities, such as filling up with gasoline ([Shearston and Hilpert, 2020](#)).

In some studies, the concentrations of VOCs and/or benzene in and around cars have been measured, but frequently the contributions of the various sources to the totals were not differentiated.

[Besis et al. \(2023\)](#) measured the concentrations of 117 VOCs in vehicular cabin air and discussed their implications for commuter exposure. The most frequently detected VOCs were alkanes, followed by organic acids, alkenes, aromatic hydrocarbons, ketones, aldehydes, sulfides, amines (diphenylamine and triethylamine; median concentration,  $0.15 \mu\text{g}/\text{m}^3$ ), phenols, mercaptans, and thiophenes. In gasoline-fuelled cars, the mean total concentration of VOCs was  $136 \mu\text{g}/\text{m}^3$ , which was lower than in passenger cars fuelled with diesel and liquefied petroleum gas (LPG). Most of the VOC concentrations were higher in the presence of exterior air ventilation.

[Chen et al. \(2014\)](#) measured the levels of VOCs and BTEX in the cabins of 38 taxis in Changsha city, the capital of Hunan Province, China. None of the tested taxis had leather trim (which might contain toluene in paint or surface coating), and all had air conditioning and used gasoline fuel. The median concentration of BTEX ranged from  $80 \mu\text{g}/\text{m}^3$  for benzene to  $217 \mu\text{g}/\text{m}^3$  for toluene, and the median TVOC concentration was  $1406 \mu\text{g}/\text{m}^3$ . The VOC level in the cars was positively associated with the interior temperature and inversely associated with the age of the vehicle.

[Esteve-Turrillas et al. \(2007\)](#) proposed a method for the assessment of air quality inside vehicles and in service stations by monitoring the BTEX level using semipermeable devices. Over sampling times of approximately 24 hours, concentrations from  $0.2$  to  $145 \mu\text{g}/\text{m}^3$  were measured for single BTEX components inside motor vehicles, and the concentration of benzene ranged from  $1.7$  to  $44 \mu\text{g}/\text{m}^3$ . During periods of exposure of 2–6 minutes during the refuelling of vehicles with gasoline, concentrations of BTEX

ranging from 0.03 to 79 mg/m<sup>3</sup> were measured, with concentrations of benzene ranging from 0.33 to 4.9 mg/m<sup>3</sup>.

[Som et al. \(2007\)](#) measured the concentrations of BTEX in passenger cars in Kolkata, India, during two time periods, when the gasoline contained 5% benzene (2001–2002), and later (2003–2004), when the benzene content had been reduced to <3%. Monitoring was performed inside and immediately outside passenger cars that were or were not fitted with catalytic converters and used various types of fuels, along two congested urban routes. When the benzene content of gasoline was 5%, the mean concentration of benzene inside the cars without a catalytic converter was found to be as high as 721.2 µg/m<sup>3</sup>. After the benzene content had been reduced to 3% and in cars with appropriately modified engines, the mean interior benzene concentration was 112.4 µg/m<sup>3</sup>. The authors concluded that commuters travelling in cars in urban Kolkata were more highly exposed to BTEX than those in many other cities of the world, owing to insufficient road space, a lack of speed, and inadequate emission control.

[Hinwood et al. \(2006\)](#) measured the concentrations of 41 VOCs in 27 locations in Perth, Australia, including a carpark located in a basement, where they found the highest 12-hour concentrations of toluene and benzene (single value, [95 and 18 µg/m<sup>3</sup>], respectively).

[Lee and Jo \(2002\)](#) measured the concentrations of MTBE, benzene, and toluene in cars and buses in commuter traffic in Taegu, the third largest city in the Republic of Korea, during both the summer and winter. Inside the cars, the median MTBE, benzene, and toluene concentrations were 28.3, 32.1, and 164 µg/m<sup>3</sup>, respectively. They found that the age of the car influenced the concentrations of these chemicals: the concentrations were lower in newer cars than in older cars.

[Vayghani and Weisel \(1999\)](#) measured the concentrations of MTBE in the air inside automobile cabins while they were being fuelled [the time of peak exposure at service stations]. The median concentrations were 360, 18, and 69 µg/m<sup>3</sup> for MTBE, benzene, and toluene, respectively. The highest interior concentrations were obtained for cars with a malfunctioning vapour recovery system, when the temperature was high, and when there was little wind. The concentrations were also higher when car windows were open during refuelling.

[Chan et al. \(1991\)](#) assessed the levels of exposure of commuters to VOCs in Boston, Massachusetts, USA. The interior median concentrations of benzene, toluene, ethylbenzene, xylene, and formaldehyde were 15.9, 28.1, 4.9, 23.4, and 4.4 µg/m<sup>3</sup>, respectively. When the mean duration of the commute was taken into account, commuting by private car was associated with the highest mean VOC concentrations (compared with walking, cycling, and subway transport), and commuting by car on urban roads was associated with higher VOC concentrations than commuting on interstate routes. It was concluded that driving can contribute approximately 10–20% to the total exposure of an individual to gasoline-related VOCs.

Overall, these studies of gasoline-related compounds, including VOCs and benzene in particular, in and around cars have pointed to several different sources of exposure, including gasoline evaporation, tailpipe emissions, and car interior furnishing materials. The determinants of the VOC concentrations were the age of the car, gasoline refuelling, the presence of an ORVR system, the ambient temperature, and the composition of the gasoline. The reported concentrations of VOCs varied from tens to thousands of micrograms per cubic metre.

*(b) Soil and groundwater*

Lead is a relevant contaminant of topsoil and deposited dust in large, urban areas. A major source of this contamination is recognized to be the traffic emissions that occurred when leaded gasoline was in use ([Mielke and Reagan, 1998](#); [Resongles et al., 2021](#); [Olowoyo et al., 2022](#); [Rubio et al., 2022](#)). In South Africa, the level of lead in soil dust was reported to be 0.08–188 µg/g, with the highest levels being found at sites with large amounts of traffic and in industrial areas ([Olowoyo et al., 2022](#)). In the city of Santa Ana, California, USA, the concentration of lead in the topsoil ranged from 20 to >230 µg/g, with the highest concentrations being found near the arterial roads ([Rubio et al., 2022](#)). [Although traffic emissions of lead were probably mainly from tailpipe exhaust, the Working Group considered that automotive gasoline evaporation was a more minor source of lead during the periods when leaded gasoline was used.]

The contamination of soil and groundwater with gasoline has also been reported. This is generally the result of fuel leaks from underground storage tanks ([Day et al., 2001](#); [Patel et al., 2004](#); [Connor et al., 2015](#); [Balseiro-Romero et al., 2016](#)) or gasoline spills, which frequently occur at service stations ([Kao and Prosser, 2001](#)). It has been reported that approximately 11% of annual oil spill accidents in Canada are gasoline spills ([Xiong et al., 2014](#)), and small gasoline leaks frequently occur at service stations, where up to 100 g per [tonne] of gasoline is lost in this way ([Khrestenko et al., 2020](#); [US EIA, 2024](#)). The concentrations of fuel-related VOCs in the topsoil and subsoil in close proximity to a service station in Spain were reported to range from undetectable to 235 µg/kg soil and undetectable to 3600 µg/kg soil for benzene and MTBE, respectively. The fate and behaviour of a fuel spill into the environment depend on a wide variety of natural processes known as weathering, including volatilization, biodegradation,

photodecomposition, chemical oxidation, bioaccumulation, dispersion, diffusion, binding to the soil, and leaching into groundwater ([Balseiro-Romero et al., 2016](#)). Furthermore, remediation of these sites can occur naturally via evaporation and natural biodegradation ([Kao and Prosser, 2001](#); [Lee and Jeon, 2018](#)).

In the United States Geological Survey's National Water-Quality Assessment (NAWQA) Program, samples of groundwater were collected between 1993 and 2002 to assess the level of contamination of major aquifers with MTBE and gasoline-related hydrocarbons ([USGS, 2025](#)). MTBE was the most frequently detected chemical; it was mostly found in areas with high population densities and where MTBE was used in gasoline. Only 13 groundwater samples from all of the study types, or 0.3%, had concentrations of MTBE that exceeded the 20 µg/L lower limit for a US EPA Drinking-Water Advisory ([Moran et al., 2005](#)). Similar results were obtained in Germany ([Kolb and Püttmann, 2006](#)).

For additional data on the contamination of soil and water with some of the oxygenated additives in gasoline, see the monographs on MTBE, ETBE, TBA, DIPE, and TAME in the present volume.

*(c) Food and consumer products*

[The Working Group noted that there was little information on the gasoline contamination of food and consumer products.]

The presence of hydrocarbons in food has been assessed in several studies, and this can have natural, especially for fruit and vegetables, and anthropogenic origins, being derived, for example, from food processing and packaging (reviewed in [Nijssen et al., 1996](#)). The concentration of (aromatic) hydrocarbons in food increases with the fat content of the food item. However, their presence has not been associated with gasoline contamination, except in the case of food sold at service stations (as reviewed by [Concawe, 2000](#)). This work showed that the



concentrations of benzene and other gasoline components in food were not increased by the presence of gasoline, whereas the concentration of toluene was up to 10 times higher in the proximity of newspapers and magazines, especially for chocolate-based sweets (Müller et al., 1990). The influence of the density of the traffic adjacent to a food shop on the hydrocarbon levels in food was unclear. In the review, the authors concluded that the levels of gasoline-related hydrocarbons in food sold by service stations were comparable to those found in food purchased from other shops (Concawe, 2000).

No data were found regarding gasoline in consumer products.

### 1.4.2 Occupational exposure

#### (a) Occupational exposure settings

Occupational exposure to automotive gasoline, a product consisting of a complex mixture of substances, has been associated with operations or jobs that are performed in both indoor and outdoor work environments (the typical hydrocarbon composition is described in Section 1.1). Operations or tasks involving gasoline exposure include the maintenance, inspection, cleaning, and filling of gasoline storage tanks and pumps; and the jobs that involve exposure are car mechanics, refuelling workers, service-station workers (including those that clean, polish, wax, and detail the interior of vehicles, cashiers, and vendors), fuel truck drivers, and traffic police officers. [The Working Group noted that the BTEX exposure of police officers probably occurs as a result of exposure to vehicle exhausts, rather than gasoline.] The exposure levels are discussed according to the job type below and provided in detail in Table S1.7 (area air concentrations), Table S1.8 (personal air concentrations), and Table S1.9 (biomonitoring) (see Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>).

Gasoline spill responders are exposed when cleaning up gasoline spills from tanks, pipelines, or accidents. [No data quantifying the exposure of gasoline spill responders were available to the Working Group.] Agricultural workers can be exposed when using gasoline as fuel for farming equipment, such as pumps, generators, and lawnmowers (US EPA, 2015). [The Working Group noted that while agricultural workers are filling these types of equipment with gasoline, their exposure would be similar to that of service station attendants who are manually filling vehicles. The agricultural workers would then be exposed to exhaust fumes while operating the equipment (US EPA, 2015), as well as to other chemicals, such as pesticides.]

Weisel (2010) described the exposure to gasoline of transportation industry workers, such as drivers [The Working Group noted that the type of driver studied was not specified, but taxi drivers were mentioned in other parts of the article], service station attendants, and car mechanics, who were exposed to gasoline fumes and could have relatively high peak exposure levels (Weisel, 2010). [The Working Group noted that drivers would have higher levels of exposure to car and truck exhaust fumes than would service station attendants and that automobile mechanics would probably be exposed to used engine oil, in addition to gasoline.]

#### (i) Service station attendants

Service station attendants are exposed to gasoline vapour released during fuelling and to spills and leaks when handling fuel pumps (Hilpert et al., 2015). [The Working Group noted that “gas station attendant”, “pump attendant”, “pump service attendant”, and “fuelling pump worker” can be used as job titles for the same job in some countries. The job title used in the present monograph is “service station attendant”.] Benzene is among the VOCs in automotive gasoline. It is classified by IARC as *carcinogenic to humans* (Group 1; IARC, 2018), and for this reason, the

concentration of benzene in gasoline is highly regulated in most countries. [The Working Group noted that benzene can thus be reasonably used as a proxy for other volatile gasoline constituents in situations in which gasoline can be expected to be the predominant source of benzene, such as at service stations. However, it must be kept in mind that there are other sources of benzene, such as air pollution, and that the proportion of the total benzene concentration measured at a service station that originates from gasoline vapour might vary.]

Service station attendants are particularly vulnerable to gasoline exposure if they manipulate the fuel, when they might inhale fuel vapour, owing to a lack of adequate risk management measures, regulatory actions, and enforcement activities (Ekpenyong and Asuquo, 2017). In addition, the presence of a high ambient temperature and insufficient personal protection may lead to service station workers being exposed to elevated levels of fuel vapour (Scheepers et al., 2019). Table S1.7 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) summarizes the area air concentrations to which service station attendants have been exposed.

In service stations that do not have vapour recovery systems or are poorly ventilated, the service station attendant filling the tank is exposed to the vapour-saturated air expelled through the filling channel around the nozzle. Those at fuelling stations equipped with vapour recovery systems, such as stage I systems for storage tanks and stage II systems for refuelling, generally experience lower exposure levels than do those at stations without such systems (see also Table S1.6, Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>). The presence and effectiveness of these vapour recovery systems influence the exposure levels. For example, service station

attendants had a range of exposure to gasoline vapour of 2.2–12.2 mg/m<sup>3</sup> (quantified as the sum of the exposures to 150 gasoline constituents) when vapour recovery systems were present and 1.3–50.5 mg/m<sup>3</sup> when they were not (Concawe, 2002). [The Working Group noted that ranges for benzene were not given in this report.] Table S1.8 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) summarizes the personal air concentrations to which service station attendants have been exposed. Few studies have been conducted in Africa, but a narrative review by Anigilaje et al. (2024) assessed the occupational health and safety challenges of service station attendants in Nigeria, and in particular BTEX exposure. They concluded that the air quality at work was generally poor for these workers.

In many European and North American countries, self-service gasoline stations predominate, with the exception of Italy and New Jersey, USA (Meyersohn, 2023). In Brazil and Thailand, service station attendants generally work in full-service stations, refuelling customers' motor vehicles (Poça et al., 2021; Lima et al., 2024). [The Working Group noted that service station attendants still refuel cars in many countries and that there was a lack of systematic data. Furthermore, in LMICs, informal workers, including street vendors, may sell their products in service stations.]

In Sri Lanka in 2019, there were 1200 service stations employing between 10 000 and 15 000 attendants (Scheepers et al., 2019). In Thailand, the number of employees at the approximately 28 000 service stations was estimated to be between 84 000 and 148 000 (CEIC, 2021). There were an estimated 150 000 fuelling outlets, in addition to 121 852 convenience stores that sold fuel, in the USA in 2025 (NACS, 2025). The US Bureau of Labour Statistics estimated the number of employees in service stations in 2023 to be the following: automotive service technicians and

mechanics, 9750; cashiers, 610 860; combined food preparation and serving workers, including for fast food, 37 200; first-line supervisors/managers of retail sales workers, 106 120; food preparation workers, 17 920; and service station attendants, 16 310. For a historical perspective, the NIOSH National Occupational Exposure Survey reported that there were 146 400 (122 047 male and 24 353 female) service station attendants during 1981–1983. In 1970, a study of service station workers was conducted in the Nordic countries (Lynge et al., 1997), in which the following numbers of service station workers were reported: in Denmark, 5250 (4055 male and 1195 female); in Norway, 3561 (3181 male and 308 female); in Sweden, 8606 (7901 male and 705 female); and in Finland, 1552 (1387 male and 165 female).

#### (ii) Gasoline tanker drivers

Gasoline tanker drivers are exposed to vapour released during the transfer of gasoline from tanker trucks to storage tanks. Stage I recovery systems, which capture and recover emissions from storage tanks, bulk plants, bulk terminals, and the loading/unloading of tankers and trucks, have been used at various stages of fuel handling, such as at service station underground storage tanks. The exposure levels of drivers have been shown to depend to a great extent on the implemented exposure reduction measures, such as stage I recovery systems (Smith et al., 1993; Hilpert et al., 2015). Lovreglio et al. (2010) measured the area air concentrations of gasoline components for 18 tanker drivers who were involved in the transport and loading (1–7 times during an 8-hour shift) and unloading (1–4 times per shift) of fuels by means of a closed-circuit system. Table S1.8 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) summarizes the personal air concentrations to which gasoline tanker drivers were exposed in this study. [The Working Group

noted that, at the present time, the transfer of gasoline from tanker trucks is mainly conducted using closed systems that include vapour recovery couplers and air interlock valves. However, the fuel tanker drivers studied by Lovreglio et al. (2010) had greater exposure to BTEX than did the service station attendants in the same study.]

#### (iii) Gasoline tank inspectors

Gasoline tank inspectors are exposed when opening and sampling underground or above-ground gasoline storage tanks. At modern service stations that include vapour control technology, exposure levels are expected to be significantly lower. In more technically advanced settings, gasoline storage tanks have sensors that automatically monitor fuel levels, water levels, and the temperature.

#### (iv) Automobile mechanics

Automobile mechanics are exposed to many petroleum-based products that are used for degreasing and cleaning purposes. Although the exact composition of these products varies, they generally include toluene, xylene, heptane, hexane, petroleum distillates, and naphtha mixtures (Fries et al., 2018). Automobile mechanics may repair fuel systems, when they would have direct contact with gasoline (Badjagbo et al., 2010). The limited data on the BTEX exposure of automobile mechanics are summarized in Table S1.7 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) with respect to the area air concentrations and in Table S1.8 (also in Annex 1) for the personal air concentrations. The presence of a functioning ventilation system and the tasks being performed, such as draining, substituting, and refuelling gasoline tanks, and cleaning up spills, influenced exposure to gasoline (Wilson et al., 2007). The peak levels of exposure were associated with specific short-term tasks (Fries et al., 2018).

*(v) Co-exposures in the workplace*

[The Working Group noted that people with certain jobs may be exposed to other substances alongside gasoline, and these are listed in [Table 1.10](#) for four occupations. No comprehensive quantitative data on the co-exposure to exhaust emissions were available for these occupations.] Exhaust fumes are a common co-exposure, in addition to gasoline, experienced by automobile mechanics while testing engines. Consequently, automobile mechanics are exposed not only to BTEX, but also particulate matter, soot, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs). In many studies, benzene was used as an exposure marker, but the contributions of gasoline vapour and traffic emissions cannot easily be determined without knowledge of the source of benzene. Service station attendants are also co-exposed to exhaust fumes from nearby traffic and waiting cars. A study in service station attendants in three states in Mexico involved the measurement of the air concentrations of components of gasoline, such as benzene and ethylbenzene, as well as formaldehyde and carbon monoxide, which are not components of gasoline ([Shinohara et al., 2024](#)). These concentrations were compared with the concentrations at a distance of 500–1000 m from the service stations, which were deemed to be the background levels, because no exposure to gasoline vapour from the service stations would be expected at these distances. This comparison showed that the concentrations of gasoline vapour components were much higher than background at the service stations (e.g. benzene, mean ranges, 0.65–1.6  $\mu\text{g}/\text{m}^3$  and 18–45  $\mu\text{g}/\text{m}^3$ , respectively), whereas the levels of general air pollution were similar at the two locations. Although the study was small (16 air measurements), the results suggested that service station attendants are exposed to more gasoline vapour, but have similar levels of exposure to traffic-related air pollutants

to individuals with other outdoor occupations in proximity to traffic.

These findings were supported by those of another study ([Fustinoni et al., 2005a](#)), which showed that traffic police officers (as representatives of workers who are mainly exposed to benzene via traffic emissions) had higher levels of exposure to benzene ( $n=77$ ; mean, 22  $\mu\text{g}/\text{m}^3$ ; range, 9–316  $\mu\text{g}/\text{m}^3$ ) than did the general population ( $n=58$ ; mean, 6  $\mu\text{g}/\text{m}^3$ ; range, <6–115  $\mu\text{g}/\text{m}^3$ ), but lower levels of exposure than service station attendants (mean, 61  $\mu\text{g}/\text{m}^3$ ). Mechanics are co-exposed to a range of carcinogens (see [Table 1.10](#)), whereas the levels of co-exposure for service station attendants and tanker drivers are more limited. Additionally, a study of the levels of benzene exposure for various occupation groups performed in Europe during 1999–2000 showed that service station attendants in Milan had the highest level of exposure (70  $\mu\text{g}/\text{m}^3$ ), followed by urban police officers (25  $\mu\text{g}/\text{m}^3$ ) and bus drivers in Genoa (20  $\mu\text{g}/\text{m}^3$ ) ([Farmer et al., 2005](#)). In contrast, the referents had much lower exposures: office and hospital workers in Milan (7  $\mu\text{g}/\text{m}^3$ ), clerks and researchers in Genoa (8  $\mu\text{g}/\text{m}^3$ ). A second part of the study was conducted in Burgas, Bulgaria, among 158 petrochemical workers and a referent population ( $n=50$ ) that was not further defined. Using a method that had an LOD of 100  $\mu\text{g}/\text{m}^3$ , the geometric means of breathing-zone benzene concentrations were 1765  $\mu\text{g}/\text{m}^3$  and <100  $\mu\text{g}/\text{m}^3$  for workers and referents, respectively. Benzene exposure was used as a proxy for gasoline exposure, but police officers and bus drivers were primarily exposed to exhaust fumes rather than gasoline. See Table S1.8 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) for details.

**Table 1.10 Non-exhaustive list of co-exposures of workers exposed to gasoline and agents classified by IARC in Group 1 or 2A**

| Agent<br>CAS No.                                                | IARC Group                  | Automobile<br>mechanics | Service<br>station<br>attendants | Gasoline<br>tanker<br>drivers | Petroleum<br>product<br>manufacturing<br>workers | References                                                                                                                                                            |
|-----------------------------------------------------------------|-----------------------------|-------------------------|----------------------------------|-------------------------------|--------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Engine exhaust emissions                                        | 1 (diesel)<br>2A (gasoline) | ✓                       | ✓                                | ✓                             |                                                  | <a href="#">EU-OSHA (2013)</a> ; <a href="#">Lim et al. (2021)</a> ; JEM <sup>a</sup> (CANJEM)                                                                        |
| Arsenic<br>7440-38-2                                            | 1                           |                         |                                  |                               | ✓                                                | <a href="#">IARC (1989)</a> ; JEM <sup>a</sup> (MatEmESp)                                                                                                             |
| Asbestos<br>12001-29-5                                          | 1                           | ✓                       |                                  |                               | ✓                                                | <a href="#">IARC (1989)</a> ; <a href="#">Paustenbach et al. (2003)</a> ; <a href="#">Salazar et al. (2015)</a> ; JEM <sup>a</sup> (CANJEM)                           |
| Cadmium<br>7440-43-9                                            | 1                           | ✓                       |                                  |                               |                                                  | <a href="#">Khan et al. (2023b)</a>                                                                                                                                   |
| Chromium(VI)<br>18540-29-9                                      | 1                           | ✓                       |                                  |                               |                                                  | <a href="#">Vitayavirasuk et al. (2005)</a> ; <a href="#">NIOSH (2018b)</a> ; JEM <sup>a</sup> (CANJEM)                                                               |
| Lead<br>7439-92-1                                               | 2A                          | ✓ <sup>b</sup>          | <sup>b</sup>                     | <sup>b</sup>                  |                                                  | <a href="#">IARC (1989)</a> ; <a href="#">Khan et al. (2023b)</a> ; JEM <sup>a</sup> (CANJEM and MatEmESp)                                                            |
| Methylene chloride<br>75-09-2                                   | 2A                          |                         |                                  |                               | ✓                                                | <a href="#">IARC (1989)</a> ; JEM <sup>a</sup> (MatEmESp)                                                                                                             |
| Nickel compounds<br>7440-02-0                                   | 1                           | ✓                       |                                  |                               |                                                  | <a href="#">Ikese et al. (2021)</a> ; JEM <sup>a</sup> (MatEmESp)                                                                                                     |
| PAHs                                                            | 2A                          | ✓                       | ✓                                | ✓                             |                                                  | <a href="#">Schoket et al. (1999)</a> ; <a href="#">Ifegwu et al. (2012)</a> ; <a href="#">Roadmap on Carcinogens (2025)</a> ; JEM <sup>a</sup> (CANJEM and MatEmESp) |
| Welding fumes                                                   | 1                           | ✓                       |                                  |                               |                                                  | <a href="#">Menicagli et al. (2018)</a> ; JEM <sup>a</sup> (MatEmESp)                                                                                                 |
| Other occupational exposures in petroleum refining <sup>c</sup> | 2A                          |                         |                                  |                               | ✓                                                | <a href="#">IARC (1989)</a>                                                                                                                                           |

CANJEM, Canadian Job-Exposure Matrix; CAS, Chemical Abstracts Service; JEM, job-exposure matrix; MatEmESp, Spanish job-exposure matrix; PAH, polycyclic aromatic hydrocarbon; ✓, exposure recorded.

<sup>a</sup> This exposure was identified for the relevant occupations in either of the following JEMs: MatEmESP or CANJEM ([Siemiatycki and Lavoué, 2018](#); [García et al., 2013](#)).

<sup>b</sup> Historical exposure to lead when leaded gasoline was used.

<sup>c</sup> Other occupational exposures during petroleum refining include a large range of chemicals present in crude oil, some of which might also have been present in gasoline in various quantities. These included a large variety of chemicals and materials not present in crude oil (see Monograph 1, Table 5; [IARC, 1989](#)).



[The Working Group noted that workers who are typically exposed to gasoline only are those involved in the direct handling of gasoline, or its transportation, storage, or use, but are not significantly exposed to combustion products, such as exhaust fumes.]

(b) *Air concentrations in the workplace*

(i) *Area air measurements*

Occupational exposure to gasoline-related VOCs has been extensively studied in various regions and industries through area air monitoring in the workplace. The details of the studies, including their sample sizes, analytical methods, and specific regional findings, can be found in the accompanying Table S1.7 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>). Studies of gasoline-exposed workers are generally conducted to measure benzene exposure, because specific exposure limits are stated in regulations (Weisel, 2010). Occupational exposure levels were usually below 0.32–3.19 mg/m<sup>3</sup>, and the level of gasoline exposure of the general population was much lower than occupational levels of exposure, ranging from <0.003 to 0.03 mg/m<sup>3</sup> (Weisel, 2010).

The studies summarized in Table S1.7 (Annex 1) were conducted in service stations worldwide, including at locations in the Islamic Republic of Iran (subsequently referred to as “Iran”), Thailand, Italy, and Brazil. These yielded various levels of exposure to BTEX. For example, the benzene concentrations in service stations were 0.3–428 µg/m<sup>3</sup>, depending on the region and sampling conditions; the maximum concentration of 428 µg/m<sup>3</sup> was measured in a study conducted in Khon Kaen, Thailand, in 2019 (Chaiklieng et al., 2021). The concentrations of toluene, ethylbenzene, and xylene were 26–1301 µg/m<sup>3</sup>, 3–105 µg/m<sup>3</sup>, and 0.1–437.5 µg/m<sup>3</sup>, respectively.

Studies of workers in automotive repair shops yielded a wider range of benzene concentrations, from 0.01 to 3160 µg/m<sup>3</sup>.

(ii) *Peak exposure measurements*

The typical tasks of service station workers in Sri Lanka have been described as the refilling of customer tanks (refilling road vehicle tanks with the nozzle approximately 0.5 m from the breathing zone) over 25 seconds, the measurement of storage tank contents (by opening the tank, inserting a dipstick into the tank, and reading the fuel line mark to determine the volume, before cleaning the measuring stick with a towel) over 45 seconds, and the refilling of main fuel storage tanks (opening the main gasoline storage tank of the station to help the truck driver connect a hose to start the refilling process) over 60 seconds (Scheepers et al., 2019). The peak exposure levels during these tasks were identified using real-time measurements of total fuel vapour concentrations. High concentrations of VOCs were found during the filling of a customer’s fuel tank (2.24 ppm), the measurement of the fuel level in a storage tank (96.4 ppm), and the refilling of the main fuel storage tank (239 ppm). During this last task, truck drivers were also exposed while connecting the hose and starting the refilling process. In addition, a review by Concawe of gasoline exposure data collected during 1993–1998 in Europe showed that road tanker drivers had high levels of exposure to many of the agents (benzene, 39.2 mg/m<sup>3</sup>; toluene, 200 mg/m<sup>3</sup>; xylenes, 160.6 mg/m<sup>3</sup>; *n*-hexane, 35.8 mg/m<sup>3</sup>; and all hydrocarbons, 1602 mg/m<sup>3</sup>) for short periods of time (<1 hour) (Concawe, 2000). Historical peak exposure measurements that were made in the Nordic countries before vapour recovery systems were introduced between the 1970s and the early 1990s have also been reported (Lyngé et al., 1997). The benzene content of gasoline at this time was 2–6% by volume. Short-term measurements of the air concentrations of

benzene were made in the late 1970s (mean, 0.27 mg/m<sup>3</sup>; range, 0.06–0.99 mg/m<sup>3</sup>; number of samples, not reported; 45 minutes); early 1980s (mean, 1.6 mg/m<sup>3</sup>; range, 0.096–3.2 mg/m<sup>3</sup>;  $n = 36$ ; 3–5 minutes), 1989 (mean, 2.3 mg/m<sup>3</sup>; range, 0.01–27.3 mg/m<sup>3</sup>;  $n = 175$ ; 0.45–5 minutes), 1995 (mean, 3.26 mg/m<sup>3</sup>; range, 0–19.94 mg/m<sup>3</sup>;  $n = 17$ ; 2–3 minutes), and 1995 (mean, 2.53 mg/m<sup>3</sup>; range, 0–17.2 mg/m<sup>3</sup>;  $n = 28$ ; 20–30 minutes). [The Working Group noted that these historical peak exposure levels were in the order of milligrams per cubic metre, whereas the mean concentrations measured in more recent studies were usually in the order of micrograms per cubic metre to tens of micrograms per cubic metre (see Table S1.8; (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>).]

### (iii) Personal air measurements

Table S1.8 (Annex 1) provides an overview of studies of the occupational exposure to gasoline-related VOCs, and specifically BTEX, measured using personal air monitoring, for various job types and geographical locations. These studies were conducted in countries including Iran, Thailand, Brazil, Sri Lanka, Spain, Italy, India, the Republic of Korea, and Malaysia, and captured a diverse range of exposure scenarios and workplace practices.

The airborne concentrations of the measured analytes varied widely between the studies. The benzene concentrations measured for service station attendants varied substantially from a minimum of <0.03 µg/m<sup>3</sup>, reported for workers in Khon Kaen, Thailand ([Chaiklieng et al., 2015](#)), to a maximum of 4088 µg/m<sup>3</sup>, for workers in Ahvaz City, Iran ([Zoleikha et al., 2017](#)). In Khon Kaen, Thailand, the measured benzene concentrations were 0.09 µg/m<sup>3</sup>–210 µg/m<sup>3</sup> ([Chaiklieng et al., 2021](#)), and those for service station attendants in Milan, Italy, were 3–3246 µg/m<sup>3</sup> ([Rota et al., 2018](#)). In Rio de Janeiro, Brazil, the benzene concentrations ranged from below the detection

limit to a peak of 928.3 µg/m<sup>3</sup> ([Figueiredo et al., 2021](#)). The toluene concentrations measured in Sri Lanka varied from undetectable to a maximum of 3100 µg/m<sup>3</sup> ([Scheepers et al., 2019](#)). The measured ethylbenzene concentrations were the highest in Ahvaz City, Iran, at 540 µg/m<sup>3</sup> ([Zoleikha et al., 2017](#)), and the xylene concentrations (sum of *o*-, *m*-, and *p*-xylene) were as high as 497 µg/m<sup>3</sup> in Rio de Janeiro, Brazil ([Figueiredo et al., 2021](#)).

Tanker drivers in Italy were exposed to benzene concentrations of 7.4–1017 µg/m<sup>3</sup> ([Lovreglio et al., 2010](#)). Other occupation groups, such as police officers, had benzene exposure levels of 3.36–129.26 µg/m<sup>3</sup> ([Fandi et al., 2020](#)). [The Working Group noted that traffic police officers are not only exposed to gasoline, but also to vehicle exhaust fumes containing BTEX. This co-exposure needs to be considered because exhaust fumes contain carcinogens other than those found in gasoline. Therefore, benzene would probably not be a good proxy for gasoline exposure for traffic police officers.]

Parking garage attendants were shown to have benzene concentrations of between 24.6 and 38.1 µg/m<sup>3</sup> ([Jo and Song, 2001](#)), and taxi drivers had concentrations of 200–300 µg/m<sup>3</sup> ([Bahrami et al., 2007](#)).

[The Working Group noted that occupational exposure to gasoline-related VOCs varies widely across different job roles. Service station attendants have consistently shown the highest levels of BTEX exposure, with concentrations up to the low milligram per cubic metre range being measured for benzene in various studies. In contrast, police officers and parking garage attendants are assumed to have negligible direct exposure to gasoline. The benzene they are exposed to would be likely to originate from the exhausts of vehicles in traffic or evaporation while they are parked. Police officers and parking garage attendants had lower concentrations of VOCs, typically <200 µg/m<sup>3</sup> for benzene. The measured concentrations of other VOCs, such

as toluene and xylenes, also showed significant variation across different regions and job types.]

Historical personal air concentration data collected in the Nordic countries during the 1970s (Lynge et al., 1997) are included in Table S1.8 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>). These samples were collected for full-service station attendants before vapour recovery systems were fitted in these countries. The benzene concentrations during this period were 2–6% by volume, and the reported mean 8-hour shift values were 0.5–1 mg/m<sup>3</sup>. [The Working Group noted that these values might be helpful in interpreting the exposure to gasoline of station attendants who are currently operating refuelling pumps that are not equipped with vapour recovery systems. However, it should be noted that the benzene concentrations in gasoline today are usually approximately 1% by volume, compared with up to 6% for the gasoline that was relevant for the study by Lynge et al. (1997).]

### (c) Occupational biomonitoring

Hydrocarbons in gasoline are easily absorbed through inhalation, the skin, and (inadvertent) ingestion (Ekpenyong and Asuquo, 2017). Air monitoring only accounts for inhalation. Biomonitoring is an approach to the assessment of gasoline exposure in workers via all possible routes, which are expected to be mainly inhalation and skin absorption. Substances in gasoline can cross the skin barrier, and thereby contribute to the total level of exposure, but exposure via this route is not captured by air monitoring. The biomonitoring of workers exposed to gasoline involves the use of various biomarkers and biological matrices. See Table S1.9 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) for the results of studies of biomonitoring.

Urinary elimination of the parent compounds is rapid, whereas the concentrations of their respective metabolites reflect exposures over the preceding hours to a day, depending on when the exposure occurred (Soleimani, 2020). Although BTEX are the most commonly monitored biomarkers of gasoline exposure, the measurement of additional hydrocarbons, including *n*-hexane (quantified in this form or as 2,5-hexanedione in urine) and oxygenated additives, such as MTBE and ETBE, can provide a broader understanding of exposures (see Section 1.3.5). Breath sampling is useful for the evaluation of recent exposure to BTEX compounds (Chen et al., 2002; Soleimani, 2020). When using biomonitoring for the assessment of gasoline exposure, the smoking habits of participants should be considered, because tobacco smoking is a source of BTEX.

#### (i) Biomarkers measured in urine

Table S1.9 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) summarizes studies on the urinary biomarker levels of various metabolites in service station and related workers, including *t,t*-MA (concentration range, <0.05–1128 µg/g creatinine), SPMA (range, 0.05–15.5 µg/g creatinine), hippuric acid (range, 0.01–11 µg/g creatinine), mandelic acid (range, 0.3–0.11 µg/g creatinine), methylhippuric acid (range, 0.04–4 µg/g creatinine), and BTEX. The studies were performed in countries including Brazil, Italy, Iran, Sri Lanka, and Thailand between 1996 and 2021, and *t,t*-MA was the most commonly measured metabolite. The sample sizes in the studies ranged from 18 to 275 workers. The results varied by geographical location and occupation type. The urinary biomarker concentrations were strongly influenced by cigarette smoking, with values being higher by up to eightfold in smokers than non-smokers (Fustinoni et al., 2005a). [The Working Group noted that the values did not

show consistent trends across studies, and consequently an overall comparison of job types could not be made. Hippuric acid and *t,t*-MA are not considered to be adequate biomarkers in most settings (see Section 1.3.5).]

(ii) *Biomarkers measured in blood*

The blood concentrations of exposure biomarkers have been measured in only a few studies. [Rekhadevi et al. \(2010\)](#) studied the blood BTX concentrations of service station attendants ( $n = 200$ ) and individuals from the general population with no history of occupational exposure to fuel, heavy metals, or any genotoxic agents ( $n = 200$ ; controls) in Hyderabad, India. These workers worked 5 hours per day, and each attendant dispensed 665 L of fuel per day, containing approximately 5–6.5% benzene by volume. The mean blood concentrations for the service station workers were 5.18 (range, 3.01–8.34)  $\mu\text{g/L}$  for benzene, 1.18 (0.65–1.69)  $\mu\text{g/L}$  for toluene, and 2.50 (1.18–4.00)  $\mu\text{g/L}$  for xylene; and these were significantly higher than those for controls, which were 2.12 (range, 1.01–4.00)  $\mu\text{g/L}$ , 1.020 (0.52–1.40)  $\mu\text{g/L}$ , and 1.296 (0.65–2.23)  $\mu\text{g/L}$ , respectively. The blood BTX concentrations for smokers in both the exposed and control groups tended to be higher than those for non-smokers, but the differences were not statistically significant.

[Pandey et al. \(2008\)](#) conducted a human biomonitoring study to evaluate the exposure of service station attendants ( $n = 39$ ) and controls ( $n = 18$ ) to benzene in gasoline fumes in Lucknow, India. The blood benzene concentrations of the service station attendants ( $7.94 \mu\text{g/L} \pm 1.45$ ) were significantly higher than those of controls ( $2.82 \mu\text{g/L} \pm 0.73$ ), whereas those of toluene and xylene did not differ (toluene,  $4.29 \mu\text{g/L}$  versus  $4.59 \mu\text{g/L}$ , and xylene,  $5.10 \mu\text{g/L}$  versus  $3.99 \mu\text{g/L}$ , for the service station attendants and controls, respectively).

(iii) *Biomarkers measured in exhaled breath*

The concentrations of biomarkers of exposure in exhaled breath have only been measured in a few studies. [Scheepers et al. \(2019\)](#) determined the end-exhaled air and urinary concentrations of BTX in service station attendants in Sri Lanka. All of the BTX components were detected in the exhaled air of both the service station attendants and control office workers. The median post-shift concentrations of toluene in the exhaled air of the service station attendants were the highest (and five times the equivalent concentrations of the controls), and there was a larger increase over the shift, compared with controls (15 times as high as in controls). For benzene, the exhaled air concentrations for the service station attendants were twice those for controls at the start of their shifts and showed an increase of fivefold during these shifts. The xylene concentrations in the exhaled air of the exposed and control participants were similar.

[Jo and Song \(2001\)](#) studied the VOC concentrations (benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, and *p*-xylene) in breath, personal, and area air samples for traffic police officers ( $n = 10$ ), parking garage workers ( $n = 8$ ), service station attendants ( $n = 10$ ), and roadside ( $n = 10$ ) and underground ( $n = 10$ ) shopkeepers in Taegu, Republic of Korea. Both the pre- and post-work breath concentrations of the workers were higher than those of a control group ( $n = 5$ ; college students). The post-work breath concentrations significantly correlated with the personal air sampling concentrations, but the pre-work breath concentrations did not. The breath concentrations of all the target compounds were higher for the underground parking garage workers than for the ground-level parking workers. The service station attendants had higher pre-shift ( $26.6 \pm 14.5 \mu\text{g/m}^3$ ) and post-shift ( $41.0 \pm 14.7 \mu\text{g/m}^3$ ) benzene concentrations; the traffic police officers, service station attendants, and roadside shopkeepers all had high



toluene concentrations; and the traffic police officers and service station attendants had high ethylbenzene, *p*-xylene, *m*-xylene, and *o*-xylene concentrations. [The Working Group noted that many of these jobs are also associated with exposure to traffic exhaust fumes. The results showed that the studied groups of workers had greater VOC exposure than did the controls and that service station attendants had higher VOC concentrations than did the workers in other jobs. The VOC concentrations in breath and personal air samples were correlated.]

### 1.4.3 Exposure of the general population

Exposure of the general population to automotive gasoline can occur via inhalation and, less frequently, via dermal exposure during various activities or in various situations including during car refuelling, from evaporative emissions from cars, from emissions from service stations (for local residents), from the use of equipment fuelled with gasoline, such as lawnmowers and other gardening equipment, and from do-it-yourself activities involving the use of gasoline for degreasing and cleaning. Few data were available for most of these types of exposure, but in general, there is wide scope for exposure. [The Working Group noted that exposure to VOCs via gasoline pumping, and to a lesser extent, via air pollution, was well documented, but that other types of exposure have not been comprehensively documented, for example, from gasoline use at home and via evaporation from cars in garages and parking spaces.]

Given that gasoline vapour is a complex mixture of hydrocarbons (see Section 1.1 for details of the composition of gasoline), exposure levels are assessed by the personal monitoring or biomonitoring of components chosen for their toxicological relevance and/or abundance. These are most commonly VOCs, particularly BTEX and some ethers, typically MTBE, ETBE, and TAME. In addition, exposure to leaded gasoline

is assessed by measuring the lead content of biological fluids.

Exposure to airborne VOCs, and particularly to BTEX, has been assessed in a number of studies in various parts of the world ([Hoffmann et al., 2000](#); [Kwon et al., 2006](#); [Hinwood et al., 2007](#); [Symanski et al., 2009](#); [Su et al., 2013](#); [Hagenbjörk-Gustafsson et al., 2014](#)). However, these types of exposure are not uniquely related to gasoline, because various sources of air pollution and personal habits can contribute, such as tailpipe emissions, industrial emissions, environmental exposure to tobacco smoke, and the use of paints and lacquers.

#### (a) *Exposure in the proximity of a service station and during refuelling*

Studies investigating the exposure to gasoline vapour of residents and/or others in the proximity of service stations are reported in Section 1.4.1 and summarized in Table S1.6 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>) ([Jo and Oh, 2001](#); [de Oliveira et al., 2007](#); [Barros et al., 2019](#)). These studies showed that residents close to service stations were exposed to gasoline vapour. [The Working Group noted that the concentration of gasoline vapour and particularly that of benzene in the residential area surrounding service stations may be elevated within 100–220 m, but rarely at a greater distance.] Studies investigating the personal exposure of consumers during refuelling are briefly reported below, and the data are summarized in [Table 1.11](#).

[Backer et al. \(1997\)](#) studied 48 customers who dispensed two types of automotive gasoline, one regular and one oxygenated with 10% ethanol, in Fairbanks, Alaska, USA, during the cold season. BTEX concentrations in the breathing zone were in the same order of magnitude for the two types of gasoline; the median blood concentrations of benzene, toluene, ethylbenzene, and xylene were



**Table 1.11 Selected studies on personal air monitoring for gasoline exposure in the general population**

| Exposure scenario | City, country, year          | No. of participants and samples                                                                    | Vapour recovery system              | Monitored chemicals                        | Sampling information                                                          | Sampling duration                 | Personal exposure (mean ± SD)                                                                                                                                                                                                                | Biological monitoring (mean ± SD)                                                                                                                                                                                                            | Reference                                   |
|-------------------|------------------------------|----------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Gasoline pumping  | Fairbanks, Alaska, USA, 1995 | 26 individuals pumping regular gasoline and 22 individuals pumping gasoline containing 10% ethanol | Stage II was not in use; Stage I NR | Benzene, toluene, ethylbenzene, and xylene | Personal monitoring, using two samplers simultaneously: 1 L/min and 0.1 L/min | NR (time taken to pump [38–53 L]) | Benzene, [1.25–0.83 mg/m <sup>3</sup> ] <sup>a, b</sup><br>Toluene, [1.60–1.44 mg/m <sup>3</sup> ] <sup>a, b</sup><br>Ethylbenzene, [0.17–0.21 mg/m <sup>3</sup> ] <sup>a, b</sup><br>Xylene, [0.77–0.52 mg/m <sup>3</sup> ] <sup>a, b</sup> | Blood sample collected < 10 min after filling with each type of gasoline<br>Benzene, 0.54–0.70 µg/L <sup>a</sup><br>Toluene, 0.74–0.85 µg/L <sup>a</sup><br>Ethylbenzene, 0.16–0.16 µg/L <sup>a</sup><br>Xylene, 0.86–0.92 µg/L <sup>a</sup> | <a href="#">Backer et al. (1997)</a>        |
| Gasoline pumping  | Helsinki, Finland, 1996      | 167 personal measurements and 8 air samples collected at the pump island                           | Stage I                             | Benzene                                    | Air sampled at the pump island<br>Personal monitoring                         | 8 h<br>20–21 refuellings          | 0.9 mg/m <sup>3</sup><br>1.0–0.77 mg/m <sup>3</sup>                                                                                                                                                                                          |                                                                                                                                                                                                                                              | <a href="#">Vainiotalo et al. (1999)</a>    |
|                   |                              |                                                                                                    |                                     | MTBE                                       | Air sampling at the pump island<br>Personal monitoring                        | 8 h<br>1 min                      | 0.16–0.07 mg/m <sup>3</sup><br>3.3 mg/m <sup>3c</sup>                                                                                                                                                                                        |                                                                                                                                                                                                                                              |                                             |
|                   |                              |                                                                                                    |                                     | TAME                                       | Air sampling at the pump island<br>Personal monitoring                        | 8 h<br>1 min                      | 0.031 mg/m <sup>3</sup><br>1.9 mg/m <sup>3c</sup>                                                                                                                                                                                            |                                                                                                                                                                                                                                              |                                             |
|                   |                              |                                                                                                    |                                     |                                            |                                                                               |                                   |                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                              |                                             |
| Gasoline pumping  | Finland, 1998                | 20                                                                                                 | Stage I                             | C3–C11 hydrocarbons<br>MTBE                | Personal monitoring<br>Personal monitoring                                    | 57 s                              | 85 mg/m <sup>3c</sup><br>15.3 mg/m <sup>3c</sup>                                                                                                                                                                                             |                                                                                                                                                                                                                                              | <a href="#">Hakkola and Saarinen (2000)</a> |
|                   |                              | 20                                                                                                 | Stage II                            | C3–C11 hydrocarbons<br>MTBE                | Personal monitoring<br>Personal monitoring                                    | 66 s                              | 18 mg/m <sup>3c</sup><br>3.4 mg/m <sup>3c</sup>                                                                                                                                                                                              |                                                                                                                                                                                                                                              |                                             |

**Table 1.11 (continued)**

| Exposure scenario | City, country, year                 | No. of participants and samples   | Vapour recovery system | Monitored chemicals                          | Sampling information                                 | Sampling duration                 | Personal exposure (mean ± SD)                                                                          | Biological monitoring (mean ± SD)                                                               | Reference                            |
|-------------------|-------------------------------------|-----------------------------------|------------------------|----------------------------------------------|------------------------------------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------------------------|
| Gasoline pumping  | North Carolina, USA, 1998–1999      | 39 participants; 130 measurements | No stage II system     | Benzene                                      | Personal monitoring during refuelling                | 3 min                             | 2.9 ± 5.8 mg/m <sup>3</sup>                                                                            | Mean ± SD exhaled breath sample collected at the end of refuelling, 160 ± 260 µg/m <sup>3</sup> | <a href="#">Egeghy et al. (2000)</a> |
| Gasoline pumping  | Baltimore, Maryland, USA, 2019–2020 | 32                                | NR                     | Benzene<br>Toluene<br>Ethylbenzene<br>Xylene | Personal monitoring by the car during the refuelling | Duration of refuelling, 0.5–6 min | 10.4 µg/m <sup>3c</sup><br>6.4 µg/m <sup>3c</sup><br>8.8 µg/m <sup>3c</sup><br>32.2 µg/m <sup>3c</sup> |                                                                                                 | <a href="#">Patton et al. (2021)</a> |

GM, geometric mean; h, hour(s); min, minute(s); MTBE, methyl *tert*-butyl ether; NR, not reported; s, second(s); SD, standard deviation; TAME, *tert*-amyl methyl ether; USA, United States of America.

<sup>a</sup> Median.

<sup>b</sup> Value extrapolated from a figure.

<sup>c</sup> Geometric mean.

approximately 0.6, 0.8, 0.16, and 0.9  $\mu\text{g/L}$ , respectively; and there were no significant differences between the two groups ([Backer et al., 1997](#)).

[Vainiotalo et al. \(1999\)](#) investigated the exposure of customers to MTBE and other oxygenated ethers (TAME and C6 alkyl methyl ethers) during refuelling at self-service stations in Finland in summer (the temperature ranged from 15 to 26  $^{\circ}\text{C}$ ). The duration of refuelling was approximately 60–70 seconds, and the overall personal exposures, stated as geometric means ( $n = 167$ ) for an adjusted 1-minute period of refuelling, were 3.3  $\text{mg/m}^3$  for MTBE and 1.9  $\text{mg/m}^3$  for TAME. [The Working Group noted that these types of exposure are infrequent and last for a period of minutes.]

[Hakkola and Saarinen \(2000\)](#) measured the exposure of customers to C3–C11 hydrocarbons and MTBE during refuelling in service stations equipped with stage I or stage II vapour recovery systems. The mean duration of refuelling was approximately 60 seconds, and the temperature during sampling was in the range of 10–17  $^{\circ}\text{C}$ . The geometric mean exposure levels of customers to C3–C11 hydrocarbons and MTBE during refuelling were 85 (range, 2.1–531) and 15.3 (range, 1.8–74)  $\text{mg/m}^3$  at the stage I service station and 18 (range, <0.2–129) and 3.4 (range, 0.2–16)  $\text{mg/m}^3$  at the stage II service station. The exposure level of customers at the stage II station during refuelling was approximately 20–25% of that at the stage I service station.

[Egeghy et al. \(2000\)](#) assessed the exposure of 39 self-service customers to benzene by performing 130 measurements during the refuelling of cars (median duration, 3 minutes) in North Carolina, USA. They found a mean benzene concentration of 2.9  $\text{mg/m}^3$  (standard deviation, 5.8  $\text{mg/m}^3$ ; range, <0.076–36  $\text{mg/m}^3$ ), a mean post-exposure exhaled breath concentration of 160  $\mu\text{g/m}^3$  (standard deviation, 260  $\mu\text{g/m}^3$ ; range, <3.2–1400  $\mu\text{g/m}^3$ ), and a correlation between the air exposure and exhaled breath concentration ( $r = 0.77$ ;  $P < 0.0001$ ).

More recently, [Patton et al. \(2021\)](#) assessed the BTEX exposure associated with gasoline pumping among 32 consumers in Baltimore, Maryland, USA; the mean temperature during the survey was 19.7  $^{\circ}\text{C}$ . The geometric mean personal air exposure levels were 10, 36, 9, and 32  $\mu\text{g/m}^3$  for benzene, toluene, ethylbenzene, and xylene, respectively.

[The Working Group noted that the levels of exposure in the study of [Patton et al. \(2021\)](#) were much lower than those in the older studies, suggesting that the implementation of the vapour recovery systems had reduced the evaporative emissions during refuelling. Few data were available on the implementation of vapour recovery systems in LMICs.]

#### (b) Residential exposure

[Mann et al. \(2001\)](#) studied five houses in the UK that had an attached or integral garage. They obtained samples over 18 months during 1998–1999 and measured mean benzene concentrations ranging from 1.9 to 2.9  $\mu\text{g/m}^3$  outside the house, 4 to 101  $\mu\text{g/m}^3$  in the garage, and 3.2 to 39.9  $\mu\text{g/m}^3$  in the room above the garage, which was the room with the highest concentrations in the house ([Mann et al., 2001](#)).

[Batterman et al. \(2007\)](#) performed a study of 15 single-family houses in Michigan, USA, and found high concentrations of gasoline-related VOCs in the attached garages (mean concentrations of benzene, toluene, ethylbenzene, and xylene, 37, 214, 28, and 152  $\mu\text{g/m}^3$ , respectively). The corresponding concentrations in the house were 2.0, 26.5, 2.3, and 11.2  $\mu\text{g/m}^3$ , respectively. The authors concluded that most of the fuel-related aromatics in the houses of non-smokers were derived from sources in the garage ([Batterman et al., 2007](#)).

The authors of both studies concluded that the presence of a garage attached to a house increases the exposure of the residents, because the evaporation of gasoline from parked cars (see

also Section 1.4.1) and the migration of VOCs ([Mann et al., 2001](#); [Batterman et al., 2007](#)).

(c) *Personal exposure to lead from gasoline containing tetraethyl lead*

In the past, the use of gasoline containing tetraethyl lead caused widespread contamination of the environment and the consequent exposure of the general population to lead. Currently, although leaded gasoline has been banned all over the world, legacy lead is still present in soils and may enter the air and/or the food chain and contaminate organisms. Several other lead sources are recognized to contribute to human exposure, such as dust from lead-based paint pigments, drinking-water from old lead pipes, coal burning, industrial emissions, mining activities, and tobacco smoke ([Skerfving and Bergdahl, 2015](#)). The elimination of lead additives from gasoline has caused a substantial decrease in lead exposure and, as a consequence, the relative importance of other sources has increased, but there is a lack of knowledge about which of these are now the predominant sources. Moreover, the relative importance of the various sources varies substantially by region ([Skerfving and Bergdahl, 2015](#)).

Exposure to VOCs has mostly been assessed through air sampling, and personal exposure to lead has mainly been assessed by measuring the lead levels in the blood. Several studies have shown high concentrations of lead in blood samples from the general population collected before or soon after the use of leaded gasoline was banned; after the ban, the concentrations gradually decreased ([Bortoli et al., 1998](#); [von Storch et al., 2003](#); [Mathee et al., 2006](#); [He et al., 2009](#); [Stroh et al., 2009](#); [Oh et al., 2017](#)). A systematic review of the studies published since the year 2000 in which the blood lead level was measured has recently been performed ([Lacerda et al., 2023](#)). The blood levels of lead around the world differed; median concentrations were 1.7 µg/dL in North America, 5.6 µg/dL in South America,

12.2 µg/dL in Africa, 2.8 µg/dL in Europe, and 2.5 µg/dL in Asia. An exponential decrease was identified in each of these regions of the world, with the concentrations before and after leaded gasoline was phased out being approximately 12 and 1.7, 13 and 2.5, 20 and 5, 6 and 1.2, and 4 and <1 µg/dL in North America, South America, Africa, Europe, and Asia, respectively. The decreases were larger for the regions that banned leaded gasoline earlier. [The Working Group considered that these data demonstrate the effectiveness of removing tetraethyl lead from gasoline for the lowering of human exposure to lead and support the evidence that use of tetraethyl lead was the major source of human exposure worldwide ([Lacerda et al., 2023](#)).]

## 1.5 Regulations and guidelines

### 1.5.1 Regulations, limits, and guidelines

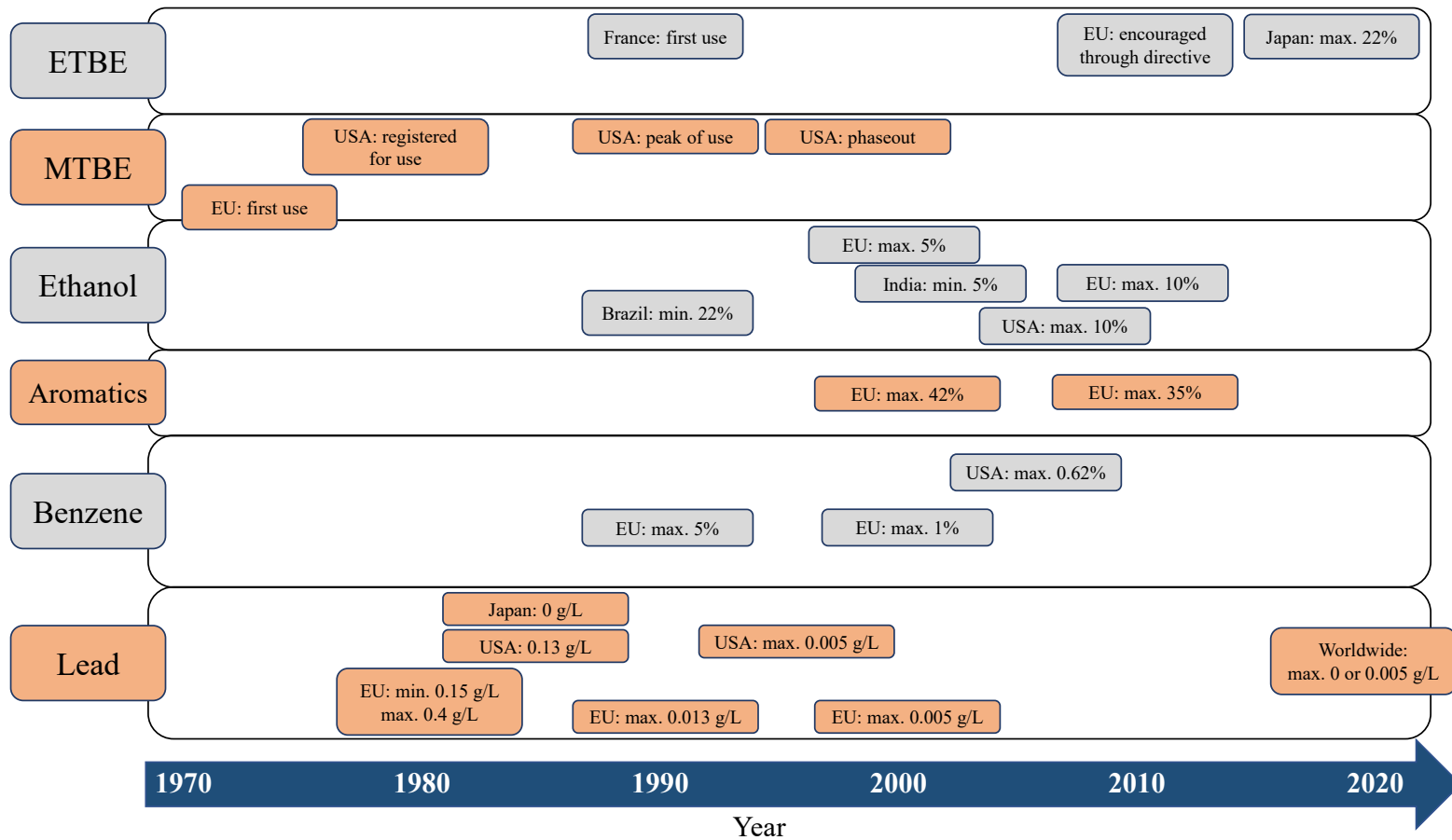
(a) *Regulations on gasoline composition*

Regulations established worldwide aim to reduce harmful emissions, protect public health, and promote environmental sustainability. The composition of gasoline and some of its physical properties, such as vapour pressure, are regulated and can differ according to the geographical setting and the season (see Section 1.1 and [Table 1.3](#) for details).

In the EU, Member States must report the volume and quality of gasoline and diesel sold in their territories ([ECHA, 2023](#)). In the USA, the Clean Air Act of 1990 ([US Senate and House of Representatives, 1990](#)) and subsequent amendments dictate fuel standards and banned leaded gasoline ([US Library of Congress, 2024](#)).

Several regulations have been introduced to limit the content of certain components in gasoline, and these have changed over time (see [Fig. 1.5](#)). The addition of lead was banned in Japan in the 1980s, followed by the USA in 1996, most European countries in the late 1990s and 2000s, and globally in 2021. The regulation

**Fig. 1.5 Overview of the changes in regulations relevant to gasoline composition over time**



ETBE, ethyl *tert*-butyl ether; EU, European Union; max., maximum; min., minimum; MTBE, methyl *tert*-butyl ether; USA, United States of America.  
 Created by the Working Group with data from [Chevron Corp \(2012\)](#).



of benzene content has become more stringent over time. In Europe, the benzene content of leaded and unleaded gasoline was limited to 5.0% by volume from 1 October 1989 ([European Commission, 1985](#)). At the present time, the typical maximum permitted concentration in many countries is approximately 1% by volume. Regulations in several countries at present encourage the blending of gasoline with ethanol, and some, such as in Brazil, require a minimum amount of ethanol in gasoline. Regulations in other countries, such as in Japan, recommend the use of other oxygenated additives, including ETBE.

*(b) Vapour recovery systems stage I and II*

Vapour recovery systems are critical for the reduction of VOC emissions in service stations (see Section 1.4.1). Although the requirements for these systems vary around the world, the most common features include stage I vapour recovery for the transfer of fuel from delivery trucks to underground storage tanks, and in some areas, stage II recovery for vehicle refuelling ([US EPA, 2002](#); [European Union, 2009](#)).

Gasoline vapour that would otherwise be vented during individual vehicle refuelling at service stations is captured in the stage II systems. These systems were first adopted in the USA and have been gradually implemented in many countries around the world, including in the EU ([European Union, 1994, 2009](#)) and China ([Sun et al., 2021](#)). Stage II vapour recovery systems were introduced in California, USA, in 1972, then started to be used in Europe during the late 1980s, first in Sweden and then in Switzerland. In Australia, the use of vapour recovery systems has been required since 1986 in the Sydney metropolitan area. In Hong Kong, Special Administrative Region of China, the regulations were amended in 2004 to require service stations to have a system to recover VOCs emitted during vehicle refuelling. In Australia, the use of vapour recovery systems in service

stations was expanded from Port Stephens to Shoalhaven and west to the Blue Mountains as a result of the Protection of the Environment Operations (Clean Air) Regulation in 2010 ([NSW EPA, 2023](#)). The EU updated its directive in 2014 to state that the gasoline vapour capture efficiency of such systems should be  $\geq 85\%$ , as certified by the manufacturer, in accordance with Standard EN 16321-1:2013 ([European Union, 2014](#)).

In Brazil, national regulations introduced in 2022 established deadlines for the installation of vapour recovery systems in service stations, according to the year of manufacture of the fuel pumps. Accordingly, for pumps manufactured in 2004, vapour recovery systems had to be installed by 31 December 2024, and for pumps manufactured between 2019 and 2022, these systems should be installed by 31 December 2033 ([Brazil Ministério do Trabalho e Previdência, 2022](#)).

*(c) Onboard refuelling vapour recovery systems*

Stage II recovery systems are increasingly being replaced by ORVR systems in vehicles ([European Committee for Standardization, 2012](#); [European Parliament and Council of the European Union, 2014](#); [California Air Resources Board, 2024](#); [US EPA, 2025b](#)). These systems return gasoline vapour displaced during refuelling to the vehicle fuel system.

In the USA, cars were first equipped with ORVR systems in the 1990s (see Section 1.4.1). After vehicles equipped with ORVR had widely permeated the market and their efficiency was shown to be  $>95\%$  ([International Council on Clean Transportation, 2011](#)), the US EPA waived the requirements for states to implement stage II gasoline vapour recovery at gasoline dispensing facilities ([US EPA, 2012a, b](#)).

*(d) Gasoline in water and soil*

The gasoline content of drinking-water is not specifically regulated, but guideline values exist for certain hydrocarbons found in gasoline,

**Table 1.12 Examples of occupational exposure limits for gasoline**

| Country <sup>a</sup>       | TWA<br>(ppm; mg/m <sup>3</sup> ) | STEL<br>(ppm; mg/m <sup>3</sup> ) | Observations                                                                                        |
|----------------------------|----------------------------------|-----------------------------------|-----------------------------------------------------------------------------------------------------|
| Norway                     | 50; 175                          |                                   | Essentially <i>n</i> -hexane.                                                                       |
| Sweden                     | 50; 180                          | 75; 250                           | Industrial gasoline and hexane (AFS, 2023).                                                         |
| Sweden                     | 200; 800                         | 300; 1200                         | Industrial gasoline and heptane (AFS, 2023).                                                        |
| Sweden                     | 200; 900                         | 300; 1400                         | Industrial gasoline and octane (AFS, 2023).                                                         |
| Kingdom of the Netherlands | 240 mg/m <sup>3</sup>            | 480 mg/m <sup>3</sup>             | Used as fuel for combustion engines. This mixture is carcinogenic if the benzene content is > 0.1%. |

ppm, parts per million; STEL, short-term exposure limit; TWA, time-weighted average.

<sup>a</sup> This table only includes exposure limits for countries that specify which gasoline component is measured.

The data were obtained from IFA (2025).

including benzene, xylene, and toluene. Soil and groundwater regulations for gasoline often exist, and the contamination of these media is usually the result of gasoline spills or leaks, including from underground pipelines and storage tanks at service stations. [The Working Group noted that these values are not specific for gasoline and that the compounds can originate from other sources. Therefore, they will not be stated here.]

#### (e) Occupational exposure limits

Occupational exposure limits for gasoline have been established in some countries (Table 1.12).

For many of the components of gasoline, separate occupational exposure limits have been established (see Table 1.13 for examples). [The Working Group noted that the data in Table 1.12 and Table 1.13 were from the GESTIS database of the German Social Accident Insurance, which is a reliable source of occupational exposure limits worldwide (IFA, 2025) and is regularly updated.] In addition, the existing occupational air concentration recommendations included the threshold limit values (TLVs) established by the American Conference of Governmental Industrial Hygienists (ACGIH) and the recommended exposure limits established by NIOSH.

#### 1.5.2 Biological limit values for some gasoline components

No biological limit value has been recommended for gasoline, but there have been such values for some gasoline components, in particular, for BTEX (see Table 1.14).

In the USA, the ACGIH recommends biological exposure indices, which are intended to be used as guidelines for the evaluation of potential health hazards in the practice of occupational hygiene.

In Germany, the Deutsche Forschungsgemeinschaft (German Research Foundation) Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, also known as the MAK Commission, provides biological values that include biological tolerance values (BATs), biological guidance values, biological reference values for workplace substances (BARs), and exposure equivalents for carcinogenic substances (EKAs). BATs are intended to protect employees from adverse effects on health at work and provide a basis to decide whether the internal dose of a substance may be harmful or not to workers. Chemical substances known or suspected to cause cancer in humans are not given BAT values because it is not possible to specify safe levels for such substances in biological materials. Therefore, these substances are analysed to detect and

**Table 1.13 Occupational exposure limits for specific components of gasoline**

| Country or region | Benzene                                |                                   | Toluene                                |                                   | Ethylbenzene                           |                                   | Xylene                                 |                                   |
|-------------------|----------------------------------------|-----------------------------------|----------------------------------------|-----------------------------------|----------------------------------------|-----------------------------------|----------------------------------------|-----------------------------------|
|                   | TWA (8 h)<br>(ppm; mg/m <sup>3</sup> ) | STEL<br>(ppm; mg/m <sup>3</sup> ) | TWA (8 h)<br>(ppm; mg/m <sup>3</sup> ) | STEL<br>(ppm; mg/m <sup>3</sup> ) | TWA (8 h)<br>(ppm; mg/m <sup>3</sup> ) | STEL<br>(ppm; mg/m <sup>3</sup> ) | TWA (8 h)<br>(ppm; mg/m <sup>3</sup> ) | STEL<br>(ppm; mg/m <sup>3</sup> ) |
| Australia         | 1; 3.2                                 |                                   | 50; 191 <sup>a</sup>                   | 150; 574 <sup>a</sup>             | 100; 434 <sup>a</sup>                  | 125; 543 <sup>a</sup>             | 80; 350 <sup>a</sup>                   | 150; 655 <sup>a</sup>             |
| Canada (Ontario)  | 0.5 ppm                                | 2.5 ppm                           | 20 ppm                                 |                                   | 20 ppm                                 |                                   | 100 ppm                                | 150 ppm                           |
| China             | 6 mg/m <sup>3</sup>                    |                                   | 50 mg/m <sup>3</sup>                   |                                   | 100 mg/m <sup>3</sup>                  | 150 mg/m <sup>3</sup>             | 50 mg/m <sup>3</sup>                   | 100 mg/m <sup>3</sup>             |
| European Union    | 0.2; 0.66 <sup>a,b</sup>               |                                   | 50; 192 <sup>a,f</sup>                 | 100; 384 <sup>a,f</sup>           | 100; 442 <sup>a,f</sup>                | 200; 884 <sup>a,f</sup>           | 50; 221 <sup>a,e,f</sup>               | 100; 442 <sup>a,e,f</sup>         |
| Israel            | 0.5; 1.6                               | 2.5; 8                            | 50; 188 <sup>a</sup>                   |                                   |                                        |                                   |                                        |                                   |
| Japan             | 1 ppm                                  |                                   | 50; 188 <sup>a</sup>                   |                                   | 50; 217 <sup>a</sup>                   |                                   | 50; 217 <sup>a</sup>                   |                                   |
| New Zealand       | 0.05; 0.16 <sup>a</sup>                |                                   | 20; 75 <sup>a,c,d</sup>                | 100; 377 <sup>a,c,d</sup>         | 20; 88 <sup>a,d</sup>                  | 40; 176 <sup>a,d</sup>            | 50; 271 <sup>a,c,d,g</sup>             |                                   |
| Singapore         |                                        | 1; 3.18                           | 50; 188                                |                                   | 100; 434                               | 125; 543                          | 100; 434                               | 150; 651                          |
| South Africa      | 1 ppm <sup>a</sup>                     | 5 ppm <sup>a</sup>                | 40 ppm <sup>a</sup>                    |                                   | 40 ppm <sup>a</sup>                    |                                   | 200 ppm <sup>a</sup>                   | 300 ppm <sup>a</sup>              |
| Switzerland       | 0.2; 0.7                               |                                   | 50; 190                                | 200; 760                          | 100; 435                               | 100; 435                          | 50; 220                                | 100; 440                          |
| United Kingdom    | 1; 3.25 <sup>a</sup>                   |                                   | 50; 191 <sup>a</sup>                   | 100; 384 <sup>a</sup>             | 100; 441 <sup>a</sup>                  | 1250; 552 <sup>a</sup>            | 50; 220 <sup>a</sup>                   | 100; 441 <sup>a</sup>             |
| USA (NIOSH)       | 0.1 ppm                                | 1 ppm <sup>h</sup>                | 100; 375                               | 150; 560                          | 100; 435                               | 125; 545                          | 100; 435                               | 150; 655                          |
| USA (OSHA)        | 1 ppm                                  | 5 ppm                             | 200 ppm                                | 300 ppm <sup>e</sup>              | 100; 435                               |                                   | 100; 435                               |                                   |

h, hour(s); NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million; STEL, short-term exposure limit; TWA, time-weighted average; USA, United States of America.

<sup>a</sup> Skin notation: substantial contribution to the total body burden possible via dermal exposure.

<sup>b</sup> Limit value was 1 ppm (3.25 mg/m<sup>3</sup>) until 5 April 2024; limit value was 0.5 ppm (1.65 mg/m<sup>3</sup>) from 5 April 2024 to 5 April 2026.

<sup>c</sup> Exposure can also be estimated by biological monitoring.

<sup>d</sup> Ototoxin.

<sup>e</sup> Ceiling limit value.

<sup>f</sup> Indicative occupational exposure limit value.

<sup>g</sup> *p*-Xylene is an ototoxin.

<sup>h</sup> Ceiling limit value (15 minutes).

Data from [IFA \(2025\)](#).

**Table 1.14 Biological limits for BTEX, according to ACGIH BEI and MAK BAT**

| Chemical | CAS No.                                         | Agency       | Biomarkers       | Timing of sample collection                                       | Type of limit value                                    | Air concentration (mg/m³)                     | Biomarker level          |                         |              |                                               |                          |
|----------|-------------------------------------------------|--------------|------------------|-------------------------------------------------------------------|--------------------------------------------------------|-----------------------------------------------|--------------------------|-------------------------|--------------|-----------------------------------------------|--------------------------|
| Benzene  | 71-43-2                                         | ACGIH        | SPMA in urine    | End of shift                                                      | BEI                                                    |                                               | 25 µg/g Cr               |                         |              |                                               |                          |
|          |                                                 |              |                  | End of shift                                                      | BEI                                                    |                                               | 500 µg/g Cr              |                         |              |                                               |                          |
|          |                                                 | DFG          | SPMA in urine    | End of shift, for long-term exposure over several previous shifts | EKA                                                    | 0.1<br>0.2<br>0.5<br>1.0<br>2.0<br>3.3<br>6.5 | 1.5 µg/g Cr (non-smoker) |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 3 µg/g Cr (non-smoker)   |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 5 µg/g Cr                |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 12 µg/g Cr               |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 25 µg/g Cr               |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 45 µg/g Cr               |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 90 µg/g Cr               |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 0.3 µg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          | BAR (non-smoker)        |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          | <i>t,t</i> -MA in urine | End of shift | EKA                                           | 1.0<br>2.0<br>3.3<br>6.5 |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 500 µg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 750 µg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 1200 µg/g Cr             |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | Benzene in urine         | End of shift            | EKA          | 0.1<br>0.2<br>0.5<br>1.0<br>2.0<br>3.3<br>6.5 | 150 µg/g Cr              |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               | 0.5 µg/L (non-smoker)    |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               | 0.8 µg/L (non-smoker)    |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               | 1.5 µg/L                 |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               | 2.75 µg/L                |
|          |                                                 | 5.0 µg/L     |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               |                          |
|          |                                                 | 7.5 µg/L     |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               |                          |
|          |                                                 | 12.5 µg/L    |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               |                          |
|          |                                                 |              | BAR (non-smoker) |                                                                   | 0.3 µg/L                                               |                                               |                          |                         |              |                                               |                          |
|          |                                                 | Ethylbenzene | 100-41-4         | ACGIH                                                             | Sum of mandelic acid and phenylglyoxylic acid in urine | End of shift                                  | BEI                      |                         | 0.15 g/g Cr  |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          |                         |              |                                               |                          |
| DFG      | Mandelic acid and phenylglyoxylic acid in urine |              |                  | End of shift                                                      | EKA                                                    | 44<br>88<br>110<br>220<br>440                 | 130 mg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 250 mg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 330 mg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 670 mg/g Cr              |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               | 1300 mg/g Cr             |                         |              |                                               |                          |
|          |                                                 |              |                  |                                                                   |                                                        |                                               |                          | BAT                     | 250 mg/g Cr  |                                               |                          |

**Table 1.14 (continued)**

| Chemical                                            | CAS No.                                              | Agency | Biomarkers                                 | Timing of sample collection                                              | Type of limit value | Air concentration (mg/m <sup>3</sup> ) | Biomarker level |
|-----------------------------------------------------|------------------------------------------------------|--------|--------------------------------------------|--------------------------------------------------------------------------|---------------------|----------------------------------------|-----------------|
| Toluene                                             | 108-88-3                                             | ACGIH  | Toluene in blood                           | Before the last shift of the working week                                | BEI                 |                                        | 0.02 mg/L       |
|                                                     |                                                      |        | Toluene in urine                           | End of shift                                                             | BEI                 |                                        | 0.03 mg/L       |
|                                                     |                                                      | DFG    | <i>o</i> -Cresol in urine                  | End of shift                                                             | BEI                 |                                        | 0.3 mg/g Cr     |
|                                                     |                                                      |        | Toluene in blood                           | Immediately after exposure                                               | BAT                 |                                        | 600 µg/L        |
|                                                     |                                                      |        | Toluene in urine                           | End of shift                                                             | BAT                 |                                        | 75 µg/L         |
|                                                     |                                                      |        | <i>o</i> -Cresol in urine after hydrolysis | End of a long-term exposure (at the end of a shift after several shifts) | BAT                 |                                        | 1.5 mg/L        |
| Xylene (all isomers; technical or commercial grade) | 1330-20-7 (isomers: 95-47-6, 106-42-3, and 108-38-3) | ACGIH  | MHA in urine                               | End of shift                                                             | BEI                 |                                        | 1.5 g/g Cr      |
|                                                     |                                                      | DFG    | MHA (all isomers) in urine                 | End of shift                                                             | BAT                 |                                        | 1800 mg/g Cr    |

ACGIH, American Conference of Governmental Industrial Hygienists; BAR, biological reference value; BAT, biological tolerance value for occupational exposure; BEI, biological exposure index; BTEX, benzene, toluene, ethylbenzene, and xylene; CAS, Chemical Abstracts Service; Cr, creatinine; DFG, Deutsche Forschungsgemeinschaft (German Research Foundation); EKA, exposure equivalent for a carcinogenic substance; MAK, Maximale Arbeitsplatz-Konzentration (maximum workplace concentration); MHA, methylhippuric acid; SPMA, S-phenyl-mercapturic acid; *t,t*-MA, *trans,trans*-muconic acid.



quantify an individual's exposure. For this purpose, the relationship between the concentration of the carcinogen in workplace air and that of the substance or its metabolites in biological material are investigated, and the EKA is derived. BARs describe the background level of a substance present at the same time in a reference population of working age who have not been occupationally exposed to this substance (Table 1.14). [The Working Group noted that *t,t*-MA and hippuric acid are not suitable for the assessment of benzene and toluene exposure, respectively (see Section 1.3.3).]

## 1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

### 1.6.1 *Quality of exposure assessment in key epidemiological studies of cancer in humans*

The epidemiological studies on gasoline exposure and cancer have employed a variety of methods to characterize the exposure to gasoline, and some involved the estimation of cumulative exposure. Studies in which exposure to gasoline was assessed using self-reporting, job-exposure matrices (JEMs), expert evaluation based on work history information, or environmental exposure during gasoline spill events or as a result of residential proximity to service stations, and studies on cancer risk for selected occupations that were considered to be good proxies for gasoline exposure, were reviewed.

[The Working Group considered that the most informative occupations to be used as proxies for gasoline exposure were service station attendants and gasoline distribution workers, because these workers experience relatively high levels of exposure to gasoline, along with relatively low and well-characterized co-exposures to other

carcinogens (see Section 1.4.2(a)(v)). Other occupations such as car mechanics and petroleum industry workers were considered to be less informative as proxies, because these workers are exposed to a variety of other carcinogens and have a large variety of gasoline exposure levels (see Table 1.10). The Working Group noted that most petroleum industry workers were not exposed to the final product, gasoline, which is why they were not considered to be an informative proxy for gasoline exposure.]

All observational epidemiological studies involve some degree of measurement error with respect to exposures, which could contribute to information bias (Berrington de González et al., 2024a). The study investigators employed various methods to reduce measurement error in their exposure estimates and identify potential information bias in their results. A critical review of the exposure assessments in key epidemiological studies is provided in Table S1.15 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>). This critical exposure assessment review is discussed below.

- (a) *Occupational exposure*
- (i) *Cohorts of service station attendants and petroleum distribution workers*

Studies that assessed occupation as a gasoline service station attendant were considered to be relevant for the present evaluation, especially those conducted during a period in which self-service gasoline refilling was uncommon (e.g. Walker et al., 1987) and in which the study setting might indicate a higher level of gasoline exposure (e.g. a small service station in the study by Lagorio et al., 1994a).

Of the studies of service station attendants, only one, which was of service station managers in Italy, recruited its study population prospectively on the basis of documented employment at

a service station ([Lagorio et al., 1994a](#)). A limitation was that the exposure assessment was only qualitative, using the proxy of occupation, and no differentiation of the participants by exposure intensity was performed.

In the UK, a prospective cohort study was based on documented employment as a petroleum distribution worker for a minimum of 12 months ([Sorahan, 2007](#); [Sorahan and Mohammed, 2016](#)). The cohort was constructed on the basis of occupation and was sensitive to exposures within the petroleum industry, but the exposure of workers to gasoline was not assessed. A limitation was the wide variation in gasoline exposure levels within the cohort that could have masked the cancer risk associated with higher levels or longer durations of exposure, as well as other unmeasured co-exposures.

Some studies conducted in petroleum workers focused on exposures to specific chemicals (benzene and total hydrocarbons), without providing specific results for gasoline. In these studies, separate analyses for tanker drivers were considered to be informative for the evaluation of gasoline, because they are thought to be a petroleum distribution population with probable exposure to gasoline ([Gun et al., 2006](#); [Schnatter et al., 2012](#)).

### (iii) *Census record-based cohorts*

In a few studies, a retrospective cohort of workers was created, and exposure to gasoline was assessed on the basis of the job title recorded in census records ([Lynge et al., 1997](#); [Lohi et al., 2008](#); [Talibov et al., 2019](#)). In a large cohort study of service station attendants identified in the 1970 censuses of four Nordic countries ([Lynge et al., 1997](#)), exposure to gasoline was inferred from the job title (service station attendant only) at the date of the census. However, the census did not ask about gasoline exposure or for the entire job history. In a cohort comprising all of the active workers in Finland identified in the 1970 census ([Lohi et al., 2008](#)), their exposure

to gasoline during their usual employment was assessed using the Finnish JEM, and the same exposure estimate was assigned to all the workers with a given job. Again, the entire work history of these individuals was not known. In the study by [Talibov et al. \(2019\)](#), the Nordic Occupational Cancer Study Cohort job-exposure matrix- (NOCCA-JEM) was used to estimate gasoline exposure on the basis of the job titles in repeat census records from four Nordic countries. The strengths of these studies were that the methods used to identify workers in relevant occupations were based on established coding systems, and most of the people coded as service station workers probably had some exposure to gasoline. However, the census forms did not collect complete job histories, and therefore some former service station workers may have been excluded from the cohort of service station workers ([Lynge et al., 1997](#)). In the studies in which records were obtained from only one or two time points, it was more likely that former service station workers were missed, and no or little information was available on the duration of employment in the specific job, which may have resulted in non-differential misclassification (e.g. [McLaughlin et al., 1987](#); [Lynge et al., 1997](#); [Vasama-Neuvonen et al., 1999](#); [Weiderpass et al., 1999](#); [Aragónés et al., 2002](#); [Guo et al., 2005](#); [Lohi et al., 2008](#); [Tarvainen et al., 2008](#)). The study by [Talibov et al. \(2019\)](#) collected information regarding a larger proportion of the participants' job history and therefore was more capable of identifying potential exposure to gasoline than were the studies in which employment was recorded at only one time point. For the studies by [Lohi et al. \(2008\)](#) and [Talibov et al. \(2019\)](#), a limitation was that a variety of occupations, without assessed co-exposures, contributed to the estimates of gasoline exposure. An additional limitation of the studies using JEMs was the lack of a sex-specific JEM, especially for those that focused on women ([Vasama-Neuvonen et al., 1999](#); [Weiderpass et al., 1999](#); [Carton et al., 2017](#)).

In all these census-based studies, the exposure assessments were crude and probably led to errors in exposure classification, which were likely to have been nondifferential with respect to cancer outcomes. This error could have led to an overestimation of risk in medium-exposure groups and an underestimation of risk in high-exposure groups, and a linear increase in risk with increases in exposure could have been missed.

### (iii) *Prospective population-based studies*

Two studies assessed occupational exposure to gasoline in prospective population-based cohorts. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study ([Neasham et al., 2011](#)), the participants were recruited from the general population without regard to their occupation and before a cancer had been diagnosed. Data from a self-administered questionnaire was used to identify their employment as a service station worker. The participants' ever exposure to gasoline was established using a proxy, and this was likely to be sufficiently accurate, but no measure of the intensity or duration of exposure to gasoline was used. The inclusion of individuals with minimal exposure to gasoline in the exposed group would be expected to attenuate the assessed risk.

In the prospective cohort study of childhood cancer by [Kirkeleit et al. \(2018\)](#), the parents' occupational exposure information was collected before their child's cancer was diagnosed. The employment history of the participants was not verified in either of these studies, but it may be reasonable to assume that a large proportion of the women who reported exposure to gasoline or exhaust fumes in the questionnaire had some level of exposure to gasoline. Limitations were that it was not possible to separate the exposure to gasoline from the exposure to exhaust fumes and that the self-reported number of days was a crude measure of cumulative exposure. Misclassification of the participants according

to their employment history would probably be nondifferential according to cancer outcomes.

### (iv) *Population-based case-control studies*

There have been several population-based case-control studies on occupational exposure to gasoline (see Table S1.15 for a study-by-study description; Annex 1). In these studies, occupational exposure to gasoline was assessed via self-reported exposure ([McKinney et al., 2008](#); [Poynter et al., 2017](#)); the proxy of being employed as service station attendant ([Band et al., 2000](#); [Colt et al., 2004](#)); or gasoline exposure estimates derived from employment histories using JEMs ([Kogevinas et al., 2003](#); [Dryver et al., 2004](#); [Barul et al., 2019](#); [Guth et al., 2023](#)) or expert assessments ([Siemiatycki et al., 1987a](#); [Partanen et al., 1991](#); [Cocco et al., 2010](#)). Two main issues would have affected the quality of the occupational gasoline exposure assessment. These were the accuracy of the information on job history and the accuracy of the estimated gasoline exposure derived from these histories.

In these case-control studies, retrospective job histories were collected using questionnaires. The quality of the information collected using the questionnaire could have depended on who provided the information (the case or control directly, or a proxy such as a spouse or next of kin). For example, in the study by [Partanen et al. \(1991\)](#), the proportion of questionnaires completed by next of kin was large (approximately two thirds), but similar for cases and controls. In the study by [Siemiatycki et al. \(1987a\)](#), both the cases and controls were patients with cancer, and < 20% of the questionnaires were completed by the next of kin. In the study by [Guth et al. \(2023\)](#), all the participants were proxy respondents for questions about their parents' jobs when they were born, which for most of the respondents was > 30 years previously. Such proxy respondents would be expected to provide less accurate information, increasing the risk of misclassification ([Stayner et al., 2024](#)). If the case participants

were more likely to recall certain exposures, or if the population control participants were less likely to do so, the consequent difference in recall quality could lead to information bias ([Stayner et al., 2024](#)). It was considered a strength of the study by [McKinney et al. \(2008\)](#) that a separate exposure assessment review was performed that estimated the probability of exposure based on job history. The review was blinded to case–control status and complemented the study respondents’ self-reports of exposure to gasoline. The blinded exposure assessment review would have been subject to misclassification error, which would probably have been comparable for the cases and controls. The mother’s self-reports were more frequently reclassified, after the exposure assessment review, for the cases than for the controls, suggesting that there was differential misclassification of gasoline exposure. The authors presented results based on both the mother’s self-reported exposures and the reclassified exposures.

In most of the population-based case–control studies, questionnaires were used to collect information about the participants’ entire job history as an initial step. Specific jobs were then coded according to the industry and occupation, often by an industrial hygienist or chemist, to identify those with potential for exposure to gasoline. When the questionnaire asked about specific exposures, gasoline was typically only one of several different substances that were included. The possibility of recall bias in the reporting of past employment could not be completely dismissed, and this would have introduced differential misclassification. However, errors introduced during the coding of probable gasoline exposure were likely to have been nondifferential by cancer outcome, especially in studies in which the coder had been blinded to the participants’ case–control status.

In some record-linkage studies conducted in Denmark, information on occupations in the Danish Supplementary Pension Fund was

used to estimate exposures (e.g. [Heineman et al., 1992](#); [Hansen, 2000](#); [Pedersen and Hansen, 2023](#)). However, a limitation was that a history of employment was not available before 1964, implying that earlier jobs could not be considered. In another Danish study using the same methodology and focusing on childhood cancer, an additional limitation of the use of the Supplementary Pension Fund was that it was not possible to ascertain the exposures of self-employed parents, because they were not adherents to this fund ([Hall et al., 2021](#)).

In some studies, results for the exposure to gasoline in combination with other exposures (e.g. to combustion products and diesel) were reported, which was considered to be a limitation (e.g. [West et al., 1995](#); [Hansen, 2000](#); [Shi et al., 2013](#)).

One study was based on self-reported personal refuelling of vehicles. This type of exposure would be expected to be far lower than those for specific occupations, thereby providing a smaller exposure contrast to assess the carcinogenic effect of gasoline.

In addition to assessing the probability of exposure to gasoline at work, semiquantitative assessments of exposure to gasoline according to the duration, intensity, and/or frequency of exposure were made in several studies. Sometimes a cumulative measure of gasoline exposure was used, in the form of a combination of the number of years in a job and the estimated intensity of exposure to gasoline in that job (based on the coder’s expertise or the use of a JEM). Estimates of cumulative exposure over time are more informative than those based on a single time point. Furthermore, the use of a JEM based on data from multiple workplaces and over multiple time periods could result in better estimates of probable workplace exposure to gasoline than could expert judgement alone. However, such matrices reflect average exposure levels, rather than maximum exposure levels, and individuals assigned to the same exposure



group may have had different exposure levels ([Stayner et al., 2024](#)). This exposure misclassification error reflected the compound error associated with each component of the exposure assessment process and would be expected to be nondifferential by cancer outcome.

Nondifferential misclassification of a dichotomous measure of gasoline exposure would usually result in a bias towards the null. However, in studies that included multiple categories of gasoline exposure (e.g. [Dryver et al., 2004](#); [Lohi et al., 2008](#); [Cocco et al., 2010](#); [Barul et al., 2018](#); [Talibov et al., 2019](#)), nondifferential misclassification of the exposure could lead to an overestimation of the risk for the medium-exposure group and an underestimation of the risk for the high-exposure group ([Stayner et al., 2024](#)). As a result, a linear increase in risk associated with increases in exposure could be missed.

#### (b) *Environmental exposure*

Studies of exposure to gasoline in residential areas have relied on measures of distance from the source of gasoline as surrogate measures of exposure, with measurements of exposures in individual residences not being available.

In the cohort study by [Talbot et al. \(2011\)](#), the assessment of gasoline exposure from contaminated groundwater was based on the distance of the residence from measured and modelled estimates of plume boundaries. The study respondents provided information on their residential history before a cancer diagnosis. The basis of the three exposure categories was well defined. The use of distance as a proxy for exposure level was likely to have introduced nondifferential exposure misclassification.

In a case-control study on childhood leukaemia, [Brosselin et al. \(2009\)](#) assessed the influence of the presence of a service station or car repair garage adjacent to a family residence. The authors described multiple efforts to monitor the quality of the interviews and check for recall bias, and these efforts lent credibility to

their findings. However, because the interviews were conducted after cancer was diagnosed in the child, the potential for differential misclassification of the exposure of mothers of cases and controls could not be completely dismissed.

In a case-control study of childhood cancer by [Mazzei et al. \(2022\)](#), the distance between the family residence and the nearest service station was assessed when cancer was diagnosed in the children. However, information about the location of service stations may have been out-of-date for the years when business census data were not collected and not particularly accurate for analyses of estimated exposures at birth. No details about the year, coding, or accuracy of the addresses of the controls and no environmental measures were provided. Without better information about the coding of the addresses of the controls, the comparability of the quality of information for the cases and controls was unknown, and misclassification of the exposure could have been differential by cancer outcome.

### 1.6.2 *Quality of exposure assessment in mechanistic studies in exposed humans*

The exposure assessment is a crucial component of mechanistic studies in exposed human populations. The various methods of assessing exposure have their strengths and limitations, which are important to consider when reviewing studies. Many mechanistic studies performed in exposed humans involved the assessment of gasoline exposure via (personal) air monitoring or biomonitoring. [The Working Group considered that occupation as a service station attendant was an adequate proxy for gasoline exposure (see Section 1.6.1). Therefore, the Working Group also reviewed many studies in which mechanistic outcomes in service station attendants were compared with those in an (unexposed) control group. Rather than a study-by-study assessment, this section provides an overall evaluation of the typical exposure assessment methods used in



the mechanistic studies described in the present monograph.]

All exposure assessment methods carry an inherent bias that is related to the representativeness of the worker groups studied. To ensure the accuracy of exposure estimates, it is essential that samples include workers in sufficiently large numbers such that, given the expected effect size, differences between groups would be expected to be statistically significant; in other words, the study has sufficient power. A similar exposure group or a homogeneous exposure group is usually created to reduce the number of samples needed, while still sufficiently describing the exposures of a group of workers defined by factors such as their tasks and work environments, the risk management measures in place, and other variables that influence exposure. However, achieving truly homogeneous exposure groups can be challenging, owing to variability in the tasks performed and the environmental conditions. Poorly designed similar exposure groups can lead to exposure misclassification in epidemiological studies, affecting the accuracy and validity of the results. This can lead to the misclassification of individuals with respect to their exposures, which may bias the results towards the null and lead to underestimation of the true relation between exposures and health outcomes.

Gasoline is a complex product with more than 150 constituents. Therefore, exposure assessments often use substances that are known to be gasoline constituents as a proxies for gasoline exposure, such as benzene. This approach is adequate when these compounds are principally derived from gasoline in a specific occupational setting or exposure scenario. Specific exposure questionnaires have also been used to ascertain gasoline exposure and any co-exposures. The exposure assessment methods ([Waters et al., 2015](#)) described in the present monograph, with their strengths and limitations, are air monitoring (both personal and area air (stationary)

monitoring), biological monitoring, self-reported exposure, modelling and estimation methods, and job title-based comparisons.

#### *(a) Air monitoring*

Several studies of diverse mechanistic outcomes have used air monitoring data, often in combination with other assessments, such as biomonitoring or job title-based assessments, to measure the exposure of participants (e.g. [Rekhadevi et al., 2010, 2011](#); [Moro et al., 2013, 2019](#)). For inhalational exposure, personal air sampling is generally considered to be the preferred method for exposure assessment, because it provides a superior representation of individual worker exposure to area air measurements. In some situations, area monitoring can be useful, but it may not capture the variation in exposure levels between workers. Area monitoring, along with direct reading instruments (which capture real-time peaks in exposure more readily), is often employed to map exposures in a workplace or to measure emissions from specific processes.

A strength of using air monitoring for exposure assessment is that it directly and accurately quantifies the current exposure to specific airborne pollutants. The air concentration data for the pollutants are objective and quantitative and have been used to assess gasoline exposure ([Chaiklieng et al., 2019](#)). Such data enable researchers to establish clear correlations between exposure levels and outcomes such as DNA damage. Personal air monitoring, in which sampling equipment is placed directly on the worker in the breathing zone to quantify inhalational exposure, is preferred to stationary (area) air monitoring, because it offers a more accurate representation of the individual's actual exposure. This method captures variations in exposure that result from personal activities, movement, and proximity to pollution sources, providing a more individualized and precise assessment.

Air monitoring should yield data that are representative of the worker group being studied, which means that a large enough number of workers from each similar exposure group must be included. These groups are defined by similar job tasks, work environments, or other exposure-related factors, which can be challenging.

A limitation is that air sampling does not consider exposure via skin contact or ingestion. In addition, air sampling over a full workday is not capable of identifying short-term peaks in exposure. Air monitoring may also not accurately reflect the exposures of different worker subgroups, because the variability in the tasks performed and the challenge of creating truly similar exposure groups can limit researchers' ability to generalize their findings to the entire workforce.

Occupational exposure limits are set by regulatory organizations and typically correspond to validated sampling and analytical methods (see Section 1.3 for details on sampling and analytical methods). Air monitoring data for many substances, including gasoline components, often follow a log-normal distribution, which means that the data are skewed, with a few very high exposure values and many low values. As a result, arithmetic means may not adequately represent the exposure levels. Instead, geometric means and geometric standard deviations better capture the distribution of the data and provide more accurate measures of the central tendency and variability of exposures ([Waters et al., 2015](#)).

#### *(b) Biological monitoring*

Many studies on mechanistic outcomes have used biomonitoring to assess the exposure of participants, sometimes in combination with other assessment tools, such as air monitoring (e.g. [Rekhadevi et al., 2010, 2011](#); [Moro et al., 2013, 2019](#)). Biomonitoring is a critical tool for assessing the exposure to chemicals that can be absorbed by inhalation and through the skin, especially when environmental sampling alone

cannot fully capture the complexity of the exposure ([Hopf et al., 2024](#)). It offers a more accurate measure of internal exposure by accounting for variations in physical effort, all routes of absorption (including via the lungs and skin), the use of personal protective equipment, environmental factors, and differences in metabolism. By capturing data on the intake through all exposure routes, individual variability, and the external factors affecting absorption, biomonitoring provides a more integrated assessment of an individual's total exposure than conventional air sampling ([White et al., 1995](#); [Hopf et al., 2025](#)).

Biological monitoring data are log-normally distributed. Therefore, geometric means and geometric standard deviations would be expected to better capture the distribution and variation of exposure data ([Hopf et al., 2025](#)).

The biomonitoring of gasoline exposure has several limitations. Biomarkers of gasoline exposure, such as benzene metabolites, typically only capture recent or short-term exposure ([Janasik et al., 2008](#)). Urinary exposure biomarkers with short half-lives (6–12 hours) reflect current exposures, as do air monitoring data. Another limitation is that some of the markers are not specific for gasoline. For instance, the urinary concentrations of benzene, SPMA, and *t,t*-MA can also be influenced by cigarette smoking. Indeed, smoking has a larger impact on metabolite concentrations than working at a service station ([Fustinoni et al., 2005a](#); [Campo et al., 2016](#)), and workers exposed to other organic solvents, such as toluene, styrene, or xylene, may have high SPMA concentrations, because these substances share metabolic pathways with benzene. Therefore, adjustment for smoking and other exposures that influence the concentrations of these exposure markers is crucial to avoid confounding. Chronic alcoholism may also influence the SPMA and *t,t*-MA concentrations, making it challenging to attribute high biomarker concentrations to exposure to gasoline or benzene, a commonly used index of gasoline exposure.

For biomonitoring to be effective, the sample studied should be representative of the group of workers being studied, meaning that it must include a large enough number of workers from each similar exposure group to permit statistically significant data to be obtained.

For gasoline exposure, information on the urinary elimination half-life of the substances of interest is fundamental to define the most appropriate timing of sample collection for biomarker measurement, depending on the anticipated gasoline exposure level ([Rahimpoor et al., 2023](#)). This typically involves quantifying parent compounds or metabolites such as *t,t*-MA (a metabolite of benzene), methylhippuric acid (a metabolite of xylene), and hippuric acid (a metabolite of toluene). Urine metabolite concentrations are corrected for creatinine concentration to account for the degree of urine dilution, but this is not necessary for the quantification of the parent compounds. In instances in which no validated method exists, sample collection should be timed according to the estimated half-life of the substance. For example, for benzene exposure, urinary metabolites such as SPMA and *t,t*-MA are commonly quantified in urine samples collected at the end of a shift. However, if workers are only exposed to benzene early in their shifts, sampling at the end of the shift could underestimate their exposure.

Urine sampling is particularly suitable for the monitoring of cumulative exposure and for substances that are metabolized, whereas breath analysis is ideal for volatile compounds and provides real-time exposure data when samples are collected immediately after exposure.

#### *(c) Self-reported exposure*

Self-reported exposure assessments are commonly used in epidemiological studies when direct measurements such as air and biological monitoring are not feasible or are too expensive. These methods rely on the recall of participants and the reporting of their exposure to certain

substances or conditions. Participants are asked to answer a series of questions regarding their occupational, environmental, and/or lifestyle exposures ([Ge et al., 2018](#)). These can include specific questions about work tasks, the use of chemicals, the use of protective equipment, their job history, and their lifestyle. This approach is associated with potential inaccuracies in reporting, and recall bias is particularly likely in retrospective studies. Information such as the job held or the duration of employment is usually collected with sufficient accuracy. However, individuals may have difficulty accurately remembering or reporting past exposures, and therefore the quality of self-reported data can vary significantly between individuals, leading to inaccuracies in the exposure assessment. Participants may either under- or overreport certain exposures, on the basis of perceived social or legal consequences, potentially skewing the data (social desirability bias).

#### *(d) Job title as a proxy for exposure*

In many studies, mechanistic outcomes have been compared between a group of exposed workers (mainly service station attendants) and a control group of individuals that are considered to be unexposed (e.g. [Pandey et al., 2008](#); [Brum et al., 2021](#)). Epidemiological studies comparing exposed workers to workers deemed to be not exposed or less exposed are susceptible to several biases that could compromise the accuracy and validity of the findings. Exposure misclassification is possible, because the controls may still be exposed to gasoline as a result of proximity to industrial areas or the use of shared workspaces, which would be expected to result in bias towards the null. Another important limitation of these studies is the existence of co-exposures. These might not be well documented; for example, the exposures may not be only to gasoline, but also to other carcinogens, and therefore the co-exposures of the two groups being compared might be quite different, leading to confounding or effect

modification. Finally, this study design does not enable the quantification of exposures, and therefore the direction and magnitude of any bias is difficult to evaluate.

The lack of personal exposure data is another limitation, because these studies typically rely on the job title or self-reporting, which fail to account for individual variability in exposure levels. Workers within the same job category may experience vastly different exposure levels, owing to factors such as differences in their specific tasks. However, the Working Group considered that the job title “service station attendant” is a good proxy for gasoline exposure, because of the relatively high exposure of such workers to gasoline, along with a relatively low level of exposure to other carcinogens (see also Section 1.6.1). These limitations would result in exposure misclassification that would be expected to be nondifferential, potentially biasing the results towards the null hypothesis.

#### (e) Conclusion

A well-designed epidemiological study that incorporates a combination of exposure assessment methods would be subject to minimal bias, be accurate, and provide a comprehensive picture of the relationship between the exposure and health outcomes. Integrating personal air monitoring or biological monitoring, while accounting for confounding variables and ensuring representative sampling of the workers of interest, is the most scientifically robust approach. This multi-method design allows for both the assessment of individual variability in exposure and the generalization of the results to the wider population, while addressing the limitations of each method, and such studies are the most informative from an exposure assessment point of view. Exposure assessments based on self-reporting or job title, if well conducted, are considered to be adequate for mechanistic studies, but less informative than quantitative methods from an exposure assessment point of view. For all these study designs,

the risk of exposure misclassification is present to some degree, but this is likely to be nondifferential, leading in expectation to a bias towards the null in the exposure–outcome relation.

## 2. Cancer in Humans

### Introduction

This section reviews studies of exposure to automotive gasoline in relation to cancer incidence in humans. In the previous evaluation of gasoline by the *IARC Monographs* programme in 1988 (Volume 45, [IARC, 1989](#)), there was *inadequate* evidence regarding cancer in humans.

A systematic search was conducted using the PubMed database to identify all cohort, nested case–control, and case–control studies assessing exposure to automotive gasoline and cancer as an outcome. The search terms and list of retrieved studies are available from: <https://hawcproject.iarc.who.int/assessment/748/>.

Studies are described in which exposure to automotive gasoline [equivalently, “petrol”, see Section 1.1] was assessed by self-reporting, JEMs, expert evaluation on the basis of work history information, environmental exposure in gasoline spill events, and from residential proximity to service stations. Additionally, studies reporting on cancer risk in selected occupations (service station attendants, land-based petroleum distribution workers), which were considered as good proxies for gasoline exposure, were selected (for the list of occupations judged to be not informative, see below). With a few exceptions ([Siemiatycki et al., 1987a](#)), “automotive gasoline” was not specifically mentioned. However, all studies in which gasoline was specified without further indication were considered for review and deemed uninformative if evidence of exposure to gasoline other than automotive gasoline was expected to be the dominant gasoline exposure

type, e.g. in studies in aircraft workers exposed to gasoline JP4 ([Radican et al., 2008](#)).

The Working Group included in their assessment cohort, nested case–control, and case–control studies and did not consider further case series, case reports, ecological studies (e.g. [Swaen and Slangen, 1995](#); [Dahlgren et al., 2008](#)), or studies that did not include cancer as an end-point, including studies that assessed cancer survival as the outcome of interest.

Six USA-based studies using proportionate mortality ratio (PMR) were retrieved ([Petersen and Milham, 1980](#); [Milham, 1983](#); [Dubrow and Wegman, 1984a, b](#); [Schwartz, 1987](#); [Leigh, 1996](#)). PMR studies were not further examined because PMR measures the proportion of deaths from a particular cause relative to the total number of deaths. Besides several other limitations (e.g. mortality instead of incidence end-points; potential misclassification of occupational and cause-of-death information), PMR results are often challenging to interpret, because PMR values for one cause of death may depend on the PMR values for other causes. The study by [Moura-Corrêa \(2023\)](#) was also considered uninformative for this evaluation because the analysis reported average annual crude rates and ratios of leukaemia mortality (including for service station attendants) on the basis of death certificate data, with valid occupations registered in only 37.5% of records. As for the PMR studies, it was based on occupations registered in the death certificates.

Employment in a wide range of occupations may imply exposure to gasoline (e.g. service station attendants, petroleum refinery and distribution workers, motor vehicle mechanics, highway maintenance workers, seafarers, or workers on marine vessels transporting petroleum). The Working Group considered that for some occupations the potential effects of gasoline could not be distinguished with reasonable confidence from those of co-exposures (see [Table 1.10](#)).

JEM and biomonitoring data indicated that automobile mechanic occupations entail exposure to gasoline (see Section 1.4.2). However, for automobile mechanics such exposure occurs only during specific tasks in the job. Although co-exposure to gasoline exhaust emissions and PAHs is a concern for both automobile mechanics and service station attendants, occupational exposure as a mechanic entails exposure to other known, possible, or probable carcinogens, such as asbestos, some metals, and welding fumes, and also entails higher exposure to exhaust fumes (see more details in Section 1.4.2). Considering these data, occupational exposure as an automobile mechanic was not considered by the Working Group as a good proxy for gasoline exposure, because exposure to gasoline would occur only in specific tasks, and the occupation is characterized by exposure to numerous other carcinogens that are usually not accounted for.

Studies reporting on risk of cancer in seafarers or workers on marine vessels transporting petroleum were excluded from the present evaluation when it was not specified whether the seafarers were transporting gasoline ([Moen et al., 1990](#); [Pukkala and Saarni, 1996](#); [Nilsson et al., 1998](#); [Pukkala, 1998](#); [Andersen et al., 1999](#); [Saarni et al., 2002](#); [Kaerlev et al., 2005](#); [Ugelvig Petersen et al., 2018, 2020](#); [Forsell et al., 2020, 2022](#)). However, the cohort of petroleum workers in the USA described in [Wong et al. \(1993, 1999\)](#), which combined both land-based and marine distribution workers, was not excluded because all workers were indicated as exposed to gasoline vapour in this study. Studies of petroleum workers that did not focus on land-based distribution workers or distribution workers exposed to gasoline ([Wong and Raabe, 2000](#); [Glass et al., 2003](#)) were not considered.

Studies on cancer in highway maintenance workers ([Bender et al., 1989](#); [Hwang et al., 1995](#)) were also considered to be uninformative, because exposure to automotive gasoline could



not be distinguished sufficiently from exposure to asphalt fumes, exhaust gas, or dust, etc.

Studies of oil refinery workers without further categorization ([Pukkala, 1998](#)) were also not further considered, given the broad range of other petroleum products that these workers could have been exposed to, and that they may not have been exposed to gasoline itself, i.e. to the final product defined as a mixture in Section 1.1. Studies of petroleum refinery workers ([Poole et al., 1993](#); [Anttila et al., 2015](#)) that examined exposure to non-aromatic liquid gasoline distillates, including hydrocarbons in the distillation range 40–200 °C, were not considered to be informative because this distillation range is too broad to be specific to gasoline (see Section 1.2). Another case–control study nested in a cohort of refinery workers in which exposure was assessed to C6–C10 aliphatic saturated hydrocarbons (components of gasoline) was considered uninformative because of the high correlation with exposure to other categories of hydrocarbons, making it difficult to distinguish the specific effect of each process stream ([Gamble et al., 1996](#)).

Studies that were considered in the present review included studies of occupational groups considered to be good proxies for gasoline exposure, namely, land-based distribution workers including tanker drivers; cohorts combining land-based and marine distribution workers where exposure to gasoline was specified; and service station attendants. It should be noted that co-exposures to potential carcinogens, such as air pollution, cannot be excluded in these occupations. In addition to studies on specific occupational groups, the review also includes all cohort or case–control studies in which exposure to gasoline was specifically assessed using self-reports, JEMs, or expert evaluation on the basis of job descriptions. In these instances, other occupational groups exposed to gasoline, such as mechanics or agricultural workers, were included.

## 2.1 Description of cohort studies and case–control studies on multiple cancer types

See [Table 2.1](#).

### 2.1.1 Record-linkage studies

There were 20 population-based record-linkage studies considered to be informative. These studies included those on the association between occupational exposure to automotive gasoline or potential proxies and risk of cancer, including from Canada ([Howe and Lindsay, 1983](#)); studies on gas station workers, using the 1970 census across the Nordic countries (Denmark, Finland, Norway, and Sweden) ([Lyngé et al., 1997](#)); and some studies conducted in each country individually, which provided additional information ([Norell et al., 1986](#); [Walker et al., 1987](#); [McLaughlin et al., 1987](#); [Aragonés et al., 2002](#); [Navas-Acién et al., 2002](#)); three nested case–control studies on workers who were likely to be exposed to gasoline on the basis of information from Danish registries ([Heineman et al., 1992](#); [Hansen, 2000](#); [Pedersen and Hansen, 2023](#)); eight Nordic census-based record-linkage studies in which the Finnish job-exposure matrix (FINJEM) was applied ([Vasama-Neuvonen et al., 1999](#); [Weiderpass et al., 1999, 2003](#); [Wesseling et al., 2002](#); [Guo et al., 2005](#); [Lohi et al., 2008](#); [Tarvainen et al., 2008](#); [Lindbohm et al., 2009](#)); and one based on the NOCCA-JEM ([Talibov et al., 2019](#)). Additional record-linkage studies deemed uninformative included the study by [Ji et al. \(2005\)](#), because exposure was grouped only into broad occupational categories (three-digit), without specifically identifying “gasoline station attendants” or providing information on exposure to automotive gasoline. Several record-linkage studies based on census data from Denmark ([Grandjean and Andersen, 1991a](#)) or Sweden ([Jakobsson et al., 1992, 1993](#); [Wilson et al., 2008](#)) were considered to be uninformative

**Table 2.1 Description of cohort studies (including nested case–control studies) on exposure to automotive gasoline and cancer**

| Reference, location, enrolment/follow-up period, study design                                                                      | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Cancer types reported, and corresponding tables <sup>a</sup>                             | Comments                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Howe and Lindsay (1983)</a><br>Record linkage study, Canada<br>1965–1969/follow-up through 1973 (mortality)<br>Cohort  | 415 201 men with occupation recorded in 1965–1969, representing 10% of the Canadian labour force. Mortality data through linkage with Statistics Canada.<br><br><i>Exposure assessment method:</i> Occupations were recorded in the national labour insurance dataset (Canadian Unemployment Insurance Commission), which covered all employers in Canada.                                                                                                                                                                     | <i>Reported in Table 2.4:</i><br>Urinary bladder plus other organs of the urinary system | <i>Strengths:</i> Large study population.<br><i>Limitations:</i> Short follow-up time with small number of deaths. Grouping by industry and occupation was crude, with no information on employment duration. No distinction between different urinary organs.                                                                               |
| <a href="#">Norell et al. (1986)</a><br>Record linkage study, Sweden<br>1960 census/follow-up, 1961–1979 (incidence)<br>Cohort     | 2 million employed men aged 20–64 yr in the 1960 census in Sweden, followed with linkage to nationwide cancer registry (1961–1979).<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of job titles derived from self-administered questionnaires (census data). No data on duration of employment.                                                                                                                                                                                                | <i>Reported in Table S2.5:</i><br>Pancreas                                               | <i>Strengths:</i> High-quality nationwide registries. High exposure in gasoline station attendants during study period because before self-refuelling by the customer was common.<br><i>Limitations:</i> An assessment of cumulative exposure was not possible.<br><i>Other comments:</i> Overlap with <a href="#">Lynge et al. (1997)</a> . |
| <a href="#">McLaughlin et al. (1987)</a><br>Record linkage study, Sweden<br>1960 census/follow-up, 1961–1979 (incidence)<br>Cohort | Population size, NR. Employed men in the 1960 census in Sweden, followed with linkage to nationwide cancer registry (1961–1979).<br><br><i>Exposure assessment method:</i> Employment data from the 1960 national census were classified by occupation and industry using revised versions of the standards of the <a href="#">International Labor Office (1958)</a> and the <a href="#">United Nations Statistical Office (1958)</a> . For specific (3-digit) employment groups, only those with ≥ 500 workers were examined. | <i>Reported in Table 2.4:</i><br>Kidney                                                  | <i>Strengths:</i> High-quality nationwide registries. High exposure in gasoline station attendants during the study period because before self-refuelling was common.<br><i>Limitations:</i> An assessment of cumulative exposure was not possible.<br><i>Other comments:</i> Slight overlap with <a href="#">Lynge et al. (1997)</a> .      |
| <a href="#">Walker et al. (1987)</a><br>Record linkage study, Sweden<br>1960 census/follow-up, 1961–1979 (incidence)<br>Cohort     | Population size, NR. Employed men in the 1960 census in Sweden, followed with linkage to nationwide cancer registry (1961–1979).<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of job titles derived from self-administered questionnaires (census data). No data on duration of employment.                                                                                                                                                                                                   | <i>Reported in Table 2.4:</i><br>Urinary bladder                                         | <i>Strengths:</i> High-quality nationwide registries. High exposure in gasoline station attendants during study period because before self-refuelling was common.<br><i>Limitations:</i> An assessment of cumulative exposure was not possible.<br><i>Other comments:</i> Slight overlap with <a href="#">Lynge et al. (1997)</a> .          |

Table 2.1 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                                                                                                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
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| <a href="#">Lyngé et al. (1997)</a><br>Service station attendants in Denmark, Norway, Sweden, and Finland<br>Census 1970/follow-up ending in 1987 (Denmark), 1991 (Norway), 1989 (Sweden), 1985 (Finland) (incidence)<br>Cohort | 16 524 men, 2445 women; workers (male and female) in the service station industry identified through the 1970 census in Denmark, Norway, Sweden, Finland. Incidence (ICD-7) through the national cancer registries.<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of job titles derived from self-administered questionnaires (census data). No data on duration of employment. Possible co-exposures mentioned but not considered in the analyses.                                                                           | <i>Reported in Table 2.3:</i><br>Lymphatic and haematopoietic NHL, NHL (CLL), multiple myeloma, HL, leukaemia, AML, all other leukaemia<br><br><i>Reported in Table 2.4:</i><br>Kidney, urinary bladder<br><br><i>Reported in Table S2.5:</i><br>Oral cavity, pharynx, oesophagus, stomach, colon, rectum, liver, gallbladder, pancreas, nose, larynx, lung<br><br><i>Reported in Table S2.6:</i><br>Breast<br><br><i>Reported in Table S2.7:</i><br>Prostate gland, brain, thyroid gland, uterine cervix, uterus, ovary, melanoma | <i>Exposure assessment critique:</i> Ever exposure to gasoline as measured by proxy was likely to be sufficiently accurate, but there was no measure of intensity or duration of exposure to gasoline. A limitation was that this study was based on information on occupation at only one point in time.<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Incidence data from cancer registers of high quality and national coverage. Large sample size.<br><br><i>Limitations:</i> Status as a service station attendant was defined by the occupation and workplace held in the census week. No information on occupational history, co-exposure to other potential carcinogens or lifestyle-related confounders (e.g. smoking, diet) other than education. |
| <a href="#">Aragónés et al. (2002)</a><br>Record linkage study, Sweden<br>Census 1970/follow-up, 1971–1984 (incidence)<br>Cohort                                                                                                | 1 779 646 men who were employed in Sweden during the 1970 census, recorded in both the 1970 and 1960 censuses, and aged 25–59 yr on 1 January 1971. A subcohort with those reporting the same occupation in 1960 and 1970 was also assembled. Incident cancer was assessed through the cancer registry. Analysis reported was for male petrol station attendants.<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of job titles derived from self-administered questionnaires (census data). No data on duration of employment. | <i>Reported in Table S2.5:</i><br>Stomach                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <i>Strengths:</i> Large cohort of service station attendants likely to be exposed to automotive gasoline during car refuelling before the introduction of self-service. High-quality nationwide registries.<br><br><i>Limitations:</i> Information on exposure was only available at one or two time points (1960 and 1970 censuses). Few cases for most of the digestive cancers. Lack of information on, and adjustment for, lifestyle factors and co-exposures.<br><br><i>Other comments:</i> Results for female petrol station workers were not reported. Partial overlap with pooled gas station worker cohort analysis by <a href="#">Lyngé et al. (1997)</a> (based on 1970 census data).                                                                          |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                         | Cancer types reported, and corresponding tables <sup>a</sup>       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
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| <a href="#">Navas-Acién et al. (2002)</a><br>(supersedes <a href="#">Jakobsson et al., 1992</a> )<br>Record linkage study, Sweden<br>1970 census/follow-up through 1989<br>(incidence)<br>Cohort | 1 779 646 (whole cohort); Swedish men employed at 1970 census, recorded also in 1960 census and still alive (and aged > 24 yr) in 1971. Recorded as retail sale of oil and gasoline (filling station attendants, number not reported) in the 1970 census or in both 1970 and 1960.<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of job titles derived from self-administered questionnaires (census data). No data on the duration of employment. | <i>Reported in Table S2.7:</i><br>Brain (glioma)                   | <i>Strengths:</i> High-quality national population-based registers, incident outcomes, long follow-up period.<br><br><i>Limitations:</i> Crude exposure assessment on the basis of one or two census years, imprecise findings, multiple comparisons.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <a href="#">Weiderpass et al. (1999)</a><br>Record linkage study, Finland<br>1970 census/follow-up, 1971–1995<br>(incidence)<br>Cohort                                                           | 892 591 (23 638 cases of incident breast cancer); active women recorded in 1970 census. Follow-up through Finnish Cancer Registry.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (FINJEM) allowing grading of exposure through estimated levels and probabilities of exposure for specific time windows.                                                                                            | <i>Reported in Table S2.6:</i><br>Breast (pre- and postmenopausal) | <i>Exposure assessment critique:</i> Strengths were that the methods used to identify gas station workers were based on established coding systems, and most people coded as gas station workers probably had some exposure to gasoline. Some former gas station workers may have been missed. The exposure classification error was probably nondifferential. A limitation was that a variety of occupations, without assessed co-exposures, contributed to estimated gasoline exposure. In addition, exposure was assessed on the basis of a single census year.<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> High-quality population-based registers, incident outcomes, data available on some personal/reproductive factors, analysis of pre- and postmenopausal disease.<br><br><i>Limitations:</i> non-gender-specific JEM, imprecise findings. |
| <a href="#">Vasama-Neuvonen et al. (1999)</a><br>Record linkage study, Finland<br>1970 census/follow-up, 1971–1995<br>(incidence)<br>Cohort                                                      | 892 591 (5072 cases); active women recorded in 1970 census. Follow-up through Finnish Cancer Registry.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM using self-reported occupational data from single census year.                                                                                                                                                                                 | <i>Reported in Table S2.7:</i><br>Ovary                            | <i>Strengths:</i> High-quality population-based registers, large number of incident outcomes, data available on some personal/reproductive factors.<br><br><i>Limitations:</i> Exposure assessment on the basis of a single census year, a non-gender-specific JEM, imprecise findings.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                      | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Cancer types reported, and corresponding tables <sup>a</sup>                | Comments                                                                                                                                                                                                                                                                                                        |
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| <a href="#">Wesseling et al. (2002)</a><br>Record linkage study, Finland<br>Census 1970/follow-up, 1971–1995 (incidence)<br>Cohort | 413 877 (693 cases); active women recorded in 1970 census (born in 1906–1945) and not in the two highest social classes (on account of minimal or no occupational exposure to the chemical and physical agents of interest). Follow-up through Finnish Cancer Registry.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (FINJEM) using self-reported occupational data from a single census year.                                                                                                                                                             | <i>Reported in Table S2.7:</i><br>Brain                                     | <i>Strengths:</i> High-quality population-based registers, large number of incident outcomes.<br><br><i>Limitations:</i> Exposure assessment on the basis of a single census year, a non-sex-specific JEM, imprecise findings, brain tumour subtypes not examined.                                              |
| <a href="#">Weiderpass et al. (2003)</a><br>Record linkage, Finland<br>1970 census/follow-up, 1971–1995 (incidence)<br>Cohort      | 413 877 (1881 cases); female workers born in 1906–1945 in Finland identified using 1970 census, excluding managers and other high administrative or clerical employees, lower administrative or clerical employees, small-scale entrepreneurs, and farmers. Follow-up through Finish cancer registry (1971–1995). 389 oesophagus, 1881 stomach, 2009 colon, 1323 rectum, 389 liver, 651 gallbladder, and 1302 pancreas cases overall.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (FINJEM) using self-reported occupational data from single census year. | <i>Reported in Table S2.5:</i><br>Stomach<br>Colon<br>Rectum<br>Gallbladder | <i>Strengths:</i> High-quality population-based registers, incident outcomes, data available on some personal/reproductive factors, analysis of pre- and postmenopausal disease.<br><br><i>Limitations:</i> Exposure assessment on the basis of a single census year, non-sex-specific JEM, imprecise findings. |
| <a href="#">Guo et al. (2005)</a><br>Record linkage study, Finland<br>1970 census/follow-up, 1971–1995 (incidence)<br>Cohort       | 667 121 (387 cases); economically active Finnish male born in 1906–1945 with occupational information collected in 1970 census. Cancer incidence through linkage with cancer registry.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (FINJEM) using self-reported occupational data from a single census year.                                                                                                                                                                                                                                              | <i>Reported in Table S2.7:</i><br>Testis                                    | <i>Strengths:</i> High-quality population-based registers, incident outcomes.<br><br><i>Limitations:</i> Exposure assessment on the basis of a single census year, small numbers of incident cases, imprecise findings.                                                                                         |



**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                           | Cancer types reported, and corresponding tables <sup>a</sup>                   | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
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| <a href="#">Lohi et al. (2008)</a><br>Record linkage, Finland<br>1970 census/follow-up, 1971–1995<br>(incidence)<br>Cohort      | 1.6 million economically active people (born in 1906–1945) identified in the 1970 census. Follow-up through Finnish Cancer Registry (1971–1995).<br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (FINJEM) allowing grading of exposure because of estimated levels and probabilities of exposure for specific time windows. | <i>Reported in Table 2.4:</i><br>Kidney<br>Urinary bladder                     | <i>Exposure assessment critique:</i> Strengths were that the methods used to identify gas station workers were based on established coding systems, and most people coded as gas station workers probably had some exposure to gasoline. Some former gas station workers may have been missed. The exposure classification error was probably nondifferential. A limitation was that a variety of occupations, without assessed co-exposures, contributed to estimated gasoline exposure.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Large number of cancer cases.<br><i>Limitations:</i> The confounding factors smoking and obesity were not assessed at an individual level, but by occupation. |
| <a href="#">Tarvainen et al. (2008)</a><br>Record linkage, Finland<br>1970 census/follow-up, 1971–1995<br>(incidence)<br>Cohort | 1.6 million economically active people (born in 1906–1945) identified in the 1970 census. Follow-up through Finnish Cancer Registry (1971–1995).<br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (FINJEM) using self-reported occupational data from a single census year.                                                  | <i>Reported in Table S2.5:</i><br>Mouth and pharynx<br>(excluding nasopharynx) | <i>Strengths:</i> High-quality population-based registers, incident outcomes, data available on some personal/reproductive factors, analysis of pre- and postmenopausal disease.<br><i>Limitations:</i> Exposure assessment on the basis of a single census year, a non-gender-specific JEM, imprecise findings.                                                                                                                                                                                                                                                                                                                                                                                                |
| <a href="#">Lindbohm et al. (2009)</a><br>Record linkage, Finland<br>1970 census/follow-up, 1971–1995<br>(incidence)<br>Cohort  | 1.2 million economically active people (born in 1906–1945) as recorded in the 1970 census. Follow-up through national cancer registry.<br><i>Exposure assessment method:</i> Occupation held for the longest time as of 1970. A JEM (FINJEM) was used for assigning estimates of exposure to gasoline and groups of hydrocarbons to each of the occupations.                                         | <i>Reported in Table S2.5:</i><br>Liver                                        | <i>Strengths:</i> High coverage and accuracy of the cancer registry. Large number of cancer cases.<br><i>Limitations:</i> The confounding factors smoking and alcohol consumption were not assessed at an individual level, but by occupation (as part of the FINJEM).                                                                                                                                                                                                                                                                                                                                                                                                                                          |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                      | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Cancer types reported, and corresponding tables <sup>a</sup> | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
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| <a href="#">Heineman et al. (1992)</a><br>Denmark<br>1970–1984<br>(incidence)<br>Nested case–control               | <p><i>Source cohort:</i> Individuals aged &gt; 18 yr or born after 1897 included in the Danish SPF.</p> <p><i>Cases:</i> 791 men diagnosed with multiple myeloma and reported to the Danish Cancer Registry.</p> <p><i>Controls:</i> 3080 population controls (from Danish central population registry), matched on sex and year of birth (4:1). Participants with a diagnosis of malignancy &gt; 91 days before matched case diagnosis date were excluded as controls.</p> <p><i>Exposure assessment method:</i> JEM: occupational hygienists assessed exposure on the basis of occupational histories obtained through linkage with Danish SPF.</p>                                                  | Reported in <a href="#">Table 2.3</a> :<br>Multiple myeloma  | <p><i>Strengths:</i> Assessment of gasoline exposure by occupational hygienists on the basis of occupational histories obtained from a registry; large sample size; focus on a specific haematolymphoid malignancy; 10-yr exposure latency; adjustment for exposure to other occupational agents.</p> <p><i>Limitations:</i> The occupational history was not available before 1964 and was more incomplete for occupations, implying that earlier jobs could not be considered.</p>                                                                              |
| <a href="#">Hansen (2000)</a><br>National Pension Fund, Denmark<br>1970–1989<br>(incidence)<br>Nested case–control | <p><i>Source cohort:</i> Danish National Pension Funds.</p> <p><i>Cases:</i> 230 men (born in 1897–1966) with first primary breast cancer identified in the Danish Cancer Registry (1970–1989) and members of the national pension fund.</p> <p><i>Controls:</i> 12 880; 56 controls (alive, cancer-free at cancer diagnosis date) per cases selected matched on birth year from members of the national pension fund.</p> <p><i>Exposure assessment method:</i> Exposure to “gasoline and combustion products” was defined as having had &gt; 3 mo of employment within companies with the trade codes of service station, vehicle maintenance, wholesale trade of gasoline, or car repair shops.</p> | Reported in <a href="#">Table S2.6</a> :<br>Breast           | <p><i>Strengths:</i> Risk factors for breast cancer related to menstrual cycle and childbearing were excluded since the cohort comprised men only. Estimates were adjusted for birth year and SES (white- vs blue-collar workers).</p> <p><i>Limitations:</i> Not possible to differentiate between exposure to gasoline and combustion products. Small number of exposed cases. No information on co-exposures to other potential carcinogenic agents and specific lifestyle factors (e.g. smoking, alcohol) other than SES (white- vs blue-collar workers).</p> |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                            | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Cancer types reported, and corresponding tables <sup>a</sup> | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                  |
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| <a href="#">Pedersen and Hansen (2023)</a><br>Denmark<br>1978–2016<br>(incidence)<br>Nested case–control | <p><i>Source cohort:</i> Danish women born after 1965 with information available on parental employment before and around the time of birth.</p> <p><i>Cases:</i> 5587 breast cancer cases (born after 1965) identified through the Danish Cancer Registry. Parental occupation retrieved through linkage with the Civil Registration System.</p> <p><i>Controls:</i> 20 per case; matched on sex and year of birth, retrieved through the Danish Civil Registration System using incidence-density sampling, had no missing paternal occupation information, alive and free of breast cancer at the time of case diagnosis.</p> <p><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a well-documented and quantitative JEM (NOCCA-DANJEM) using employment history as recorded in the nationwide pension fund registry (SPF).</p> | <p>See Table S2.6:<br/>Breast</p>                            | <p><i>Strengths:</i> Large-scale study, high-quality registry data, detailed information on parental occupational history in specific time windows of exposures, incident cases, data available on hormonal subtypes.</p> <p><i>Limitations:</i> Lack of personal and other reproductive information, lack of occupational information before 1964 and on parental leave or on potential changes in work tasks, non-sex-specific JEM.</p> |

Table 2.1 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                                                                                                                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Cancer types reported, and corresponding tables <sup>a</sup> | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
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| <a href="#">Talibov et al. (2019)</a><br>Nordic Occupational Cancer Study, Finland, Iceland, Norway, Sweden/follow-up through 2003 in Denmark and Norway, 2004 in Iceland, and 2005 in Finland and Sweden<br>(incidence)<br>Nested case-control | <p><i>Source cohort:</i> Individuals aged 30–64 yr on 1 January of the year after the first available census in which they participated (1960, 1970, 1980/1981, and/or 1990 censuses).</p> <p><i>Cases:</i> colon, 181 709; rectum, 109 227; colorectal cancer identified through linkage with national cancer registries.</p> <p><i>Controls:</i> 1 454 680; 5 controls (free of colorectal cancer and living in the country at case diagnosis) per case matched on country, sex, and year of birth.</p> <p><i>Exposure assessment method:</i> Nationwide occupational cohorts from four countries; estimated cumulative exposure on the basis of job titles using a JEM and time of occupation held.</p> | Reported in Table S2.5:<br>Colon and rectum                  | <p><i>Exposure assessment critique:</i> Strengths were that the methods used to identify gas station workers were based on established coding systems, and most people coded as gas station workers probably had some exposure to gasoline. Some former gas station workers may have been missed. The exposure classification error was probably nondifferential. Another strength was the country-specific JEM. A limitation was that a variety of occupations, without assessed co-exposures, contributed to estimated gasoline exposure.</p> <p><i>Design and analysis:</i></p> <p><i>Strengths:</i> Large sample size and full coverage of population for both sexes. Complete ascertainment of incident cancer cases through high-quality national registers. Long follow-up time. Harmonized occupational data. Adjustment of occupational co-exposure to other carcinogenic agents. Adjustment for relevant lifestyle factors on a group level in a subgroup (Finland).</p> <p><i>Limitations:</i> Lack of confounder control for lifestyle factors on an individual level (e.g. tobacco smoking, alcohol, diet, obesity).</p> |
| <a href="#">Preston-Martin (1989)</a><br>LA County, USA<br>1972–1985<br>(incidence)<br>Cohort                                                                                                                                                   | <p>8612 White men (aged 25–64 yr) (2448 gliomas, 810 meningiomas); incident cases of brain cancer (PIR analysis) in 1975–1985 among LA County residents identified through the Cancer Surveillance Program (reporting, 95% complete).</p> <p><i>Exposure assessment method:</i> Occupation abstracted from hospital charts.</p>                                                                                                                                                                                                                                                                                                                                                                            | Reported in Table S2.7:<br>Brain (meningioma and glioma)     | <p><i>Strengths:</i> Data on cancer incidence instead of mortality.</p> <p><i>Limitations:</i> Crude occupational data, few exposed cancer cases, multiple comparisons.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                     | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                                                                                  | Comments                                                                                                                                                                                                                                                                                                                                                                                                           |
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| <a href="#">Schnatter et al. (1993)</a><br>Update of <a href="#">Schnatter et al. (1992)</a><br>Distribution workers, Canada<br>1964–1983<br>(mortality)<br>Cohort                | 6672; all active male employees and living retirees as of 1 January 1964 or new male employees (1964–1983) with ≥ 1 yr of employment in marketing and distribution for petroleum industries in Canada. Mortality data from Statistics Canada, National Death Index, or other sources (regional registries). There were 4889 marketing or distribution workers ever exposed to hydrocarbons.<br><br><i>Exposure assessment method:</i> Industrial hygienists determined whether a job/function/department combination at a given location would entail hydrocarbons exposure. | <i>Reported in Table 2.3:</i><br>NHL, multiple myeloma, all leukaemia, myeloid/lymphatic leukaemia<br><i>Reported in Table 2.4:</i><br>Kidney, urinary bladder<br><i>Reported in Table S2.5:</i><br>Lung, oesophagus, stomach, large intestine (colon), rectum, pancreas<br><i>Reported in Table S2.7:</i><br>Melanoma, prostate gland, brain | <i>Exposure assessment critique:</i> No assessment of exposure to gasoline. Exposure solely inferred from work in the marketing and transportation sector.<br><i>Strengths:</i> Assessment of hydrocarbon exposure frequency.<br><i>Limitations:</i> No information on potential confounders. Left truncation of the cohort. HWSB because of 1-yr exposure criteria.<br><i>Other comments:</i> Industry-sponsored. |
| <a href="#">Schnatter et al. (1996)</a><br>Update of <a href="#">Schnatter et al. (1992)</a><br>Distribution workers in Canada<br>1964–1983<br>(mortality)<br>Nested case–control | <i>Source cohort:</i> see <a href="#">Schnatter et al. (1993)</a> cohort.<br><i>Cases:</i> 14 with leukaemia recorded on death certificate.<br><i>Controls:</i> 55; 4 controls per case, frequency-matched on decade of birth, alive at the time of case death.<br><i>Exposure assessment method:</i> See <a href="#">Schnatter et al. (1993)</a> .                                                                                                                                                                                                                          | <i>Reported in Table 2.3:</i><br>Leukaemia                                                                                                                                                                                                                                                                                                    | <i>Strengths:</i> Analysis restricted to participants exposed to hydrocarbons.<br><i>Limitations:</i> Small sample size; co-exposure to other potential occupational carcinogens was probable. No analysis by leukaemia subtype.                                                                                                                                                                                   |



Table 2.1 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                                                               | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                     | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <a href="#">Sorahan (2007)</a><br>Update of <a href="#">Rushton (1993a)</a><br>UK<br>Enrolment, 1946–1975/follow-up, 1951–2003 (mortality), 1971–2003 (incidence)<br>Cohort | 16 477 male workers in petroleum distribution centres first employed in 1946–1974 with $\geq 1$ yr employment and some employment after 1951.<br><i>Exposure assessment method:</i> Occupational cohort of oil refinery or petroleum distribution workers with a minimum of 12 mo of employment.<br><i>Exposure assessment method:</i> Exposure defined as ever employed as petroleum distribution worker. No information on duration or on specific exposures, but job titles were available. | <i>Reported in Table 2.3:</i><br>HL, NHL (lymphosarcoma), multiple myeloma, leukaemia (lymphoid, myeloid, monocytic), CLL, AML, ANLL<br><i>Reported in Table 2.4:</i><br>Kidney and other urinary system, urinary bladder<br><i>Reported in Table S2.5:</i><br>Oesophagus, stomach, small intestine, large intestine, rectum, liver, gallbladder, pancreas, peritoneum, nose and sinus, larynx, lung and bronchus, pleura<br><i>Reported in Table S2.6:</i><br>Male breast cancer<br><i>Reported in Table S2.7:</i><br>Melanoma, brain, thyroid gland, prostate gland, testes | <i>Exposure assessment critique:</i> Ever employment for $\geq 12$ mo was probably classified correctly. The exposure assessment was limited because it was only sensitive to exposure to petroleum products but was not specific to gasoline.<br><i>Design and analysis:</i><br><i>Strengths:</i> Large study population. Long follow-up period. Cancer incidence data were presented in addition to cancer mortality.<br><i>Limitations:</i> No information on lifestyle potential confounders or co-exposures. HWSB because of 1-yr exposure criteria.<br><i>Other comments:</i> A later cohort follow-up (1951–2011) focused only on MDS is reported ( <a href="#">Sorahan and Mohammed, 2016</a> ). Industry-sponsored. |
| <a href="#">Rushton and Romaniuk (1997)</a><br>UK (distribution workers cohort)<br>Enrolment, NR to 1975/follow-up, 1993 (incidence and mortality)<br>Nested case–control   | <i>Source cohort:</i> Male workers in petroleum distribution centres.<br><i>Cases:</i> 90 men who died from leukaemia or were registered with a diagnosis of leukaemia in the cancer register, which was available since 1971.<br><i>Controls:</i> 354; 4 controls for each case were randomly selected from the cohort, within an interval of 3 yr from the case's date of birth.<br><i>Exposure assessment method:</i> See <a href="#">Sorahan (2007)</a> .                                  | <i>Reported in Table 2.3:</i><br>NHL (CLL), leukaemia, leukaemia (lymphoid), AML and monocytic leukaemia, CML                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <i>Exposure assessment critique:</i> See <a href="#">Sorahan (2007)</a> .<br><i>Design and analysis:</i><br><i>Strengths:</i> Relatively large sample size; analysis by haematolymphoid malignancies.<br><i>Limitations:</i> Possible co-exposure with other occupational carcinogens and lack of control for occupational confounders.<br><i>Other comments:</i> The Working Group considered it informative to report, in addition to <a href="#">Sorahan (2007)</a> , results on duration of employment from this previous study on the same population.                                                                                                                                                                  |

Table 2.1 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Cancer types reported, and corresponding tables <sup>a</sup>                                | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <a href="#">Sorahan and Mohammed (2016)</a><br>Update of <a href="#">Rushton (1993b)</a><br>UK<br>1995–2011<br>(incidence)<br>Cohort | 16 467 workers in petroleum distribution centres, first employed in 1946–1974 with ≥ 1 yr employment and some employment after 1951. Incidence data on MDS were available from 1995 to 2011.<br><br><i>Exposure assessment method:</i> Occupational cohort of oil refinery or petroleum distribution workers with a minimum of 12 mo of employment. No information on duration or on specific exposures, but job titles were available.                                                                                                                                                                   | <i>Reported in Table 2.3:</i><br>MDS                                                        | <i>Exposure assessment critique:</i> Ever employment for ≥ 12 mo was probably classified correctly. The exposure assessment was limited because it was only sensitive to exposure to petroleum products but was not specific to gasoline.<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Relatively large sample size; analysis focused on a specific haematolymphoid malignancy.<br><br><i>Limitations:</i> Possible co-exposure with other occupational carcinogens and lack of control for occupational confounders.                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <a href="#">Wong et al. (1993)</a><br>USA<br>Enrolment 1946–1985/<br>follow-up through<br>30 June 1989<br>(mortality)<br>Cohort      | Gasoline distribution employees (≥ 1 yr) at land-based terminals or on marine vessels, (9026 land-based; 9109 marine) between 1946 and 1985. Outcome data from: Social Security Administration, Death Master File, National Death Index, participating companies.<br><br><i>Exposure assessment method:</i> Potential exposure to downstream gasoline identified on the basis of job titles, work location, and department recorded in company records. Local company contracts and a parallel exposure assessment study were used to identify job titles with potential exposure to downstream gasoline. | <i>Reported in Table S2.5:</i><br>Lung<br><br><i>Reported in Table S2.6:</i><br>Male breast | <i>Strengths:</i> Detailed employment history information, available industrial hygiene data, long-term employment, long period of follow-up.<br><br><i>Limitations:</i> Small number of male breast cancer deaths among workers with low exposure, and small numbers of other cancer deaths, fatal end-points, co-exposure to other hydrocarbons and crude oil, lack of data on other potential personal-level confounders. HWSB because of 1-yr exposure criteria.<br><br><i>Other comments:</i> Study sponsored by the American Petroleum Institute. The Working Group noted the overlap with <a href="#">Wong et al. (1999)</a> . Male breast cancer results were, however, only reported in <a href="#">Wong et al. (1993)</a> , for other cancer sites the updated cohort was used. Lung cancer analysis by latency and by duration of employment in <a href="#">Wong et al. (1993)</a> is additionally reported, as such analysis was absent in the 1999 publication. |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                                                                                                                        | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <a href="#">Wong et al. (1999)</a><br>(supersedes <a href="#">Wong et al., 1993</a> )<br>USA<br>Enrolment, 1946–1985/follow-up, NR (mortality)<br>Cohort and nested case–control | <p><i>Source cohort:</i> Marine vessel and land-based terminal petroleum distributors (see <a href="#">Wong et al., 1993</a>) exposed to gasoline.</p> <p><i>Cases:</i> 35 leukaemia, 13 AML, 11 multiple myeloma, 12 kidney; the nested case–control study was restricted to land-based workers; cases identified through underlying and contributory causes of death listed on death certificates (ICD-8).</p> <p><i>Controls:</i> 5 controls per case; individuals alive at the time of case death and either alive at the end of follow-up or dead from causes other than the cancers of interest (leukaemia, AML, multiple myeloma, kidney cancer). Matching on company, date of birth, sex.</p> <p><i>Exposure assessment method:</i> Job titles, work location, and department as recorded in company records. An exposure assessment study was used to identify gasoline exposure and estimate cumulative total hydrocarbon exposure (<a href="#">Smith et al., 1993</a>).</p> | <p><i>Reported in Table 2.3:</i><br/>Leukaemia, AML, multiple myeloma</p> <p><i>Reported in Table 2.4:</i><br/>Kidney</p> <p><i>Reported in Table S2.5:</i><br/>Oesophagus, stomach, large intestine, liver and biliary tract, pancreas, larynx, lung</p> <p><i>Reported in Table S2.7:</i><br/>Prostate gland, testes, brain and other CNS, thyroid and other endocrine glands</p> | <p><i>Strengths:</i> Quantitative exposure assessment, including dose–response analysis.</p> <p><i>Limitations:</i> Use of mortality rather than morbidity data, the lack of information on smoking and other risk factors. HWSB because of 1-yr exposure criteria.</p> <p><i>Other comments:</i> in addition to the nested case–control study, cohort analysis (SMR for various cancer) site is reported. Study sponsored by the American Petroleum Institute.</p>                                                                                          |
| <a href="#">Gun et al. (2006)</a><br>Petroleum distribution, Australia (Health Watch)<br>1980–2000 (incidence)<br>Cohort                                                         | <p>16 547 men and 1356 women; employees of Australian Institute of Petroleum companies enrolled through a survey interview in 1980–1983, 1985, and/or 2000. Mortality from National Death Index and incidence from National Cancer Statistics Clearing House (included cancer registries in various states except Victoria and South Australia, which were separately used for additional cancer identification).</p> <p><i>Exposure assessment method:</i> Tanker drivers employed by the Australian Institute of Petroleum identified through survey interview.</p>                                                                                                                                                                                                                                                                                                                                                                                                                  | <p><i>Reported in Table 2.4:</i><br/>Kidney</p>                                                                                                                                                                                                                                                                                                                                     | <p><i>Strengths:</i> Mortality and incidence data presented.</p> <p><i>Limitations:</i> Results reported for the entire cohort of petroleum industry workers (petroleum distribution workers not identified, with exception of the analysis for kidney cancer in tanker drivers). Exposure assessment for total hydrocarbon exposure, not limited to gasoline. A few results were presented for tanker drivers with probable exposure to gasoline and these were considered in the present evaluation.</p> <p><i>Other comments:</i> Industry-sponsored.</p> |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Cancer types reported, and corresponding tables <sup>a</sup>                | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <a href="#">Schnatter et al. (2012)</a><br>UK, Australia, Canada<br>Canada, 1964–1983;<br>UK, 1950–1997;<br>Australia, 1981–1999<br>(incidence)<br>Nested case–control | <p><i>Source cohort:</i> Male petroleum workers: 1-yr employment inclusion criteria for UK and Canada, 5 yr for Australia.</p> <p><i>Cases:</i> 370 cases of haematolymphoid malignancies (through mortality or cancer incidence registry). AML (<math>n = 60</math>), CLL (<math>n = 80</math>), CML (<math>n = 28</math>), MDS (<math>n = 29</math>), and MPD (<math>n = 30</math>).</p> <p><i>Controls:</i> 1587; cancer-free at case diagnosis, five age-matched (Australian study) or four age- and company-matched (Canadian and UK studies).</p> <p><i>Exposure assessment method:</i> Tanker drivers as identified in company records.</p> | Reported in <a href="#">Table 2.3</a> :<br>NHL (CLL), AML, MDS,<br>CML, MPD | <p><i>Design and analysis:</i></p> <p><i>Strengths:</i> Lymphatic and haematopoietic cancer subtypes and a large sample size.</p> <p><i>Limitations:</i> As the analysis was conducted internally in the cohort, workers in the reference category were also exposed to gasoline to a certain extent, with a potential bias towards the null of the OR estimates for tanker drivers. The analyses on tanker drivers were not adjusted for potential confounders, including smoking and other occupational co-exposures, introducing potential bias in an unpredictable direction.</p> <p><i>Other comments:</i> The study focused on exposure to benzene, most of its results were not considered for the present evaluation, including those on duration of employment because the Australian cohort was not restricted to distribution workers (and study-specific results were not presented). Only the analysis for “tanker drivers” was considered relevant for the gasoline evaluation as they were thought to represent a petroleum distribution population with probable exposure to gasoline.</p> <p>Overlap with the UK and Canadian distribution cohorts (<a href="#">Schnatter et al., 1996</a>; <a href="#">Sorahan, 2007</a>; <a href="#">Sorahan and Mohammed, 2016</a>).</p> |

Table 2.1 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                                                                                                                      | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                   | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
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| <a href="#">Lagorio et al. (1994a)</a><br>Gas station attendant cohort, Latium, Italy<br>Enrolment, 1980/ follow-up, 1981 to July 1991 (residents in Rome), June 1992 (residents in other municipalities)<br>(mortality)<br>Cohort | 2665 (including 357 women) from 3272 stations; service gas station managers in the Latium region, identified through a nationwide survey in 1980, who were alive as of 1 January 1981. Vital status through municipality registry of residence and cause of death through linkage with national or regional mortality registry, or through review of death certificates. Loss to follow-up, 6%.<br><br><i>Exposure assessment method:</i> A nationwide survey of Italian gas station managers that reported information on employment, and gas station characteristics were used to derive proxies for exposure. | <i>Reported in Table 2.3:</i><br>Lymphatic and haematopoietic, NHL, leukaemia<br><i>Reported in Table 2.4:</i><br>Kidney, urinary bladder<br><i>Reported in Table S2.5:</i><br>Oral/pharyngeal combined, oesophagus, stomach, colon, liver and bile ducts, pancreas, larynx, lung<br><i>Reported in Table S2.6:</i><br>Breast<br><i>Reported in Table S2.7:</i><br>Corpus uteri, prostate gland, CNS, melanoma | <i>Exposure assessment critique:</i> Ever exposure to gasoline as measured by proxy was likely to be sufficiently accurate, but there was no measure of intensity or duration of exposure to gasoline. A strength was the focus on smaller-sized stations with presumed higher levels of exposure.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Cohort members were probably involved in refuelling activities.<br><i>Limitations:</i> Relatively small sample size (100 expected cancer deaths) and short follow-up period; lack of control for confounders, including smoking and alcohol. |
| <a href="#">Alavanja et al. (2003)</a><br>Agricultural Health Study, Iowa and North Carolina, USA<br>1993–1999<br>(incidence and mortality)<br>Cohort                                                                              | 55 332 (566 prostate cancer cases); private and commercial pesticides applicator and female spouses enrolled in 1993–1997 through questionnaire. Cancer incidence through state cancer registries and vital status through National Death Index. Prevalent cases were excluded ( $n = 414$ ).<br><br><i>Exposure assessment method:</i> Self-reported gasoline exposure.                                                                                                                                                                                                                                         | <i>Reported in Table S2.7:</i><br>Prostate gland                                                                                                                                                                                                                                                                                                                                                               | <i>Strengths:</i> Prospective design, incident prostate cancer cases, comprehensive cancer registry data used to ascertain cases.<br><br><i>Limitations:</i> Short follow-up time, crude exposure assessment, gasoline exposure ascertained for nonfarm jobs in a subset of the main cohort completing the take-home questionnaire, small number of exposed cases.                                                                                                                                                                                                                                      |



**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                     | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
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| <a href="#">Patel et al. (2004)</a><br>Area of a gasoline spill, PA, USA<br>1990–2000<br>(incidence)<br>Cohort  | 663 people living for ≥ 6 mo (1990–2000) in the US EPA remediation area affected by the Tranguch gasoline spill. Within each household identified in the area, all individuals aged > 18 yr were interviewed. Cancer identified through self-reporting in the questionnaire (2001–2002, retrospective design), later validated through medical records. Participation rate, 63% (full questionnaire completion).<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a combination of ranges of air measurements, modelled estimates of groundwater contamination with benzene, and residence distance to the spill allowed to define classification for the potential of exposure (high, medium, and low). Number of persons and years of exposure were also calculated. | <i>Reported in Table 2.3:</i><br>NHL<br><i>Reported in Table 2.4:</i><br>Urinary bladder<br><i>Reported in Table S2.5:</i><br>Stomach, colon, lung<br><i>Reported in Table S2.6:</i><br>Female breast<br><i>Reported in Table S2.7:</i><br>Uterine cervix, prostate gland, brain | <i>Exposure assessment critique:</i> The basis of the three exposure categories of exposure was well defined. The use of distance as a proxy for exposure was likely to introduce nondifferential exposure.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Residents had lived in the area for a long time. Unique study of potential exposure to gasoline vapours in a community setting.<br><i>Limitations:</i> Small number of cancers. Although this study collected information on potential confounders such as smoking and chronic diseases, this information was not used in the analysis.<br><br><i>Other comments:</i> Later follow-up for leukaemia only in <a href="#">Talbot et al. (2011)</a> , hence results for leukaemia were reported only from <a href="#">Talbot et al. (2011)</a> . |
| <a href="#">Talbot et al. (2011)</a><br>Area of a gasoline spill, PA, USA<br>1990–2000<br>(incidence)<br>Cohort | 625 (living participants at interview); update of <a href="#">Patel et al. (2004)</a> , see <a href="#">Patel et al. (2004)</a> for cohort description. In this follow-up study, cancer cases identified through the PA cancer registry were added (validating those identified in the questionnaire and adding those not identified through the questionnaire).<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of a combination of ranges of air measurements, modelled estimates of groundwater contamination with benzene, and residence distance to the Tranguch gasoline spill were used to define classification for the potential of exposure (high, medium, and low). Number of persons and years of exposure were also calculated.                             | <i>Reported in Table 2.3:</i><br>Leukaemia, AML                                                                                                                                                                                                                                  | <i>Exposure assessment critique:</i> The basis from the three exposure categories of exposure was well defined. The use of distance as a proxy for exposure was likely to introduce nondifferential exposure.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Although historical data were limited, residents had resided in the area for a long time.<br><i>Limitations:</i> Small number of cancers.                                                                                                                                                                                                                                                                                                                                                                                                   |

Table 2.1 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                                                                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Cancer types reported, and corresponding tables <sup>a</sup>                                              | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
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| <a href="#">Neasham et al. (2011a)</a><br>EPIC cohort, Europe<br>Enrolment 1992–2000/<br>follow-up until 2006<br>(incidence)<br>Cohort                                                 | 218 968 men and women aged 35–70 yr at recruitment to the EPIC cohort; restricted to centres with information on occupational history (Denmark, Greece, Germany, Italy, Spain, and the UK). Follow-up through cancer registries, vital statistics (mortality), in Germany and Greece: a combination of methods including health insurance records, cancer and pathology registries, and active follow-up.<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of job titles. A standardized self-administered questionnaire was used to collect information on occupational history.                                                                                                                                                                                                                           | <i>Reported in Table 2.3:</i><br>NHL, NHL (excluding multiple myeloma/plasmacytoma), multiple myeloma, HL | <i>Exposure assessment critique:</i> The questionnaire was sensitive for the identification of employment as a gasoline station attendant, but there was no measure of intensity or duration of exposure to gasoline.<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Large cohort, with in total 13 cases of lymphatic and haematopoietic tumours among gasoline station attendants; adjustment for alcohol and smoking.<br><br><i>Limitations:</i> It was unknown whether the workers were exposed to refuelling activities; no results were available on neoplasms other than lymphatic and haematopoietic tumours.                                                             |
| <a href="#">Ekenga et al. (2015)</a><br>Sister Study, USA<br>Enrolment, 2004–<br>2009/follow-up, end<br>date NR, but reported<br>mean follow-up was<br>5.2 yr<br>(incidence)<br>Cohort | 47 640 women aged 35–54 yr and breast cancer-free at recruitment, with a sister with breast cancer diagnosis. Women who had never worked outside the home were excluded. Outcome data were self-reported through annual questionnaire. All self-reported cases were included in the analysis (98% agreement between self-reported cases and medical records, in participants whose medical records were obtained).<br><br><i>Exposure assessment method:</i> Self-reported information collected during a structured CATI. Participants were followed from the baseline interview until a breast cancer diagnosis, death, or end of follow-up. Participants provided detailed information about their lifetime exposure to specific agents and materials categories on the basis of quartile cut-points of cumulative lifetime exposure. | <i>Reported in Table S2.6:</i><br>Breast                                                                  | <i>Exposure assessment critique:</i> The study's reliance on self-reported data could lead to measurement error and inaccuracies. Exposure misclassification was likely to be nondifferential. Cumulative lifetime exposure estimates were a strength of this study.<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Large sample size.<br><br><i>Limitations:</i> Gasoline was grouped with “other petroleum products” in the exposure assessment. The time period of exposure was not ascertained, thus the lag between exposure and diagnosis was unknown. Breast cancer was obtained from self-report, with no additional linkage to cancer registries to obtain missed cases. |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Cancer types reported, and corresponding tables <sup>a</sup>                       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
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| <a href="#">Kirkeleit et al. (2018)</a><br>Norwegian Mother and Child Cohort (MoBa), Norway<br>Enrolment, 1999–2008/follow-up, through December 2014<br>(incidence)<br>Cohort | 113 754 children, 94 428 mothers, and 75 995 fathers; offspring cohort of the MoBa cohort which included Norwegian women who were pregnant in 1999–2008. All women in Norway at 17 wk of gestation were asked to participate (acceptance rate, 41%). Fathers were not asked to participate until recruitment had already begun; 87.3% were asked to participate and of those, 82.9% consented. Childhood cancer follow-up through linkage with Norway cancer registry.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of self-reported and parent-reported exposures to gasoline. Exposures were categorized on the basis of number of days of exposure. Information on probability of co-exposure to other carcinogenic agents was collected and excess risk ascribed for each. | <i>Reported in Table 2.2:</i><br>Childhood leukaemia (all subtypes), childhood ALL | <i>Exposure assessment critique:</i> It may be reasonable to assume a large proportion of women who reported exposure to gasoline or exhaust on the questionnaire had some exposure to gasoline. However, a limitation was that it is not possible to separate exposure to gasoline from exposure to exhaust, and self-reported number of days was a crude measure of cumulative exposure.<br><br>Prospectively collected exposure information, limiting recall bias, with any misclassification expected to be nondifferential and bias results to the null.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Prospective study.<br><br><i>Limitations:</i> Low participation rate in the cohort, and low prevalence of exposed mothers, causing low statistical power and imprecise risk estimates. Given the small sample size of cancers, the reliance on traditional statistical significance testing to choose relevant confounders yielded no confounders for adjustment. Smoking status (associated with childhood leukaemia) was missing for 60% of fathers. Informativeness was limited by not differentiating between gasoline and exhaust, with biases potentially going in either direction. |

**Table 2.1 (continued)**

| Reference, location, enrolment/follow-up period, study design                                              | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Cancer types reported, and corresponding tables <sup>a</sup>                                                                                                                                                                                                                                                                          | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
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| <a href="#">Siemiatycki et al. (1987a)</a><br>Montreal, Canada<br>1979–1985<br>(incidence)<br>Case–control | <i>Cases:</i> Multiple cancer sites; patients (men, aged 35–70 yr) with a cancer diagnosed at one of the 19 participating hospitals. Response rate, 82%.<br><i>Controls:</i> Other cancer sites as controls; Controls were selected from cases of other cancer types. No matching.<br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of occupational history. Experts estimated exposure to gasoline on the basis of the degree of exposure and the number of years of exposure. | <i>Reported in Table 2.3:</i><br>NHL<br><i>Reported in Table 2.4:</i><br>Kidney, urinary bladder<br><i>Reported in Table S2.5:</i><br>Stomach, colon, rectum, lung<br><i>Reported in Table S2.7:</i><br>Prostate gland<br>Melanoma<br>Stomach<br>Lung<br>Oral/pharyngeal combined<br>Hypopharynx<br>Larynx<br>Oral cavity, oropharynx | <i>Exposure assessment critique:</i> Semiquantitative assessment on the basis of several occupations, including mechanics, service station attendants, and agricultural workers. A limitation was that a variety of occupations, without assessed co-exposures, contributed to estimated gasoline exposure.<br><i>Design and analysis:</i><br><i>Strengths:</i> Analysis of incident cases.<br><i>Limitations:</i> Small numbers of included and exposed cases.<br><i>Other comments:</i> In total, 462 participants (12.4%) were coded as having any exposure to automotive gasoline. The percentage coded as exposed with high confidence was 10.4%, with high frequency, 2.3% (relative) high concentration 3.9%, and with > 20 yr exposure, 4.4%. Additional details from <a href="#">Siemiatycki et al. (1987b)</a> . |

AML, acute myeloid leukaemia; ANLL, acute non-lymphoblastic leukaemia; CATI, computer-assisted telephone interview; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CNS, central nervous system; EPIC, European Prospective Investigation into Cancer; FINJEM, Finnish Job-Exposure Matrix; HL, Hodgkin lymphoma; HWSB, healthy-worker survivor bias; LA, Los Angeles; ICD, International Classification of Diseases; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; mo, month(s); MoBa, Norwegian Mother and Child Cohort; MPD, myeloproliferative disorder; NHL, non-Hodgkin lymphoma; NOCCA-DANJEM, Nordic Occupational Cancer Study Job Exposure Matrix for Denmark; NR, not reported; OR, odds ratio; PA, Pennsylvania; PIR, proportionate incidence ratio; SES, socioeconomic status; SMR, standardized mortality ratio; SPF, Supplementary Pension Fund; UK, United Kingdom; USA, United States of America; US EPA, United States Environmental Protection Agency; vs, versus; wk, week(s); yr, year(s).

<sup>a</sup>The supplementary tables are presented in Annex 2 (Supplementary tables for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>).

because of considerable overlap with a larger pooled study by [Lynge et al. \(1997\)](#), which had better identification of gasoline station attendants.

Among those record-linkage studies that were relevant for the assessment of automotive gasoline exposure, the earliest was published by [Howe and Lindsay \(1983\)](#). This was a cohort established using a 10% sample of the Canadian labour force, which collected data on sex, year of birth, and current occupation and industry from all employers in Canada in 1965–1969. Census industry and occupation codes were used to describe the individual's occupational history ([Howe and Lindsay, 1983](#)). The final cohort comprised 415 201 men followed for mortality from 1965–1973. Women were excluded because of the small number of deaths during the follow-up period. Although none of the 12 occupational divisions listed was relevant for the evaluation of automotive gasoline, specific occupations were listed on a three-digit level and included service station attendants. Standardized mortality ratios (SMRs) were computed based on death rates for both the Canadian population and the complete occupational cohort. Only an overall SMR for the cancer types in excess was reported. [The Working Group noted the short follow-up time with low number of deaths, and that the grouping by industry and occupation was crude, no information being provided on employment duration. The study was considered to be less informative for the present evaluation of automotive gasoline.]

The majority of the other record-linkage studies were conducted in the Nordic countries. The unique personal identification number introduced in the 1960s for all individuals born, residing in, or working in the Nordic countries enables the linkage of data from various high-quality nationwide registries with information at an individual level, including on demographics and occupation from national population censuses, cases of incident cancer from the

national cancer registries, and mortality from cause-of-death registries. Also, mandatory registration in the nationwide registries means that individuals cannot opt out, preventing any voluntary dropouts. However, in general there is likely to be nondifferential misclassification of exposure status, since most of the record-linkage studies are based on national population censuses in which the occupational categories are broad and defined on the census day, with no information on duration of or shift in occupation in between censuses, and do not differentiate between specific jobs and occupational exposures within a job. Finally, most often there was a lack of individual-level data on lifestyle factors.

[Norell et al. \(1986\)](#) conducted a cohort study using linkage of the 1960 census in Sweden, comprising approximately 2 million men with information on age, sex, county, and branch of industry, including work in petrol stations. Cases of incident pancreatic cancer were retained from the Swedish Cancer Register for follow-up in 1961–1979. The reference population was employed men in the same population. A similar design was followed in studies by [McLaughlin et al. \(1987\)](#) and [Malker et al. \(1987\)](#), which were also based on 1960 census data from Sweden and reported on risk for cancers of the kidney and urinary bladder, respectively, during follow-up from 1961 to 1979. [The Working Group noted that this study complemented a later study of service station workers by [Lynge et al. \(1997\)](#), described below, and found it to offer some additional informational value regarding gasoline station attendants because it was conducted before the introduction of self-service in gasoline stations in Sweden and included cases that were deceased before the 1970 census used by [Lynge et al. \(1997\)](#).]

[Lynge et al. \(1997\)](#) established a cohort of 19 000 service station workers identified from the 1970 population and housing censuses in Denmark, Norway, Sweden, and Finland. The cohort of 16 524 men and 2 445 women was



followed through a period of 15–20 years (1970–1991, depending on the country), resulting in 288 533 and 42 889 person-years, respectively. Service station workers were identified by a combination of codes for self-reported industry (International Standard Industrial Classification of All Economic Activities, ISIC 1958) and occupation (mainly International Classification of Occupations, ISCO 58). The cohort of service station workers comprised a combination of self-employed and salaried employees, as well as family workers. Incident cancer cases were ascertained by linkage with the national cancer registries, using the International Classification of Diseases 7th edition (ICD-7). By the end of follow-up, 1309 incident cancer cases had been observed. Standardized incidence ratios (SIRs) were estimated using national incidence rates for comparison (total, census, and economically active in census, depending on the country). [The Working Group noted the large sample size and the use of incidence rather than mortality outcomes as strengths. Limitations included categorization as a gasoline station filling attendant on the basis of occupation and industry from the 1970 census, hence inferring exposure from a single point in time and not permitting an assessment of cumulative exposure. Also, transition to self-service fuelling in the Nordic countries began in the 1970s and into the 1980s, introducing uncertainty regarding temporal patterns of exposure to gasoline vapour. No information on potential confounders (e.g. smoking and alcohol consumption) or co-exposure to other occupational carcinogens was provided. However, the authors stated that the service station workers were not likely to smoke more than the general population and referred to an unpublished survey of men in Sweden, conducted in 1963, in which 51% of service station workers reported being smokers compared with 53% of all other men.]

Data from the Swedish censuses in 1960 and 1970, including job title information, were linked to the Swedish cancer registry to facilitate occupational cancer studies. Two record-linkage studies ([Aragonés et al., 2002](#); [Navas-Acién et al., 2002](#)) were considered to add complementary information to the study by [Lyngé et al. \(1997\)](#). Both studies ([Aragonés et al., 2002](#); [Navas-Acién et al., 2002](#)) included all Swedish individuals who were employed at the time of the 1970 census, had been recorded in the 1960 census, and were still alive and in the age range 25–59 years on 1 January 1971. The two studies included 1 779 646 men (the studies also included women, but no analysis for filling station attendants was presented for the women in the cohort). [Aragonés et al. \(2002\)](#) identified 8763 cases of stomach cancer among men in the study population within the follow-up period (1971 through 1989). Within the same follow-up period, [Navas-Acién et al. \(2002\)](#) identified 3363 gliomas and 1166 meningiomas in men. [The Working Group noted the use of high-quality national population-based registers, use of incident outcomes, and a long follow-up period. The Working Group further noted that categorization as a gas station filling attendant was based on occupation and industry from the 1960 and 1970 censuses, hence inferring exposure from a single point in time and not permitting an assessment of cumulative exposure. However, for both studies, the Working Group noted that at the time of the censuses it was likely that the gasoline station attendants were exposed to gasoline during car refuelling since this was before self-service by the customer became normal practice. The exposure misclassification including lack of duration of exposure (years of employment) and failure to identify the most highly exposed participants may have resulted in biased risk estimates towards the null. Despite overlap with the study by [Lyngé et al. \(1997\)](#), the Working Group considered these studies to be informative because they included additional 1960 census data and comparisons

with other occupations, and the results for brain subtype risk were reported only by [Navas-Acién et al. \(2002\)](#).]

Eight census-based record-linkage studies in Nordic countries have focused on specific industries and/or occupations with relevance for exposure to automotive gasoline, and they applied the FINJEM for a more detailed exposure assessment that included gasoline as one of 43 occupational chemical exposures ([Vasama-Neuvonen et al., 1999](#); [Weiderpass et al., 1999, 2003](#); [Wesseling et al., 2002](#); [Guo et al., 2005](#); [Lohi et al., 2008](#); [Tarvainen et al., 2008](#); [Lindbohm et al., 2009](#)). The FINJEM encompasses chemical, biological, physical, physiological, ergonomic, and psychosocial factors for 311 occupations during the period 1945–1997, which for the chemical factors is divided into the subperiods 1945–1959, 1960–1984, 1985–1994, and 1995–1997 ([Kauppinen et al., 1998](#)). The FINJEM is primarily based on two key metrics – proportions of the exposed by occupation (P, in percentage) and annual level (L) of exposure among the exposed persons for the respective occupational agents. For gasoline, the unit was given in parts per million (ppm) of benzene. P and L can be combined to calculate cumulative exposure ( $P \times L \times \text{duration of exposure}$ ), enabling the estimation of an exposure–response relation. The period-specific estimates allowed for the assessment of changes over time, whereas the wide range of exposures enabled the consideration of co-exposures. In the FINJEM, the estimated number of workers occupationally exposed to gasoline in Finland was 11 000 (0.5% of those employed), with a mean level of exposure of 0.15 ppm benzene in 1995–1997 ([Kauppinen et al., 1998](#)). The FINJEM contains occupation-specific estimates for smoking (proportion of daily smokers), alcohol consumption (average in grams per week), obesity (average body mass index, BMI), and dietary fat (proportion of regular butter users) ([Pukkala et al., 2005](#)). These data originate from annual surveys on health behaviour of the Finnish adult population carried out

by the Finnish National Public Health Institute during 1978–1991.

A cohort of people born in 1906–1945 (approximately 1.2 to 1.6 million participants, depending on the study) who participated in the national census in Finland in 1970 was used to assess the association between occupation and risk of cancer of the urinary bladder (10 277 cases) and kidney (9954 cases) ([Lohi et al., 2008](#)), mouth and pharynx (2708 cases) ([Tarvainen et al., 2008](#)), and liver (2474 cases) ([Lindbohm et al., 2009](#)), respectively. Occupation held as of 1970 was used to categorize the individuals into 311 occupational categories that were further divided into two to nine industrial subcategories, whereafter the FINJEM ([Kauppinen et al., 1998](#); [Pukkala et al., 2005](#)) could be applied. Since occupational histories were not available, exposure for each 5-year birth cohort was assumed to start in the year when the average age of the birth cohort was 20 years and to end at the earlier of the midyear of the observation period or at age 65 years. Cumulative exposure for the respective agents was a product of the duration of exposure reported by the participants, and the prevalence and level of exposure retrieved from the FINJEM. One of the exposure metrics used was annual average exposure (high, middle and low, none) to gasoline for the periods 1945–1959 and 1960–1984. Cases of incident cancer were ascertained through the Finnish Cancer Registry (follow-up, 1971–1995).

Using the same method for sampling of study population and exposure assessment as described above, four studies of economically active women and one study of men participating in the 1970 census in Finland have been conducted to investigate the association between occupational exposure and risk of ovarian cancer (892 591 women, 5072 cases) ([Vasama-Neuvonen et al., 1999](#)), breast cancer (892 591 women, 23 638 cases) ([Weiderpass et al., 1999](#)), cancer of brain and nervous system (413 877 women, 693 cases) ([Wesseling et al., 2002](#)), gastrointestinal cancer

(413 877 women, 1881 cases) ([Weiderpass et al., 2003](#)), and testicular cancer (667 121 men; 387 cases) ([Guo et al., 2005](#)). Adjustment of estimates according to social class differed between the studies: socioeconomic status based on participant or spouse's occupation (upper white-collar [workers who generally have a managerial or administrative role]; clerical; skilled blue-collar [workers who generally perform skilled manual tasks]; unskilled workers; and farmers) ([Guo et al., 2005](#); [Tarvainen et al., 2008](#); [Lindbohm et al., 2009](#)); social class (four categories based on occupation, education, and industrial status) ([Vasama-Neuvonen et al., 1999](#); [Weiderpass et al., 1999](#); [Lohi et al., 2008](#)); and by analysing only a subsample of the population (industry and service sector) ([Wesseling et al., 2002](#); [Weiderpass et al., 2003](#)). In some of the studies ([Weiderpass et al., 2003](#); [Lohi et al., 2008](#); [Tarvainen et al., 2008](#); [Lindbohm et al., 2009](#)), the risk estimates were adjusted for smoking (proportion of daily smokers) and obesity (average BMI) (only in [Lohi et al., 2008](#)) on an occupational aggregate level that was based on an annual survey on health behaviour of the adult population of Finland during 1978–1991 (non-gender-specific). [The Working Group noted that, in addition to the strengths of the Nordic record-linkage studies described above, a notable strength was the comprehensive coverage for both genders, allowing for reliable risk estimates for female workers as well as male. Also, use of the FINJEM (which is not gender-specific) allowed estimation of gasoline exposure, in addition to providing some contrast in cumulative exposure across occupational groups. Inclusion of multiple co-exposures in the analysis of the association between gasoline and the various cancer types was not performed for any of the studies assessing the estimate of gasoline exposure using FINJEM. However, the Working Group also noted that the occupational categories used were crude and were assigned average exposures, resulting in potential nondifferential misclassification of

exposure because of the inability of FINJEM to account for exposure variability within occupations, biasing the effect estimate towards null. Furthermore, since occupational history before and after the census was missing, the same occupation was imputed backward and forward in time, assuming a stable work population. Overall, nondifferential misclassification would generally bias the estimates towards the null. Finally, as with most other register-based studies, there was a lack of individual-level data on lifestyle factors and co-exposure to other carcinogenic agents, which hampered confounder control, potentially leading to false associations. However, although such data were not available on an individual level, some of the studies used FINJEM data on smoking and obesity at the group level, by occupation. Adjusting risk estimates for lifestyle factors at this level can introduce biases, potentially distorting the true relationship between risk factors and outcomes. For example, if an occupation has a high prevalence of smoking, adjusting on the basis of group data may inaccurately reflect the risk for non-smokers exposed to gasoline in that occupation.]

Also based on the Nordic censuses is the NOCCA, a collaborative project focused on occupation-specific cancer risk in Denmark, Finland, Iceland, Norway, and Sweden ([Pukkala et al., 2009](#); preceded by [Andersen et al., 1999](#)). NOCCA includes data on 14.9 million people born between 1896 and 1960, who were aged 30–64 years during the population and housing censuses in 1960, 1970, 1980/1981, and/or 1990. The participants have been followed in the respective national cancer registries for up to 45 years (1961–2005) and the data include 2.8 million cases of incident cancer. In the study by [Pukkala et al. \(2009\)](#), participants were classified by occupation reported at the first census in which they participated, whereafter the occupations were converted to 53 occupational categories (ISCO-58) and one category of economically inactive people. Information on lifestyle factors on an

occupational level was available for Finland (see above), but the cohort does not contain information on potential confounders on an individual level (lifestyle factors and co-exposure to carcinogenic agents). The estimates are standardized according to country, age, sex, and time period. [From the description of the 53 occupational categories, the Working Group considered that none of the occupational categories in the study by [Pukkala et al. \(2009\)](#) was informative individually for the evaluation of automotive gasoline. Later analyses using the NOCCA cohort were also available ([Talibov et al., 2019](#), described below) and were considered more informative for the present evaluation because the NOCCA-JEM was used, rather than crude exposure to occupational categories.]

The NOCCA-JEM was developed from the FINJEM described above ([Kauppinen et al., 2009](#)) and provides country-specific occupational exposure estimates for 24 chemical factors, including gasoline (in parts per million of benzene), across four time periods (1945–1959, 1960–1974, 1975–1984, and 1985–1994). As for FINJEM, the NOCCA-JEM assigns prevalence of exposure (P, in percentage) and annual average level of exposure (L) among the exposed people for the occupational agents, including gasoline, for 311 occupational groups. Occupational groups found to be exposed to gasoline were service station attendants, printers, occupation in graphics, and machine and engine mechanics, but the product of P and L ( $P \times L$ ) was highly dependent on the time period, being the highest for service station attendants in the earlier period ([Talibov et al., 2019](#)). [Talibov et al. \(2019\)](#) conducted a case-control study nested within the NOCCA study ([Pukkala et al., 2009](#)), using the NOCCA-JEM ([Kauppinen et al., 2009](#)) and assessing the association between occupational exposure to gasoline and risk of colorectal cancer. A total of 181 709 cases of colon cancer and 109 227 cases of rectal cancer diagnosed between 1961 and 2005 (in Finland, Iceland, Sweden, and Norway) were

matched on country, birth year, and sex. In the exposure assessment,  $P \times L$  for gasoline exposure was assigned to each year over the duration of the employment period of the study participants, after which these product terms were summed to estimate cumulative exposures ([Talibov et al., 2019](#)). [The Working Group noted the large number of participants and cases, the accuracy and completeness of the incidence data, the long follow-up time enabling assessment of long-term exposures and latency periods of cancer, the harmonized occupational data retrieved from nationwide census records, and the use of a country-specific JEM as strengths. The NOCCA-JEM allowed estimation of gasoline exposure, in addition to providing some contrast in the cumulative exposure, across occupational groups. Another strength was the complete coverage of the population for both sexes, enabling reliable risk estimates for female workers as well as male (non-gender-specific JEMs). However, the Working Group also noted that the occupational categories used were crude and were assigned an average exposure, resulting in a potential misclassification of exposure because of the inability of the NOCCA-JEM to account for exposure variability within occupations. The estimated cumulative exposure was further based on the occupations reported on the available census dates (one to four), lacking information on when the participants changed occupation and exposure status between the censuses, causing further misclassification, biasing the effect estimate towards the null. The lack of exposure data on an individual level and lack of data on temporal changes limited causal inferences. The Working Group noted that gasoline was included in the JEM using the proxy benzene (ppm) because of the high correlation between the two, for most occupations. Using benzene as a proxy for gasoline exposure when estimating risk of cancer could lead to biased risk estimates, depending on cancer type. For cancer types for which benzene is a known risk factor (e.g. leukaemia),



an overestimation of risk is likely, particularly if workers involved in printing were using benzene for cleaning. Furthermore, the benzene content of gasoline has decreased over the years, and it is likely that the correlation between gasoline exposure and benzene exposure has decreased with time. This might lead to a misclassification of exposure over time. The Working Group also noted that benzene would not be a good proxy for gasoline exposures among printers in the early time periods, because benzene was used to clean ink presses during the early time period. Finally, as for most other record-linkage studies, there was a lack of information on lifestyle factors and co-exposure to other carcinogenic agents on an individual level, reducing the possibility for confounder control, generating potential bias that could create a false association or mask a true association.]

[Heineman et al. \(1992\)](#) conducted a nested registry-based case-control study on incident multiple myeloma identified from the Danish Cancer Registry among men diagnosed between 1970 and 1984. For each case, four controls were sampled randomly from the population registry, matching on sex and year of birth. Cases and controls with a previous diagnosis of a malignancy were excluded. Information on the occupational history for each study participant was obtained from the Supplementary Pension Fund, which since 1964 has registered all wage-earners aged 16–66 years who were employed for  $\geq 10$  hours per week by any one employer. The linkage was successful in identifying information on either occupation or industry for 90% of cases and 85% of controls. Information on type of industry was available for any job from 1964 onwards, and information on the most recent job title (occupation) was available from the participant's most recent tax record. Using this information, four industrial hygienists created a JEM (none, possible, probable, or unknown) for 20 categories of substances and 27 specific substances, including gasoline, as well

as duration ( $< 5$  years and  $\geq 5$  years). Analyses on occupations and industries were restricted to cases and controls with entries for the corresponding variable. None of the analysed specific occupations was of relevance for the present evaluation. The total number of cases and controls for which information was available on whether they were exposed to gasoline was not explicitly reported in the article. Information allowed for adjustment by occupational exposure to engine exhausts, phthalates, and vinyl chloride. [The Working Group noted as a strength that this study was based on occupational histories obtained through record-linkage and expert exposure assessment. Further strengths included the study's focus on a specific haematolymphoid malignancy, namely multiple myeloma; it was little affected by non-participation; considered a 10-year latency in the analysis; and had a large sample size. Information on the type of industry in which the participants were employed was not available before 1964 and, for occupations, the information was less complete because only the job title recorded in the most recent tax record was considered, implying that earlier jobs could not be considered. This could lead to nondifferential misclassification of the exposure, with potential dilution of the associations.]

[Hansen \(2000\)](#) linked the Danish national pension fund and Danish Cancer Registry for a nested case-control study on male breast cancer incidence among workers exposed to gasoline and vehicular combustion products as a proxy for PAHs. The cases consisted of 230 male workers (born between 1897 and 1966) and diagnosed between 1970 and 1989. For each case, 56 controls (alive and free of cancer at date of cancer diagnosis) were randomly selected and matched on birth year (total, 12 880). Controls were drawn from members of the national pension fund. The occupational history of the cases and controls (dates of start and end of employment, company, and a five-digit industry code) were ascertained from the pension fund. The last job title was used



for categorizing the participants into five socioeconomic groups and finally into white- and blue-collar workers. Exposed individuals were defined as: a blue-collar worker with  $\geq 3$  months of employment within the trade codes service station, vehicle maintenance, wholesale trade of gasoline, or car repair shops. The risk estimates for breast cancer were adjusted for birth year and socioeconomic status (white- and blue-collar workers), but did not control for other potential confounders, such as alcohol consumption. [The Working Group noted that, although an effort was made to restrict the exposed group to workers with probable exposure to gasoline vapour and combustion products as a proxy for PAHs, the design did not allow for distinguishing the two exposures. Also, the number of exposed cases was low, and there was a lack of information on lifestyle factors (e.g. alcohol consumption, obesity). However, since male breast cancer is rare, the Working Group considered that the study was nevertheless informative for the present evaluation.]

[Pedersen and Hansen \(2023\)](#) conducted a register-based nested case–control study of parental occupational exposure and breast cancer risk among female offspring. Each of 5587 cases of incident breast cancer identified using the Danish Cancer Registry and with information on maternal or paternal occupational history was matched on sex and year of birth to 20 female cancer-free controls using the Danish Civil Registration System. Cases born after 1965 were included in the study, because information on parental employment was accessible from 1964 through 2014, and were followed until 2016. As such, cases were aged up to 51 years in the study. Information on estrogen receptor status was available for 82% of cases. Industry code was used to link maternal and paternal employment with the Danish version of the NOCCA-JEM to obtain probability and mean intensity level information over the four different periods for gasoline, as well as the other specific

occupational chemical exposures included in the study. The most common industries in which mothers might be exposed to gasoline included automobile and motorcycle businesses, manufacture of other machinery, and iron shipyards. The most common industries in which fathers might be exposed to gasoline included automobile and motorcycle businesses, manufacture of other machinery, and forge- and machine-repair workshops. A total of 3.4% of case participants and 3.2% of control participants had mothers who had ever been occupationally exposed to gasoline, and 15.2% of cases and 15.3% of controls had fathers who had ever been occupationally exposed. Occupational parental exposure to gasoline among the offspring ranged from 10.9% to 11.1% for cases and 11.7% to 12.1% for controls. [The Working Group noted that this was a large-scale study based on high-quality registry data, with detailed information on parental occupational history in specific time windows of exposure, as well as data on estrogen receptor status of disease. Limitations included the lack of information on personal lifestyle and reproductive factors, occupational information before 1964, parental leave, and potential changes in work tasks; and that the JEM was not gender-specific.]

### *2.1.2 Proportionate incidence ratio study on brain cancer, Los Angeles County, USA*

See [Table 2.1](#).

[Preston-Martin \(1989\)](#) examined occupation and industry groups among all cases of primary tumours of the brain, cranial nerves, and meninges in White men aged 25–64 years, in Los Angeles County, USA, in 1972–1985, based on the Cancer Surveillance Program register. Reporting of benign and malignant tumours was estimated to be 95% complete. Information on occupation and industry at the time of diagnosis was abstracted from hospital charts. More than 90% of cases were histologically confirmed. Age-adjusted proportionate incidence ratios

(PIRs) were calculated by selected occupations, including garage and gas station attendants, to provide the ratio of the number of observed cases to that expected based on that of the population of the USA in 1970. A total of 59.4% of cases were gliomas and 19.7% were meningiomas. [The Working Group noted that occupational data, based on occupational title, were captured at the time of diagnosis only.]

### 2.1.3 Petroleum distribution cohorts

See [Table 2.1](#).

[Schnatter et al. \(1993\)](#) conducted an industry-funded examination of the mortality experience of petroleum marketing and distribution workers at a petroleum company in Canada; this study superseded a previous study by [Schnatter et al. \(1992\)](#) that covered a more diverse range of workers. The latter study population consisted of all active employees and living retirees on 1 January 1964 and all new employees hired between 1 January 1964 and 31 December 1983. One year of work in the marketing or distribution operating segment of the company was necessary for a worker to be included in the cohort, and women were excluded because few were exposed to finished petroleum products; 6672 male workers were thus included in the cohort. Using work history records, industrial hygienists assigned each job entry a frequency of exposure to hydrocarbons classified as “daily exposed”, “exposed less than daily” and “non-exposed”. Information on cause of death from 1964 through 1983 was obtained from Statistics Canada as well as the United States National Death Index, with an estimated 2.4% underascertainment. SMRs were calculated by comparing the observed number of cause-specific deaths with the expected number based on Canadian population mortality rates. Results were presented according to hydrocarbon exposure frequency, length of employment, latency years, and for the group of tank truck drivers.

Poisson regression analyses were also performed to examine the effect of hydrocarbon exposure frequency on the risk of death from selected causes of cancer. This cohort was the basis of several additional analyses, including a nested case-control study on leukaemia ([Schnatter et al., 1996](#)), which was considered additionally to the estimations presented in [Schnatter et al. \(1993\)](#) for the continuous analysis by year of employment.

[The Working Group noted several strengths of this study, including information on work history and assessment of hydrocarbon exposure frequency. The use of Poisson regression analysis for some cancer outcomes, including leukaemia, to eliminate the healthy-worker bias expected in SMR analyses, was also a strength, although these analyses were hampered by small numbers. The main limitations included the reliance on mortality rather than morbidity data (which may be particularly important for cancers with a high survival rate, such as cancer of the urinary bladder), the lack of information on hydrocarbon exposure intensity, the lack of complete work histories for 50% of the workers, and potential bias from left truncation (also known as “prevalent exposure”, which occurs when follow-up begins after exposure has begun; [Berrington de González et al., 2024b](#)) of the cohort that may have induced downward bias. No information on non-occupational factors was available.]

[Sorahan \(2007\)](#) investigated mortality and cancer incidence in an industry-sponsored cohort study of male workers employed in eight oil refineries and 476 petroleum distribution centres in the UK. The cohorts were re-defined from those initially presented by [Rushton and Alderson \(1981, 1983\)](#) and included a further 5 years of mortality data and previously unreported data on cancer incidence. The cohort of petroleum distribution workers, presumed to distribute automotive gasoline (i.e. the cohort of principal interest in the present monograph), consisted of 16 477 men first employed

in the period 1946–1974. All participants had a minimum of 12 months employment (1946–1974) with some employment after 1 January 1951. The observed numbers of cause-specific deaths from 1951 to 2003 and site-specific cancer registrations from 1971 to 2003 were compared with expected numbers based on national mortality and cancer incidence rates in the UK. The SMRs were additionally presented by period of commencing employment, year of hire, and occupation for all causes of death, and for cancers of the pleura, leukaemia and acute non-lymphocytic leukaemia (ANLL) only. Standardized registration ratios (SRRs) for myelodysplastic syndrome (MDS) were presented in a separate publication in which follow-up was extended to 2011 ([Sorahan and Mohammed, 2016](#)). Additionally, the Working Group considered the continuous analysis per year of employment duration presented within the case-control study on chronic lymphocytic leukaemia (CLL) and leukaemia (including acute myeloid leukaemia, AML; monocytic leukaemia; and chronic myeloid leukaemia, CML) nested in the UK distribution workers cohort ([Rushton and Romaniuk, 1997](#)) [The Working Group noted the large size of the cohort of petroleum distribution workers and the long follow-up period, which allowed the calculation of reasonably precise risk estimates for the most frequent cancers. The availability of data on morbidity, in addition to mortality, was also a valuable feature, particularly for cancers that have high survival rates. Although risk estimates by occupation, duration of employment, or latency period were provided for a few cancers, no such information was available for the most frequent cancer sites. The Working Group considered that the study was prone to the healthy-worker effect (HWE), and some healthy-worker survivor bias (HWSB) because of the restriction to employees with 1 year of employment, which was consistent with the lower SMR for all-cause mortality in distribution workers.]

[Wong et al. \(1993\)](#) formed an industry-funded retrospective cohort of 18 135 primarily (98.3%) male marketing and marine distribution workers from four companies who had potential exposure to vapour from gasoline, which was presumed to be automotive gasoline. The study examined potential associations between occupational exposure to gasoline vapour and mortality from kidney cancer or leukaemia. Mortality from multiple myeloma, other cancers, and other causes of death was of secondary interest. Workers at either land-based terminals ( $n=9026$ ) or on marine vessels ( $n=9109$ ) from 1946 to 1985 who were primarily exposed to gasoline vapour were included and were followed for mortality until 30 June 1989. Workers were identified from employment records, which included information on job titles and processes. Attempts to verify cohort completeness for more recent time periods were made through related company files and contacts. [Wong et al. \(1999\)](#) subsequently reported results for the cohort analysis (the end of follow-up was not reported but appeared likely to coincide with that for the analysis by [Wong et al., 1993](#)), as well as for a nested case-control study of mortality from leukaemia, AML, multiple myeloma, and kidney cancer and used more refined job categories and exposure estimates among land-based workers only. [Given the overlap between these studies, the Working Group decided to consider the cohort and nested case-control results from the study by [Wong et al. \(1999\)](#) for leukaemia, AML, multiple myeloma, and kidney cancer, and the results from the study by [Wong et al. \(1993\)](#) for male breast cancer and, for lung cancer, the analysis by duration of exposure and latency (since these were not covered by [Wong et al., 1999](#)). The Working Group noted that details regarding the extended analysis and follow-up of the cohort were not specified.] Detailed information on the exposure estimation used by [Wong et al. \(1993\)](#) was provided by [Smith et al. \(1993\)](#). Company contacts identified job titles and locations with

potential exposure to gasoline in the distribution chain. The exposure definition included workers with potential exposure to gasoline vapour for  $\geq 1$  year, including displaced vapour through tasks involving loading and offloading of gasoline, evaporation from open liquids from spills or open tanks, and contamination of work clothing through contact or splashes.

In contrast to exposure estimates for earlier time periods, those for the later part of the study (mid-1970s or later) were based on measurement data. Using exposure measurements collected by the four companies (1975–1985) and information on historical working conditions, time-weighted average (TWA) exposures were assigned to the job categories over four time periods (before 1950, 1950–1964, 1965–1974, and 1975–1986). For marine distribution workers, two job categories were created (deck and non-deck), with TWA exposures by time period (before 1980 and 1980–1985) assigned for deck personnel on inland waterway barges only. In the nested case-control studies, 5 controls (who were alive at the time of case death) were individually matched to each case on company, date of birth, and sex. For the nested case-control component in [Wong et al. \(1999\)](#), the analysis was performed by job category as well as by quantitative exposure estimates, including cumulative exposure (ppm-years of exposure to total hydrocarbons or benzene), peak exposure (cumulative frequency of peak exposure to total hydrocarbons, defined as an episode of exposure greater than 500 ppm for 15–90 minutes), duration of exposure, duration of employment, and year of first exposure (1948 or earlier versus 1949 or later) ([Wong et al., 1999](#)).

Vital status was determined through national social security and mortality databases as well as via the companies themselves. Underlying and contributing causes of death from death certificates were coded by a trained nosologist according to the International Classification of Diseases 8th revision (ICD-8). SMRs were

calculated in comparison to the general population of the USA. In addition, to compensate for a potential HWE, internal comparisons were conducted through univariable and multivariable conditional logistic regression models within the nested case-control studies. In the nested case-control studies, 5 controls (who were alive at the time of case death) were individually matched to each case on company, date of birth, and sex ([Wong et al., 1999](#)). Deaths for which death certificates were not available were redistributed according to cause-specific proportions among certificates with deaths registered. There was a total of 1081 deaths from all malignant neoplasms, as well as 35 deaths from leukaemia (all cell types), 11 deaths from multiple myeloma, and 12 deaths from kidney cancer ([Wong et al., 1999](#)). The mean duration of exposure was  $> 20$  years among cases and controls.

[The Working Group noted as strengths the detailed information on employment history and exposure metrics constructed using available data on industrial hygiene, as well as the long-term employment of included workers and long follow-up. Limitations included potential HWEs in the external comparison analysis because the cohort was restricted to those with  $\geq 12$  months of employment ([Dement et al., 1997](#)). There were also small numbers of deaths for several cancer sites and reliance on fatal rather than incident end-points. Although the cohorts were assumed to be primarily exposed to gasoline vapour, workers may also have been exposed to other hydrocarbons (e.g. crude oil for marine employees), and potential differential or nondifferential misclassification may be of concern. Although some information was available on patterns of smoking habits, alcohol consumption, and socioeconomic status on a group level, the study lacked such data on an individual level. The lack of information on exposure to other potential carcinogenic agents, as well as lifestyle factors, could potentially create false associations, masking true effects or exaggerating non-existent ones.]



[Gunes et al. \(2006\)](#) conducted an industry-sponsored cohort study on mortality and cancer incidence among employees in the petroleum industry in Australia. The cohort was composed of 16 547 men and 1356 women enrolled through a survey interview in 1980–1983, and three further surveys were carried out between 1985 and 2000. Data on tobacco smoking were collected during the interviews. The participation rates for each survey were 92%, 92%, 84%, and 73%. Cause-specific mortality was determined up to 31 December 2001 using the National Death Index, and cancer incidence was determined up to 31 December 2000 by searches in cancer registration databases. SMRs and SIRs were calculated using the sex-, age- and period-specific mortality and incidence rates in Australia. SIR trends according to period of first employment, duration of employment, latency, and exposure to hydrocarbons were tested, adjusting for smoking. [The Working Group noted that exposure to gasoline cannot be inferred from the data provided in this paper on petroleum-industry workers without distinction between refinery, distribution, and other sectors. Results for tanker drivers who were probably exposed to gasoline were given for kidney cancer, and those were therefore considered by the Working Group.]

The two case-control studies on lymphatic and haematopoietic malignancies nested in cohorts of petroleum distribution workers in Canada ([Schnatter et al., 1996](#)) and the UK ([Rushton and Romaniuk, 1997](#)) and the cohort study of mortality and incidence in 16 910 workers in petroleum distribution, refining, and upstream processes in Australia ([Glass et al., 2003](#)) were pooled by [Schnatter et al. \(2012\)](#) in an industry-funded study. Before pooling the studies, [Schnatter et al. \(2012\)](#) updated the follow-up of the cohorts using mortality and cancer incidence registries to identify new cases of lymphatic and haematopoietic malignancies and select new matched controls (the case:control ratio was 1:5 in the Australian cohort and

1:4 in the Canadian and UK cohorts). All cases diagnosed were reassessed by two histopathologists. The pooled study included 60 cases of AML (241 controls), 28 cases of CML (122 controls), 80 cases of CLL (345 controls), 29 cases of MDS (129 controls), and 30 cases of myeloproliferative disease (MPD) (124 controls). [The Working Group considered the results for ever-versus-never working as a tanker driver for  $\geq 1$  year as a proxy for an exposure-response analysis within the cohorts, because tanker drivers are expected to have higher exposure levels to gasoline than do other distribution workers. [Schnatter et al. \(2012\)](#) has reported that tanker drivers are exposed to higher levels of benzene than are other distribution workers, and tanker drivers have been reported in the literature to have higher levels of exposure to gasoline (Section 1.4.2). The Working Group did not consider the other results to be relevant for the present evaluation, because they focused on exposure to benzene, including the results on duration of employment, given that the Australian cohort was not restricted to distribution workers (and results were not presented stratified by study in [Schnatter et al., 2012](#)). The Working Group noted the clear definition of lymphatic and haematopoietic cancer subtypes as a strength. Because the analysis was conducted internally in the cohort, workers in the reference category were also exposed to gasoline to a certain extent, with the odds ratios (ORs) for tanker drivers being potentially biased towards the null. The analyses for tanker drivers were not adjusted for potential confounders (such as smoking and other occupational co-exposures), introducing potential bias in an unpredictable direction. However, the authors reported that information on smoking was available from interview and workplace records for 51% of the cohorts, and that the *P* value associated with the positive dose-response relation between exposure to benzene and MDS became stronger after adjustment for smoking, suggesting that smoking was not a strong positive confounder.]



#### 2.1.4 Service station workers cohort, Latium, Italy

See [Table 2.1](#).

A cohort of 2665 service station managers in the Latium region, Italy was identified through a survey of service stations in 1980 and was followed from the beginning of 1981 until mid-1992 (1 year earlier if the cohort member was resident in Rome) for vital status and cause of death ([Lagorio et al., 1994a](#)). The initial survey included the managers of 3272 stations, which, according to the authors, corresponded to the expected number of stations in the Latium region.

The members of the cohort included 81.4% of the respondents to the original survey, because records for non-identifiable individuals (i.e. commercial houses) or persons who were not alive at the beginning of the follow-up were excluded. At study entry, cohort members were aged on average 46 years, and their mean duration of employment was 14 years. There were 357 (13.9%) women. Vital status was obtained from demographic files, and information on cause of death was obtained through record linkage with the national or regional mortality files or, when linkage was unsuccessful, from death certificates available at the municipality level. Loss to follow-up was 6%, and for 4% of the deceased individuals there was no information on cause of death.

The survey in 1980 collected information on duration of employment and workload at the station. Most (65%) of the managers worked in small stations, where the average number of employees was 1.87, as opposed to large stations where the average was 2.95 employees. Small stations, however, had higher sales of gasoline per full-time employee, corresponding to a higher level of exposure. An environmental monitoring survey was carried out between December 1991 and November 1992 on a random sample of 111 cohort members in order to collect detailed working information and vapour samples at the

breathing zone of the workers ([Lagorio et al., 1993](#)), and this information was used to attempt to predict exposure to aromatic hydrocarbons in the full cohort. However, the multivariable model explained only a small proportion of the variance, and a quantitative exposure assessment was not used in the study.

During the study period, 83 deaths from malignant neoplasms were observed (78 among men). SMRs for all malignancies and for selected cancer sites were calculated using sex-, age- and period-specific regional mortality rates. Results were also stratified by small and large stations.

[The Working Group noted that it was likely that the cohort members were exposed to refuelling activities, because self-service stations were still rare in the study area when the study was carried out, and most of the gas station workforce included self-employed workers. As discussed in Section 1.4.2, this was a unique characteristic of this study as self-service gasoline stations are very common in several contexts, including many countries in Europe and in North America. The cohort, however, was relatively small, with a total of about 100 expected cancer deaths. There was also left truncation, as almost 60% of the workers had  $\geq 10$  years of employment at the beginning of follow-up, but there was no attempt to analyse data by time since the beginning of employment. This selection may have introduced downward bias from a possible HWE. In addition, short-term workers with higher levels of exposure would not contribute to the cohort. Likewise, 40% of the workers had a duration of employment of  $< 10$  years, and no latency was considered in the analysis, although the follow-up duration was relatively short. Finally, there was no information on important potential confounders, including smoking and alcohol consumption. This was discussed by [Lagorio et al. \(1994a\)](#), who dismissed both potential positive confounding by smoking, because there was no excess mortality for lung cancer in the cohort, and potential positive confounding by alcohol consumption,

because participants in the 1990 survey had a lower prevalence of wine consumption than did the population of Italy.]

### 2.1.5 *Agricultural Health Study, USA*

See [Table 2.1](#).

In the Agricultural Health Study (AHS) in the USA, the authors recruited largely private (farmers or nursery workers) as well as commercial (pest control or other businesses) pesticide applicators who were licensed to apply restricted-use pesticides between 1993 and 1997 in Iowa and North Carolina, and also the spouses of farmer applicators. Recruitment was performed at pesticide-certification testing sessions ([Alavanja et al., 1996](#)). Participation consisted of completion of a detailed baseline questionnaire (the response rate ranged from 74% to 82% in the first year). Although the focus of the AHS was exposure to pesticides, information on other occupational agents in non-farm jobs, including gasoline (no/yes), was also captured in a supplemental, longer take-home questionnaire. Approximately 50% of participants who had completed the enrolment questionnaire completed the supplemental questionnaire ([Alavanja et al., 1996](#)). The characteristics of people who had completed the take-home questionnaire were generally similar to those of people who did not ([Tarone et al., 1997](#)). Incident cancer cases during follow-up were identified through linkage with cancer registries in Iowa and North Carolina, and vital status was obtained through National Death Index files.

Within this cohort, the association between risk of prostate cancer and gasoline exposure was explored by [Alavanja et al. \(2003\)](#). However, this analysis was not the main focus of the publication, which was aimed to explore the association between prostate cancer risk and exposure to 45 agricultural pesticides. The overall cohort included 55 332 male applicators, of whom 566 developed prostate cancer during follow-up

(1993–1999). The analysis of gasoline exposure included 22 423 cohort members of whom 292 were diagnosed with prostate cancer. Participants with a history of prostate cancer were excluded.

[The Working Group noted the prospective study design and analysis of incident prostate cancer outcomes based on comprehensive cancer registry data as strengths of the study. Limitations included the short follow-up time of the study (average follow-up, 4.3 years), crude exposure assessment, ascertainment of gasoline exposure or nonfarm jobs in a subset of the main cohort, and the small number of exposed cases ( $n = 24$ ).]

### 2.1.6 *Residents affected by a gasoline spill in Pennsylvania, USA*

See [Table 2.1](#).

[Patel et al. \(2004\)](#) conducted a retrospective cohort study in two municipalities (Hazle Township and Hazleton) in Pennsylvania, USA, to investigate the health effects of a gasoline spill from underground gasoline storage tanks of a gasoline and tyre-retreading station (the “Tranguch gasoline spill”). The spill, or leakage, released an estimated 50 000–900 000 gallons [approximately 190 000–3 400 000 L] of gasoline, probably between 1990 and 2000, although the years of leakage could not be confirmed. The spill resulted in groundwater contamination, with gasoline fumes entering homes because of vapour migration through the sewer system. The Pennsylvania Department of Environmental Protection and the US EPA conducted indoor air sampling in homes in the region, but cumulative exposure could not be calculated because of the lack of historical data. The US EPA sampled indoor air in the home, and 20% of participants provided these reports to the study investigators; of these, benzene levels ranged from undetectable to 140  $\mu\text{g}/\text{m}^3$ . [The Working Group noted that there may be other indoor sources of benzene.] The US EPA and United States Army Corps of Engineers used exposure modelling to

define the affected area, which consisted of 347 exposed households. The cohort was constructed using municipal lists, tax records, and residential histories of people who had resided in the area for  $\geq 6$  months between 1 January 1990 and 31 December 2000. The study response rate was 79.3% of households, resulting in 663 study participants across 275 households. Participants were 99% Caucasian [White], 51.7% female, and had a mean age of 49.2 years. Most (75%) had resided in their home for  $> 20$  years. Data were collected via questionnaire and medical record review, with cancers confirmed via physician report and the Pennsylvania Cancer Registry. All household members were invited to participate in the study, and death certificates were obtained for deceased persons. Researchers attempted to conduct interviews with next of kin when the participant was deceased or unable to respond. Age-adjusted SIRs were calculated and compared with Pennsylvania average annual cancer rates.

This study was updated by [Talbott et al. \(2011\)](#) to encompass an additional year of follow-up and to conduct a more detailed analysis using each participant's time period of residence (1990–1994, 1995–2001, 1990–2001) and proximity to the underground gasoline plume. Homes having exposure defined as high were those directly over the plume or directly adjacent (up to one house away), those with medium exposure were from two houses away up to three blocks away, and those with low exposure were more than three blocks away. Age- and sex-adjusted SIRs were compared with leukaemia rates state-wide and in Luzerne County. No formal medical visits to detect aplastic anaemia or leukaemia among exposed residents were conducted as part of this study. Pennsylvania Department of Health provided free complete blood tests beginning in 2001 for all residents in the study area (400 individuals underwent the tests, and no cases of leukaemia were identified in this way).

[The Working Group noted that the strengths of this study included the historical monitoring of air and groundwater and the modelling of the exposure area. Although the exposure period was not clearly identified, most study participants had resided in their homes for many years. Informativeness was limited by the small number of cancers.]

[The Working Group noted that an SIR analysis in this population was also published in several reports from the Pennsylvania Department of Health, which at the time of the meeting for the present volume (2025) were no longer available on the website of the Pennsylvania Department of Health. Some of these reports (e.g. one dated 2002) were cited in the studies by [Patel et al. \(2004\)](#) and [Talbott et al. \(2011\)](#). The Working Group was able to retrieve a copy of a report dated 2001 ([Pennsylvania Department of Health, 2001](#)) and decided to rely on the results reported in the published scientific paper, because the Working Group could not determine whether this report was in its final form, because later reports were referenced by [Patel et al. \(2004\)](#) and [Talbott et al. \(2011\)](#).]

### 2.1.7 *European Prospective Investigation into Cancer and Nutrition (EPIC) cohort*

See [Table 2.1](#).

The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort is a large prospective cohort into which participants were recruited from the general population, including convenience subgroups such as blood donors and screening participants, in more than 20 centres in 10 European countries ([Riboli et al., 2002](#)). The study was established to investigate diet, lifestyle, and metabolic factors in relation to risk of cancer, as well as of other chronic diseases such as cardiovascular disease and diabetes. Cohort members were recruited between 1992 and 2000 (approximately 521 000 participants, mainly aged 35–70 years at inclusion) and were followed

using cancer registries in seven countries, or a combination of other methods in the remaining three countries. For most study centres, information on diet, lifestyle factors (smoking, alcohol consumption, and physical activity), and socioeconomic factors were collected by self-administered questionnaires, and blood samples, blood pressure, and anthropometric measures (height and weight) were collected at a physical examination. Self-reported information from a job questionnaire was available at baseline for participants from Denmark, Germany, Greece, Spain, four centres in Italy, and one centre in the UK. Cohort members reported whether they had ever worked in any of 52 occupations considered a priori to be associated with an excess risk of cancer, including employment as a gasoline station worker.

Within the EPIC cohort and with end of follow-up in 2006, [Neasham et al. \(2011a\)](#) studied the association between occupation and risk of lymphoma, focusing on incidence of non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma. Multiple myeloma and plasmacytoma were analysed both separately and grouped together with NHL. Further stratification by NHL subgroup was hampered by the limited number of events for most of the occupations studied, including gasoline station attendants. Internal analyses in the cohort were carried out using Cox models stratified on centre and adjusted for sex, educational level, smoking, and alcohol consumption. As reported in an erratum published together with the article ([Neasham et al., 2011b](#)), a total of 218 968 individuals without missing values were included in the analysis. During the study period, 6 cases of NHL, 5 cases of multiple myeloma or plasmacytoma, and 2 cases of Hodgkin lymphoma occurred among gasoline station attendants out of a total of 663 cases of NHL, 143 cases of multiple myeloma or plasmacytoma, and 39 cases of Hodgkin lymphoma among all occupations combined.

In a parallel analysis of occupational risk of leukaemia, based on the EPIC cohort, [Saberi Hosnijeh et al. \(2013\)](#) did not report results for gasoline station attendants because there were fewer than 5 exposed cases, and hence this study was not considered further in the present monograph.

[The Working Group noted that exposure to automotive gasoline could be assessed in this study ([Neasham et al., 2011b](#)) only on the basis of the self-reported information on ever working as a gasoline station attendant. There was no attempt to study time since beginning of employment, latency, or duration of employment, and no information was presented on job tasks or the presence of carcinogenic agents at the workplace. The lack of full occupational history could potentially cause underestimation of the exposure prevalence for the occupations of interest. This crude exposure assessment probably caused nondifferential misclassification, which was likely to result in a bias towards the null. The strengths of the EPIC cohort included that it covered the general population, had a prospective design, a very large sample size, and adjusted for important confounders, including alcohol consumption and smoking. No results were available on neoplasms other than lymphatic and haematopoietic malignancies.]

### 2.1.8 Sister Study, USA

See [Table 2.1](#).

The Sister Study is a prospective nationwide cohort in the USA into which were enrolled 50 884 cancer-free sisters of women with breast cancer from 2004 to 2009. At baseline, participants were aged between 35 and 74 years, 83.7% were non-Hispanic White, and 85% had at least some university education. Recruitment occurred via word of mouth and flyers to breast-cancer support and advocacy organizations, hospitals, mammography centres, churches, unions and trade organizations, and via direct



mail, email, and media. Data were collected via computer-assessed telephone interview. At baseline, 98% of participants had previously undergone mammography ([Sandler et al., 2017](#)). The baseline questionnaire collected information about workplace exposure to specific agents, including “gasoline and other petroleum products”, hence gasoline was grouped with other petroleum agents in the questionnaire. For each agent, participants were queried about lifetime use (ever/never), frequency of use, and duration of use.

The cohort was followed through annual health updates and biennial questionnaires, with diagnosis of breast cancer being collected via self-report. When participants reported a breast cancer diagnosis, researchers asked permission to obtain related medical records. Agreement between self-report and medical records was > 98% for the occurrence of first primary breast cancer.

In the Sister Study cohort, [Ekenga et al. \(2015\)](#) calculated cumulative lifetime occupational exposure to gasoline and other petroleum products in days, and quartile cut-points were assigned, with never use as the reference group (mean follow-up, 5.2 years). Researchers constructed directed acyclic graphs (DAGs) to identify confounding factors. Risk of breast cancer was estimated via hazard models that included adjustment for race/ethnicity, educational attainment, income, parity, and age at first birth.

[The Working Group noted that this study had a large sample size, yet only 4% of participants were ever occupationally exposed to gasoline or petroleum products. Information on the proportion of participants lost to follow-up was not provided. The study relied on self-reported exposure, and there was no additional assessment by industrial hygienists, although the prospective data collection suggests that any misclassification would have been nondifferential. Breast cancer was self-reported, and no additional efforts were

made to identify missed cases. Informativeness was limited by the grouping of gasoline with other petroleum agents. Additionally, the time period of exposure was not collected, hence the lag between exposure and breast cancer diagnosis was unknown and could not be explored in the analysis.]

### 2.1.9 Norwegian Mother and Child Cohort Study (MoBa)

See [Table 2.1](#).

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based cohort established with the aim of collecting exposure data to study a variety of serious diseases, including childhood leukaemia ([Kirkeleit et al., 2018](#)). The establishment of the cohort is described in detail by [Magnus et al. \(2006, 2016\)](#). Participants were recruited from 50 of the 52 maternity hospitals in Norway. Pregnant people attending antenatal care were invited to participate before an ultrasound examination performed in week 17 of pregnancy, which was offered as a part of standard antenatal care. From 1999 to 2008, 112 908 pregnant participants were recruited, representing a response rate of 41%. Compared with the general population, a higher proportion of participants were older (> 30 years), nulliparous, non-smoking, and took folic acid supplements during pregnancy ([Magnus et al., 2006](#); [Nilsen et al., 2009](#)). In a questionnaire administered at week 17 of pregnancy, the participants were asked about employment and leisure-time exposures, and whether they had been in contact with “gasoline or exhaust (does not apply to filling your own car)” in the previous 6 months. Later, fathers-to-be were also recruited; 87.3% were asked to participate and of those, 82.9% consented ([Magnus et al., 2006](#)). The demographics of participating and non-participating fathers-to-be were not reported. Fathers-to-be were similarly asked about contact with gasoline or exhaust in the 6 months before conception.



The questionnaires also included questions on demographic characteristics, lifestyle factors, and socioeconomic status. Study materials were written in Norwegian, limiting the sample to people who could read that language.

In a study assessing the association between parental exposure to “gasoline or exhaust” and childhood leukaemia, incident cancer cases were ascertained via linkage to the national cancer registry ([Kirkeleit et al., 2018](#)). The study included 113 754 children, 94 428 mothers, and 75 995 fathers. To identify higher exposures to gasoline or exhaust representing occupational exposures, investigators defined higher exposure as occurring for  $\geq 30$  days in the 6-month period. By December 2014, 70 cases of leukaemia had occurred among children in the cohort; of these, 52 were acute lymphoblastic leukaemia (ALL) and 16 were AML. The mean age at diagnosis was 3.7 years. Authors estimated the risk of cancer using Cox proportional hazard analysis. Only unadjusted estimates were reported in the results section of the publication. The authors tested whether confounders including birth weight, maternal intake of folate, parental and maternal grandmother’s smoking, parental age, parental consumption of alcohol, and socioeconomic status (level of education and marital status) were associated with leukaemia risk; none of these were found to be associated with leukaemia risk and thus none were considered to be strong confounders. Maternal smoking and birth weight were taken into account in a sensitivity analysis (excluding mothers who smoked or with unknown smoking status at week 17 of pregnancy) and did not change results.

[The Working Group considered that the strengths of this study included the prospectively collected exposure information, limiting recall bias, with any misclassification expected to be nondifferential and to bias results to the null. The Working Group noted the low participation rate in the cohort and the low prevalence of exposed mothers, leading to imprecise risk

estimates. Given the small sample size of cancers, the reliance on traditional statistical-significance testing to choose relevant confounders yielded no confounders for adjustment. Information on smoking status, which has been associated with childhood leukaemia ([IARC, 2012](#)), was missing for 59.6% of fathers-to-be. Exposures were self-reported by parents. The informativeness of the study was limited because there was no differentiation between gasoline and exhaust, with biases potentially going in either direction.]

### *2.1.10 Case-control studies with multiple cancer sites*

See [Table 2.1](#).

[Siemiatycki et al. \(1987a\)](#) conducted a population-based case-control study of 20 types of cancer among men aged 35–70 years in Montreal, Canada, that was designed to generate hypotheses regarding new occupational cancer risks. A detailed description of the study methodology was available in a separate manuscript ([Siemiatycki et al., 1987b](#)). Information regarding about 300 of the most common occupational exposures in the past was captured from interviews with 3726 cancer patients diagnosed in 19 participating hospitals (response rate, 82%). The exposure assessment was conducted via interviews probing job history that were translated into semiquantitative information on degree of exposure by trained chemists and hygienists. Information on the number of years of exposure to the agent was also captured. Two main exposure indices were constructed: cumulative exposure (based on cumulated concentration, frequency, and confidence that exposure took place) and average level of exposure (dividing cumulative exposure by duration). Exposure to automotive gasoline was presumed to be to leaded gasoline; although more recent exposures may also have encompassed unleaded gasoline, these were not differentiated. In total, 462 participants (12.4%) were considered as having any exposure

to automotive gasoline. The percentage of participants exposed with high confidence was 10.4%, including those with exposure at high frequency 2.3%, at (relative) high concentration, 3.9%, and with >20 years of exposure, 4.4%. The main occupations for which exposure to automotive gasoline was coded at any level were mechanics and repairmen (24.7%), sales (mainly service station attendants) (15.4%), farming (14.3%), forestry (10.0%), and motor transport (7.4%). The largest numbers of cases were represented by cancer of the lung ( $n=857$ ), bladder ( $n=486$ ), and prostate gland ( $n=452$ ). There were also 250 cases of stomach cancer, among the cancers at a range of other sites that were investigated in relation to automotive gasoline. The reference group consisted of the other patients with cancer included in the study (with the exception of cases of lung cancer, which were not used as referents), in a conservative study design.

Stratified Mantel-Haenszel analysis was performed in a first screening step to examine associations of any level for any duration versus never exposure, and of substantial (based on the median cumulative index of exposure) versus never exposure, stratified by personal and occupational factors. Selected associations were then further examined using logistic regression according to four exposure subgroups that were based on level (median cumulative index) and duration of exposure (10-year cut-point) adjusted for personal and job factors. [The Working Group noted the detailed exposure assessment procedure undertaken that allowed automotive gasoline to be classified as a separate subgroup of occupational exposure. The analysis of use of automotive gasoline by mechanics and repairmen compared with unexposed workers regardless of occupation was uninformative for the present evaluation (see Introduction, Section 2). There were also relatively small numbers of exposed cases and a large number of comparisons performed because of the hypothesis-generating nature of the study; some associations may thus

be chance findings. The use of patients with other cancers as the reference group may lead to some degree of underestimation of the strength of estimated associations.]

Two additional studies using data from this case-control study in Montreal focused on two specific outcomes: melanoma ([Fritschi and Siemiatycki, 1996](#)) and stomach cancer ([Parent et al., 1998](#)) (see Sections 2.7.3(b) and 2.5.1(b), respectively).

The Investigation of occupational and environmental CAuses of REspiratory cancers (ICARE) study examined associations of occupational solvents and petroleum-based solvent exposures, including gasoline, and risk of cancer at various sites, including the head and neck in women ([Carton et al., 2017](#)), and the oral cavity and oropharynx, hypopharynx, larynx, and lung in men ([Mattei et al., 2016](#); [Barul et al., 2018, 2019](#)). These studies are detailed in Section 2.5.

## 2.2 Childhood lymphatic and haematopoietic malignancies and other childhood cancers

See [Table 2.2](#).

### 2.2.1 Introduction

One cohort and six case-control studies investigated the relation between childhood haematopoietic cancers and parental exposure to automotive gasoline ([Shu et al., 1988](#); [Infante-Rivard et al., 2005](#); [McKinney et al., 2008](#); [Bailey et al., 2011](#); [Shi et al., 2013](#); [Jiang et al., 2016](#); [Kirkeleit et al., 2018](#)), and an additional five case-control studies reported on a variety of non-haematopoietic paediatric cancers (hepatoblastoma, Wilms tumour, neuroblastoma, and germ cell tumours; [Kantor et al., 1979](#); [De Roos et al., 2001](#); [Tsai et al., 2006](#); [Janitz et al., 2017](#); [Hall et al., 2021](#)).

**Table 2.2 Epidemiological studies on exposure to automotive gasoline and childhood lymphatic and haematopoietic malignancies and other childhood cancers**

| Reference, location, enrolment/follow-up period, study design                                                                                                                  | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                             | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                   | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Kirkeleit et al. (2018)</a><br>Norwegian Mother and Child Cohort (MoBa), Norway<br>Enrolment, 1999–2008/<br>follow-up, through December 2014 (incidence)<br>Cohort | 113 754 children, 94 428 mothers, and 75 995 fathers; offspring cohort of the MoBa cohort which include Norwegian women who were pregnant in 1999–2008. All women in Norway at 17 wk of gestation were offered to participate (41% acceptance rate). Childhood cancer follow-up through linkage with Norway cancer registry.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Childhood leukaemia (all subtypes)                   | Maternal exposure to “gasoline or exhaust” (excluding filling their own car) in the 6 mo before wk 17 of pregnancy (HR):                     |                         |                        | None                  | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Prospective design.<br><i>Limitations:</i> Small sample size. Inability to account for exhaust.<br><br>See also <a href="#">Table 2.1</a> .<br><br><i>Other comments:</i> The stratum of “non-smoker mothers” excluded mothers with unknown smoking status. |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                                                                                        | 51                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                                                                                         | 5                       | 2.59 (1.03–6.48)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 1–29 days                                                                                                                                    | 2                       | 2.09 (0.51–8.58)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 30–180 days                                                                                                                                  | 3                       | 3.08 (0.96–9.86)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Trend-test <i>P</i> value, 0.032                                                                                                             |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood leukaemia (all subtypes)                   | Maternal exposure to “gasoline or exhaust” (excluding filling their own car) in the 6 mo before wk 17 of pregnancy, non-smoker mothers (HR): |                         |                        | None                  |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                                                                                        | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                                                                                         | NR                      | 2.71 (0.97–7.55)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood ALL                                        | Maternal exposure to “gasoline or exhaust” (excluding filling their own car) in the 6 mo before wk 17 of pregnancy (HR):                     |                         |                        | None                  |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                                                                                        | 39                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                                                                                         | 4                       | 2.71 (0.97–7.58)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood cancer (leukaemia, all subtypes)           | 1–29 days                                                                                                                                    | 1                       | 1.37 (0.19–9.94)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 30–180 days                                                                                                                                  | 3                       | 4.03 (1.25–13.0)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Trend-test <i>P</i> value, 0.027                                                                                                             |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Paternal exposure to “gasoline or exhaust” (excluding filling their own car) in the 6 mo before conception (HR):                             |                         |                        | None                  |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                                                                                        | 35                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                                                                                         | 8                       | 1.34 (0.84–2.15)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 1–29 days                                                                                                                                    | 6                       | 1.93 (0.81–4.60)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 30–180 days                                                                                                                                  | 2                       | 0.75 (0.18–3.11)       |                       |                                                                                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Trend-test <i>P</i> value, 0.74                                                                                                              |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                          |

Table 2.2 (continued)

| Reference, location, enrolment/ follow-up period, study design                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                           | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                   | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                                           | Comments                                                                                                                                           |
|------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Kirkeleit et al. (2018)</a><br>(cont.)                                             |                                                                                                                                                                                                                                                                                                                                                      | Childhood ALL                                        | Paternal exposure to “gasoline or exhaust” (excluding filling their own car) in the 6 mo before conception (HR):             |                         |                        | None                                                                                                                                                                            |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Never                                                                                                                        | 26                      | 1                      |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Ever                                                                                                                         | 8                       | 1.34 (0.78–2.31)       |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | 1–29 days                                                                                                                    | 6                       | 2.60 (1.07–6.32)       |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | 30–180 days                                                                                                                  | 2                       | 1.01 (0.24–4.24)       |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Trend-test <i>P</i> value, 0.27                                                                                              |                         |                        |                                                                                                                                                                                 |                                                                                                                                                    |
| <a href="#">Shu et al. (1988)</a><br>Shanghai, China<br>July 1974 to June 1986<br>Case-control | <i>Cases:</i> 309; all children (aged < 15 yr) with childhood leukaemia diagnosis in the Shanghai urban area.<br><br><i>Controls:</i> 608; randomly selected from Shanghai urban area (2:1 ratio) matched on sex and birth calendar year.<br><br><i>Exposure assessment method:</i> self-reported occupational exposure through telephone interview. | Childhood leukaemia                                  | Maternal occupational exposure to gasoline during pregnancy, children with at least one maternal occupational exposure (OR): |                         |                        | Age, sex, birth weight, birth order, born in rural area, chloramphenicol and syntomycin usage, prenatal and paternal preconception X-ray exposure, and mother’s age at menarche | <i>Strengths:</i> High response rates: 99.7% for cases, 98.4% for controls.<br><br><i>Limitations:</i> Limited information on exposure assignment. |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Never                                                                                                                        | NR                      | 1                      |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Ever                                                                                                                         | 38                      | 1.6 (0.8–3.1)          |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      | Childhood ALL                                        | Maternal occupational exposure to gasoline during pregnancy, children with at least one maternal occupational exposure (OR): |                         |                        |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Never                                                                                                                        | NR                      | 1                      |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Ever                                                                                                                         | 21                      | 1.7 (1.0–3.0)          |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      | Childhood ANLL                                       | Maternal occupational exposure to gasoline during pregnancy, children with at least one maternal occupational exposure (OR): |                         |                        |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Never                                                                                                                        | NR                      | 1                      |                                                                                                                                                                                 |                                                                                                                                                    |
|                                                                                                |                                                                                                                                                                                                                                                                                                                                                      |                                                      | Ever                                                                                                                         | 13                      | 2.1 (1.1–4.3)          |                                                                                                                                                                                 |                                                                                                                                                    |

**Table 2.2 (continued)**

| Reference, location, enrolment/follow-up period, study design                               | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                 | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Infante-Rivard et al. (2005)</a><br>Quebec, Canada<br>1980–2000<br>Case-control | Cases: 790 cases of incident childhood ALL were identified from hospitals with regional coverage. Mothers responded to the questionnaire. Participation rate, 93.1%.<br><br>Controls: 790; controls mothers matched for age and sex of the child were identified from registers representing the population of the area. Participation rate, 86.2%.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment based on the parent's self-reported occupational history (2 yr prenatal to birth). Level of exposure was estimated by an industrial hygienist on the basis of job-exposure levels and duration. | Childhood ALL                                        | Maternal occupational exposure to leaded gasoline during pregnancy (OR):                         |                         |                        | Sex of child, age of child, maternal age, maternal level of schooling | <i>Exposure assessment critique:</i> The categorization in leaded and non-leaded gasoline might have led to misclassification as it may coincide with changes in gasoline vapour control and exposure levels over time. Misclassification of gasoline exposure was probably nondifferential.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Well-designed population-based case-control study.<br><i>Limitations:</i> Imprecise results. |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | No                                                                                               | NR                      | 1                      |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Exposed                                                                                          | NR                      | 4.14 (0.46–37.16)      |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Childhood ALL                                        | Maternal occupational leaded gasoline exposure from 2 yr before pregnancy up to birth (OR):      |                         |                        |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | No                                                                                               | NR                      | 1                      |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Exposed                                                                                          | NR                      | 5.09 (0.59–43.65)      |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Childhood ALL                                        | Maternal occupational exposure to unleaded gasoline during pregnancy (OR):                       |                         |                        |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | No                                                                                               | NR                      | 1                      |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Exposed                                                                                          | NR                      | 0.83 (0.22–3.10)       |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Childhood ALL                                        | Maternal occupational exposure to unleaded gasoline from 2 yr before pregnancy up to birth (OR): |                         |                        |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | No                                                                                               | NR                      | 1                      |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Exposed                                                                                          | NR                      | 0.90 (0.30–2.71)       |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |



Table 2.2 (continued)

| Reference, location, enrolment/ follow-up period, study design               | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                              | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------|------------------------|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">McKinney et al. (2008)</a><br>UKCCS<br>1991–1996<br>Case–control | <i>Cases:</i> 1881; childhood leukaemia or lymphoma (age 0–14 yr) in the UKCCS. Participation rate, 87%.<br><br><i>Controls:</i> 3742; two controls per case matched on age, sex, and region. Participation rate, 64%.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment of gasoline exposure on the basis of occupational history. Personal interviews with parents of case and control children collected data on occupational history. Exposure status (exposed, partially exposed unexposed) was assigned by an expert occupational hygienist, on the basis of exposure probability and level. | Childhood ALL                                        | Maternal occupational exposure to petrol (self-reported) (ever vs never) (OR):              |                         |                        | Age, sex, region, SES, other occupational exposure | <i>Exposure assessment critique:</i> Several steps were taken to enhance the quality of information on parental exposure to gasoline and other exposures. The proportion of mothers' self-reports of gasoline exposure that were reclassified after the exposure assessment review differed between cases and controls, suggesting differential misclassification of gasoline exposure. A strength was that study results were presented on the basis of both the self-reported exposures by the mothers and the reclassified exposures.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Very large sample size. Population-based study design.<br><br><i>Limitations:</i> Only for 33% of cases and 36% of controls was exposure rated as “certain”. |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | In preconception period                                                                     | 78                      | 1.6 (1.2–2.2)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | During pregnancy                                                                            | 24                      | 2.1 (1.2–3.6)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood ALL                                        | Postnatal                                                                                   | 30                      | 2.2 (1.3–3.5)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Maternal occupational exposure to petrol (expert reviewed occupation) (ever vs never) (OR): |                         |                        |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | In preconception period                                                                     | NR                      | 0.8 (0.4–1.4)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | During pregnancy                                                                            | NR                      | 0.9 (0.2–3.3)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood HL                                         | Postnatal                                                                                   | NR                      | 2.4 (0.8–6.9)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Maternal occupational exposure to petrol (expert reviewed occupation), OR (ever vs never):  |                         |                        |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | In preconception period                                                                     | NR                      | 0.6 (0.1–4.9)          |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                     | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                       | Exposed cases or deaths | Risk estimate (95% CI)                            | Covariates controlled                                                   | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|---------------------------------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">McKinney et al. (2008)</a><br>(cont.)                                                              |                                                                                                                                                                                                                                                                                | Childhood NHL                                        | Maternal occupational exposure to petrol (self-reported), OR (ever vs never):<br>In preconception period                                         | NR                      | 3.7 (1.8–7.5)                                     | Age, sex, region, SES, other occupational exposure                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                                                                                                                |                                                                                                                                                                                                                                                                                | Childhood NHL                                        | Pregnancy<br>Maternal occupational exposure to petrol (expert reviewed occupation) (ever vs never) (OR):<br>In preconception period<br>Postnatal | NR<br>NR                | 4.4 (1.9–10.4)<br>1.1 (0.4–3.2)<br>3.0 (0.6–14.5) |                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <a href="#">Bailey et al. (2011)</a><br>Aus-ALL study, Australia<br>July 2003 to December 2006<br>Case-control | <i>Cases:</i> 416; childhood (aged < 15 yr) ALL cases identified through all 10 paediatric oncology centres in Australia and had reached remission.<br><br><i>Controls:</i> 1361; recruited by random-digit dialling, frequency-matched on age, sex, state of residency (3:1). | Childhood ALL                                        | Maternal refuelling with petrol in the year before or during the pregnancy (OR):<br>No refuelling with petrol<br>Any petrol refuelling           | NR<br>NR                | 1<br>1.00 (0.71–1.41)                             | Age, sex, state, maternal education, version of the fuel question asked | <i>Strengths:</i> National population-based study. Recruitment from all paediatric oncology centres in Australia.<br><br><i>Limitations:</i> Participation was lower in controls: 80.2% of cases and 70.3% of controls. They only recruited cases who were in remission.<br><br><i>Other comments:</i> Additional methodological details from <a href="#">Milne et al. (2010)</a> . Examination of personal refuelling of vehicles would yield far lower exposure than occupational studies. |
|                                                                                                                |                                                                                                                                                                                                                                                                                | Childhood ALL                                        | Frequency of maternal refuelling with petrol in the year before or during pregnancy (OR):<br>No refuelling with petrol<br>Low<br>High            | NR<br>NR<br>NR          | 1<br>0.87 (0.59–1.27)<br>1.10 (0.76–1.58)         |                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description Exposure assessment method                                                                                                                                                                                                                                                                                                                                                        | Cancer type (histopathology), incidence or mortality    | Exposure category or level                                                                                                 | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                   | Comments |
|----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-------------------------------------------------------------------------|----------|
| <a href="#">Bailey et al. (2011)</a><br>(cont.)                | <i>Exposure assessment method:</i> Mothers' questionnaire contained questions about the number of times per month that a vehicle was refuelled with different types of fuel (petrol [gasoline], diesel, and liquefied petroleum gas) for non-occupational use in the year before the pregnancy and during pregnancy. Fathers were asked similar questions about the year before conception of the index child. | Childhood ALL (immunophenotype and cytogenetic subtype) | Maternal refuelling with petrol in the year before or during the pregnancy (vs did not refuel a vehicle with petrol) (OR): |                         |                        | Age, sex, state, maternal education, version of the fuel question asked |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Pre-B-cell                                                                                                                 | 271                     | 0.93 (0.65–1.32)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | T-cell                                                                                                                     | 32                      | 2.60 (0.76–8.90)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Normal karyotype                                                                                                           | 107                     | 1.21 (0.70–2.07)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Any genetic feature                                                                                                        | 195                     | 0.93 (0.62–1.40)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Chromosomal deletions                                                                                                      | 51                      | 1.30 (0.59–2.86)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | ETV6-Runx-1 t(12;21)                                                                                                       | 46                      | 1.29 (0.56–2.98)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Hyperdiploidy                                                                                                              | 82                      | 0.54 (0.33–0.89)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Trisomy (or greater) 21 (somatic)                                                                                          | 48                      | 0.47 (0.25–0.87)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                | Childhood ALL                                           | Paternal refuelling with petrol in the year before conception (OR):                                                        |                         |                        | Age, sex, state, paternal education, version of the fuel question asked |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | No refuelling with petrol                                                                                                  | NR                      | 1                      |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Any petrol refuelling                                                                                                      | NR                      | 0.90 (0.54–1.51)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                | Childhood ALL                                           | Frequency of paternal refuelling with petrol in the year before conception (OR):                                           |                         |                        |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | No refuelling with petrol                                                                                                  | NR                      | 1                      |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | Low                                                                                                                        | NR                      | 0.87 (0.51–1.47)       |                                                                         |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                |                                                         | High                                                                                                                       | NR                      | 0.99 (0.56–1.73)       |                                                                         |          |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method | Cancer type (histopathology), incidence or mortality    | Exposure category or level                                                                                    | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                   | Comments |
|----------------------------------------------------------------|------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-------------------------------------------------------------------------|----------|
| <a href="#">Bailey et al. (2011)</a><br>(cont.)                |                                                            | Childhood ALL (immunophenotype and cytogenetic subtype) | Paternal refuelling with petrol in the year before conception (vs did not refuel a vehicle with petrol) (OR): |                         |                        | Age, sex, state, paternal education, version of the fuel question asked |          |
|                                                                |                                                            |                                                         | Pre-B-cell                                                                                                    | 258                     | 0.86 (0.50–1.46)       |                                                                         |          |
|                                                                |                                                            |                                                         | T-cell                                                                                                        | 25                      | 0.99 (0.21–4.61)       |                                                                         |          |
|                                                                |                                                            |                                                         | Normal karyotype                                                                                              | 98                      | 0.82 (0.38–1.75)       |                                                                         |          |
|                                                                |                                                            |                                                         | Any genetic feature                                                                                           | 183                     | 0.91 (0.49–1.67)       |                                                                         |          |
|                                                                |                                                            |                                                         | Chromosomal deletions                                                                                         | 43                      | 0.70 (0.26–1.92)       |                                                                         |          |
|                                                                |                                                            |                                                         | ETV6-Runx-1 t(12;21)                                                                                          | 42                      | 0.63 (0.20–1.93)       |                                                                         |          |
|                                                                |                                                            |                                                         | Hyperdiploidy                                                                                                 | 82                      | 0.76 (0.35–1.65)       |                                                                         |          |
|                                                                |                                                            |                                                         | Trisomy (or greater) 21 (somatic)                                                                             | 46                      | 1.03 (0.34–3.09)       |                                                                         |          |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                  | Cancer type (histopathology), incidence or mortality | Exposure category or level                           | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                                                                                                | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|-------------------------|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Shi et al. (2013)</a><br>Shanghai, China<br>2009–2010<br>Case–control | <i>Cases:</i> 201; childhood acute leukaemia cases (aged < 15 yr) identified in three Shanghai hospitals.<br><br><i>Controls:</i> 201; sex-, age-matched children recruited in the orthopaedic department of the hospitals where cases were recruited.<br><br><i>Exposure assessment method:</i> Self-reported occupational exposure collected through in-person interview. | Childhood acute leukaemia                            | Maternal gasoline/diesel 3 mo before pregnancy (OR): |                         |                        | Maternal age, education level, alcohol consumption history, smoking, passive smoking, and residential location<br><br>Paternal age, education level, alcohol consumption history, smoking, passive smoking, and residential location | <i>Strengths:</i> Multivariable confounder adjustment, including by rural vs urban area, which should partially account for air pollution exposure.<br><br><i>Limitations:</i> Small number of exposed cases and controls, leading to imprecise estimates. For mothers, the pre-pregnancy period may not be the most relevant time period. Data collected by self-report. Hospital-based controls including children from orthopaedics department. Limited information on exposure assessment, including how gasoline exposure was assigned. Gasoline and diesel included together in analyses. No co-adjustment for other chemical exposures. Participation rates were not reported. |
|                                                                                   |                                                                                                                                                                                                                                                                                                                                                                             |                                                      | No                                                   | 188                     | 1                      |                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                                                                                   |                                                                                                                                                                                                                                                                                                                                                                             | Childhood acute leukaemia                            | Yes                                                  | 13                      | 2.8 (0.5–15.5)         |                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                                                                                   |                                                                                                                                                                                                                                                                                                                                                                             |                                                      | Paternal gasoline/diesel 3 mo before pregnancy (OR): |                         |                        |                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                                                                                   |                                                                                                                                                                                                                                                                                                                                                                             |                                                      | No                                                   | 165                     | 1                      |                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                                                                                   |                                                                                                                                                                                                                                                                                                                                                                             |                                                      | Yes                                                  | 36                      | 0.9 (0.4–1.7)          |                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |



**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level                              | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                                                                                       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |  |
|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| <a href="#">Jiang et al. (2016)</a><br>Shenzhen Children's Hospital, China<br>2015–2016<br>Case–control | Cases: 71; ALL cases in children (aged 0–14 yr) identified in the Department of Hematology and Oncology.<br><br>Controls: 142; matched (2:1) on sex and age and recruited in the orthopaedic department.<br>Exclusion: HIV infection for both cases and controls.<br><br><i>Exposure assessment method:</i> Self-reported parental exposure to gasoline/diesel. | Childhood ALL                                        | Paternal gasoline/diesel exposure (OR):                 |                         |                        | Resided in a newly painted residence within the past 10 yr, maternal exposure to chemicals during pregnancy, paternal exposure to hair dyes and other dyes, Burning of garbage in the surroundings of the child's residence | <i>Strengths:</i> Both parents completed questionnaires. The model co-adjusted for home chemical exposures.<br><br><i>Limitations:</i> It is not clear if proxy respondents were used. Exposure data was self-reported; limited information available on exposure assessment, including whether parents were asked specifically about gasoline; no information about occupational co-exposures; small number of exposed cases and controls, leading to imprecise estimates. For mothers, the pre-pregnancy period may not be the most relevant time period. |  |
|                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                 |                                                      | No                                                      | 129                     | 1                      |                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |
|                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                 | Childhood ALL                                        | Yes                                                     | 13                      | 8.15 (2.68–24.83)      | None                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |
|                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Maternal (pre-pregnancy) gasoline/diesel exposure (OR): |                         |                        |                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |
|                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                 |                                                      | No                                                      | 63                      | 1                      |                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |
|                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Yes                                                     | 8                       | 4.22 (1.08–16.54)      |                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                           | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                     |
|---------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------|
| <a href="#">Harrison et al. (1999)</a><br>West Midlands, UK 1990–1994<br>Case–control | <p><i>Cases:</i> 130 leukaemias, 251 solid tumours; childhood leukaemia or solid tumour cases (aged 0–15 yr) recorded in the West Midlands Cancer Intelligence Unit.</p> <p><i>Controls:</i> 130 leukaemias, 251 solid tumours; childhood leukaemia cases served as controls for solid tumour and vice versa.</p> <p><i>Exposure assessment method:</i> Locations of petrol stations obtained from local authorities. Should be a comprehensive list of operational petrol stations. Time period of the assessment: address at diagnosis.</p> | Childhood leukaemia                                  | Distance from petrol station (from address at diagnosis) (OR):<br>> 100 m<br>< 100 m | 122<br>8                | 1<br>1.99 (0.73–5.43)  | None                  | <p><i>Strengths:</i> Population-based.</p> <p><i>Limitations:</i> Use of solid tumour cases as controls.</p> |

**Table 2.2 (continued)**

| Reference, location, enrolment/follow-up period, study design                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                        | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Brosselin et al. (2009)</a><br>ESCALE, France<br>2003–2004<br>Case-control | <i>Cases:</i> 765; cases of acute leukaemia (aged < 15 yr) identified through French paediatric hospitals with the support of a national childhood registry, excluding children who had died or who were receiving hospital palliative care.<br><br><i>Controls:</i> 1681; randomly selected from French population (using French national phone directory) ensuring a comparable distribution in terms of age and sex to the case distributions.<br><br><i>Exposure assessment method:</i> Qualitative assessment on the basis of personal interviews with mothers using structured questionnaires with questions related to petrol stations in the close proximity of the residence. | Acute childhood leukaemia                            | Residence next to a petrol station only between conception and diagnosis (OR):<br>Never garage or petrol station | 689                     | 1                      | Age, sex, number of children < 15 yr living in the household | <i>Exposure assessment critique:</i> The study authors described multiple efforts to monitor the quality of the interviews and check for recall bias, and these efforts lent credibility to their findings. Because the interviews were conducted after the child's cancer diagnosis; however, the potential for differential misclassification of exposure between mothers of cases and controls could not be completely dismissed.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Recruitment from all paediatric oncology hospitals in France. Questionnaire asked specifically about petrol stations.<br><br><i>Limitations:</i> Controls were younger than cases. Case families moved more often than controls. |
|                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood ALL                                        | Ever                                                                                                             | 19                      | 2.1 (1.1–4.0)          |                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Childhood AML                                        | Residence next to a petrol station only between conception and diagnosis (OR):<br>Never garage or petrol station | NR                      | 1                      |                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                                                             | 16                      | 2.0 (1.0–4.0)          |                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Residence next to a petrol station only between conception and diagnosis (OR):<br>Never garage or petrol station | NR                      | 1                      |                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                                                             | 3                       | 2.5 (0.7–8.8)          |                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

Table 2.2 (continued)

| Reference, location, enrolment/ follow-up period, study design                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                           | Cancer type (histopathology), incidence or mortality | Exposure category or level                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                                                                                                                             | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------|-------------------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Mazzei et al. (2022)</a><br>Switzerland<br>1985–2015<br>Case–control | <i>Cases:</i> 1880 leukaemias, 1290 CNS tumours; children (aged ≤ 15 yr) with cancer identified through the Swiss Childhood Cancer Registry.<br><br><i>Controls:</i> 18 800 (for leukaemia), 12 900 (for CNS tumours); controls (10 per case) matched on sex, age, and year identified through the national census.<br><br><i>Exposure assessment method:</i> The minimum distance was calculated from the cases' and controls' residence to the nearest gasoline station, using geocoded addresses. | Childhood leukaemia                                  | Residential proximity to petrol stations at birth (OR):     |                         |                        | Sex, age, year, ambient level of NO <sub>2</sub> , distance to the nearest highway, socioeconomic position of the immediate neighbourhood area, degree of urbanization of the municipality of residence, years of existence of a general cantonal cancer registry | <i>Exposure assessment critique:</i><br>The comparability of the quality of information of residential history between cases and controls was unknown, differences of quality would lead to differential misclassification. Nondifferential misclassification may occur because information about gasoline station location may have been out-of-date for years when business census data were not collected and less accurate for analyses of exposure at birth.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Population-based study. Adjusted for traffic exhaust using NO <sub>2</sub> .<br><br><i>Limitations:</i> Small sample size close to petrol stations. Misclassification of residences possible because residential history was obtained at the time of national census. |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | 0–50 m                                                      | 3                       | 0.52 (0.16–1.67)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 50–100 m                                                  | 13                      | 0.90 (0.51–1.60)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 100–250 m                                                 | 78                      | 0.92 (0.72–1.17)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 250–500 m                                                 | 194                     | 1.00 (0.84–1.18)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 500 m                                                     | 1254                    | 1                      |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Childhood leukaemia                                  | Residential proximity to petrol stations at diagnosis (OR): |                         |                        |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | 0–50 m                                                      | 6                       | 1.08 (0.46–2.51)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 50–100 m                                                  | 15                      | 0.86 (0.50–1.46)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 100–250 m                                                 | 100                     | 1.01 (0.81–1.25)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 250–500 m                                                 | 240                     | 1.05 (0.90–1.22)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 500 m                                                     | 1519                    | 1                      |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Childhood CNS cancer                                 | Residential proximity to petrol stations at birth (OR):     |                         |                        |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | 0–50 m                                                      | 3                       | 1.11 (0.33–3.68)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 50–100 m                                                  | 6                       | 1.65 (0.28–1.49)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 100–250 m                                                 | 50                      | 0.88 (0.65–1.20)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 250–500 m                                                 | 121                     | 0.94 (0.76–1.15)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 500 m                                                     | 866                     | 1                      |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Childhood CNS cancer                                 | Residential proximity to petrol stations at diagnosis (OR): |                         |                        |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | 0–50 m                                                      | 5                       | 1.30 (0.51–3.35)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 50 to 100 m                                               | 10                      | 0.92 (0.48–1.78)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 100 to 250 m                                              | 63                      | 0.89 (0.68–1.17)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 250 to 500 m                                              | 155                     | 0.97 (0.80–1.17)       |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                      | > 500 m                                                     | 1057                    | 1                      |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

Table 2.2 (continued)

| Reference, location, enrolment/ follow-up period, study design                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                         | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                                                 | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Malavolti et al. (2023)</a><br>Modena and Reggio Emilia, Italy<br>1998–2019<br>(incidence)<br>Case–control | Cases: 182 leukaemias (including 148 ALL); newly diagnosed cases of childhood cancer (aged 0–14 yr) in Modena and Reggio Emilia identified through the Italian hospital-based registry of childhood malignancies, available from the Italian Association of Paediatric Hemato-Oncology.<br><br>Controls: 726; controls matched 4:1 on sex, year of birth, and province of residence at diagnosis, randomly selected from those enrolled in the National Health Service directory of the Modena and Reggio Emilia provinces, in which all residents are compulsorily registered. | Childhood leukaemia                                  | Residential proximity to petrol stations at diagnosis (RR):                        |                         |                        | Sex, age, province of residence, fuel supply within the 1000 m buffer, PM <sub>10</sub> , ELF-MF from high-voltage power lines, indoor transformer stations, urban area, arable crops | <i>Exposure assessment critique:</i> The study used strong methods like geocoding, GIS, and blinded assessments to improve exposure assessment, also considering proximity to petrol stations and fuel supply as key exposure metrics. However, limitations such as historical data gaps, and residential mobility, could lead to exposure misclassification.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Population-based, objective exposure assessment, reports on leukaemia subtypes. Adjustment for PM <sub>10</sub> .<br><i>Limitations:</i> Lack of information on paternal smoking. |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | < 50 m                                                                             | 3                       | 2.2 (0.5–9.4)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 50 to < 200 m                                                                      | 20                      | 1.0 (0.5–1.9)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 200 to < 500 m                                                                     | 58                      | 0.9 (0.5–1.5)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 500 to < 1000 m                                                                    | 53                      | 0.9 (0.6–1.5)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | ≥ 1000 m                                                                           | 48                      | 1                      |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Childhood leukaemia                                  | Fuel supply (L/day) at petrol stations within 250 m of the home at diagnosis (RR): |                         |                        |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | No petrol stations within 250 m                                                    | 146                     | 1                      |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | < 150 L/day                                                                        | 4                       | 1.4 (0.4–4.4)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 150–999 L/day                                                                      | 21                      | 1.2 (0.7–2.1)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | ≥ 1000 L/day                                                                       | 11                      | 1.6 (0.7–3.3)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Childhood ALL                                        | Residential proximity to petrol stations at diagnosis (RR):                        |                         |                        |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | < 50 m                                                                             | 3                       | 2.9 (0.6–13.4)         |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 50 to < 200 m                                                                      | 16                      | 1.2 (0.5–2.5)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 200 to < 500 m                                                                     | 47                      | 1.0 (0.6–1.7)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | 500 to < 1000 m                                                                    | 44                      | 1.0 (0.6–1.7)          |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | ≥ 1000 m                                                                           | 38                      | 1                      |                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |



**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                        | Exposed cases or deaths | Risk estimate (95% CI)                               | Covariates controlled                                                                                                                                                                 | Comments |
|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| <a href="#">Malavolti et al. (2023)</a><br>(cont.)             | <i>Exposure assessment method:</i> Residential addresses of children and locations of petrol stations were geocoded using GIS. Adjustments were made for potential confounders such as PM <sub>10</sub> concentrations, magnetic fields, presence of indoor transformer stations, urban area, arable crops, maternal age, ethnicity, and parental income. | Childhood ALL                                        | Fuel supply (L/day) at petrol stations within 250 m of the home at diagnosis (RR):<br>No petrol stations within 250 m of the home<br>< 150 L/day<br>150–999 L/day<br>≥ 1000 L/day | 118<br>4<br>18<br>8     | 1<br>1.6 (0.5–5.0)<br>1.4 (0.8–2.5)<br>1.5 (0.6–3.8) | Sex, age, province of residence, fuel supply within the 1000 m buffer, PM <sub>10</sub> , ELF-MF from high-voltage power lines, indoor transformer stations, urban area, arable crops |          |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Cancer type (histopathology), incidence or mortality | Exposure category or level                                   | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                   |
|----------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------|-------------------------|------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Norzaee et al. (2024)</a><br>Hospital-based, Islamic Republic of Iran<br>2016–2021<br>Case-control | <i>Cases:</i> 428 leukaemias, 428 lymphomas; children (aged 1–15 yr) with childhood leukaemia or lymphoma from three hospitals in Tehran.<br><br><i>Controls:</i> 428 healthy children visiting paediatric clinic in the same hospital as cases for non-blood-related diseases (fractures, osteoarticular disorders, trauma, injuries, gastrointestinal diseases, infections, and poisoning). Matching not mentioned.<br><br><i>Exposure assessment method:</i> GIS-based methods using land use information (including petrol station); however, no information on sources of the land use information (e.g. time frame). | Childhood leukaemia                                  | Distance from residence to petrol station at diagnosis (OR): |                         |                        | NR                    | <i>Strengths:</i> Included patients from all children's hospitals in Tehran. No concerns for participation bias. Confounders taken from medical records.<br><br><i>Limitations:</i> Source of petrol station information was not provided. |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | No petrol station within 100 or 500 m                        | NR                      | 1                      |                       |                                                                                                                                                                                                                                            |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Within 100 m                                                 | NR                      | 2.12 (0.98–4.58)       |                       |                                                                                                                                                                                                                                            |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Childhood lymphoma (type not specified)              | Within 500 m                                                 | NR                      | 1.44 (0.92–2.24)       |                       |                                                                                                                                                                                                                                            |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Distance from residence to petrol station at diagnosis (OR): |                         |                        |                       |                                                                                                                                                                                                                                            |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | No petrol station within 100 or 500 m                        | NR                      | 1                      |                       |                                                                                                                                                                                                                                            |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Within 100 m                                                 | NR                      | 1.05 (0.46–2.43)       |                       |                                                                                                                                                                                                                                            |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Within 500 m                                                 | NR                      | 1.01 (0.63–1.62)       |                       |                                                                                                                                                                                                                                            |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                       | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Cancer type (histopathology), incidence or mortality | Exposure category or level                           | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                         | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|-------------------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Janitz et al. (2017)</a><br>HOPE study, USA<br>2000–2008<br>Case–control | Cases: 383 cases of hepatoblastoma in children (aged < 15 yr) recruited at Children's Oncology Group institutions across the USA. Inclusion: birth in the USA, birth mother speaking English or Spanish, and physician permission to contact cases.<br><br>Controls: 387; controls drawn from birth certificates registry, frequency-matched on year of birth, region of birth, sex, birth weight.<br><br>Exposure assessment method: mothers were asked about jobs held from 1 yr before becoming pregnant through the index child's birth. Collected self-report of occupational exposures to gasoline at the job at least once per week for 6 mo in a row. Industrial hygienist also classified exposures on the basis of job titles. | Hepatoblastoma                                       | Maternal occupational gasoline/diesel exposure (OR): |                         |                        | Year of birth, region of birth, sex, birth weight, household income, maternal race<br><br>Year of birth, region of birth, sex, birth weight, household income | <i>Strengths:</i> Detailed exposure assessment and industrial hygienist review.<br><br><i>Limitations:</i> Concerns for non-representativeness of controls. Response rate was 60.7% for cases, 51.3% for controls. In comparison to the population of the USA, controls included larger numbers of White participants and fewer Black and Hispanic participants; mothers were older and had a higher level of educational attainment.<br><br><i>Other comments:</i> Communication with authors confirmed that in this paper the exposure was “gasoline/diesel”, which limited the informativeness of the study. Additional details from: <a href="#">Puumala et al. (2012)</a> . |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Unlikely                                             | 718                     | 1                      |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Possible                                             | 18                      | 0.51 (0.18–1.44)       |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Likely                                               | 17                      | 1.03 (0.36–2.97)       |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Hepatoblastoma                                       | Possible/likely                                      |                         | 0.72 (0.34–1.50)       |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Paternal occupational gasoline/diesel exposure (OR): |                         |                        |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Unlikely                                             | 489                     | 1                      |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Possible                                             | 128                     | 1.05 (0.68–1.61)       |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Likely                                               | 124                     | 0.87 (0.55–1.36)       |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                      | Possible/likely                                      | 252                     | 0.96 (0.68–1.35)       |                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                        | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                    | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                       | Comments                                                                                                                                                                                                                                                                       |
|----------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">De Roos et al. (2001)</a><br>Hospital-based, 139 hospitals in the USA and Canada<br>May 1992 to April 1994<br>Case-control | <i>Cases:</i> 538 cases of neuroblastoma (children aged < 19 yr) diagnosed in 139 hospitals.<br><i>Controls:</i> 504, matched on date of birth; identified through random-digit dialling.<br><i>Exposure assessment method:</i> Self-reported parental exposure reviewed by industrial hygienist. Proxy respondents for father's interview were not asked occupational questions. | Neuroblastoma                                        | Maternal occupational gasoline exposure in the 2-yr period before birth (self-reported) (OR):                 |                         |                        | Date of birth, child's age, maternal race, maternal age, maternal education | <i>Strengths:</i> Industrial hygienist coding blind to case-control status and parents' gender.<br><i>Limitations:</i> 73% response rate among cases, 74% among controls. 14.2% of case and 31.7% of control paternal interviews occurred via proxy interview with the mother. |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | No                                                                                                            | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | Exposed                                                                                                       | 14                      | 1.6 (0.6–3.8)          |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   | Neuroblastoma                                        | Maternal occupational gasoline exposure in the 2-yr period before birth (industrial hygienist's review) (OR): |                         |                        |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | No                                                                                                            | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | Exposed                                                                                                       | 3                       | 0.8 (0.2–4.2)          |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   | Neuroblastoma                                        | Paternal occupational gasoline exposure in the 2-yr period before birth (self-reported) (OR):                 |                         |                        |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | No                                                                                                            | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | Exposed                                                                                                       | 77                      | 0.8 (0.5–1.2)          |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   | Neuroblastoma                                        | Paternal occupational gasoline exposure in the 2-yr period before birth (industrial hygienist's review) (OR): |                         |                        |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | No                                                                                                            | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                                                                |
|                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                   |                                                      | Exposed                                                                                                       | 45                      | 0.8 (0.5–1.3)          |                                                                             |                                                                                                                                                                                                                                                                                |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design                              | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                         | Cancer type (histopathology), incidence or mortality | Exposure category or level                                             | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Kantor et al. (1979)</a><br>CT, USA<br>1953–1973<br>(incidence)<br>Case–control | <p><i>Cases:</i> 149 individuals (aged ≤ 19 yr) with Wilms tumour, reported in the CT tumour registry.</p> <p><i>Controls:</i> 149; individuals without cancer matched on sex, race, year of birth drawn from the Connecticut State Health Department files of birth certificates.</p> <p><i>Exposure assessment method:</i> Paternal occupation as recorded in the child's birth certificate.</p> | Wilms tumour                                         | No. of individuals with father working as a service station attendant: |                         |                        | None                  | <p><i>Strengths:</i> Population-based and included all cases in the tumour registry who were born in that state. Although occupation was self-reported, exposure was based on prospectively collected records. Overall, a simple and low-cost study but provided good information.</p> <p><i>Limitations:</i> Occupation at birth was collected, which may not match occupation in the periconception period. Comparability of cases and controls was said to be good but was not shown. Birth weight should not be expected to be comparable between cases and controls.</p> |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                    |                                                      | Cases (number)                                                         | 3                       | NC                     |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                    |                                                      | Control (number)                                                       | 0                       | NR                     |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

**Table 2.2 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                  | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                       | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                          |
|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Tsai et al. (2006)</a><br>CA, FL, NJ, MI, North Carolina, and PA, USA<br>1992–1995<br>Case-control | Cases: 303 children (aged 0–9 yr) with newly diagnosed Wilms tumour, as identified in cancer registries. Participation rate, 86%.<br><br>Controls: 575; matched on age and race (2:1) in each state. Recruited using random-digit dialling.<br><br><i>Exposure assessment method:</i> Maternal interview (< 1% of fathers interviewed). Self-reported exposures. | Wilms tumour                                         | Maternal (occupational and/or household) gasoline exposure during pregnancy (OR):         |                         |                        | Age, race, state      | <i>Strengths:</i> Large sample size.<br><i>Limitations:</i> Case mothers were older and more highly educated than controls, higher family income. |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                  |                                                      | No                                                                                        | 230                     | 1                      |                       |                                                                                                                                                   |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                  |                                                      | Yes                                                                                       | 5                       | [0.76 (0.26–2.17)]     |                       |                                                                                                                                                   |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                  | Wilms tumour                                         | Maternal (occupational and/or household) gasoline exposure during 2-yr study period (OR): |                         |                        |                       |                                                                                                                                                   |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                  |                                                      | No                                                                                        | 230                     | 1                      |                       |                                                                                                                                                   |
|                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                  |                                                      | Yes                                                                                       | 5                       | [0.60 (0.22–1.67)]     |                       |                                                                                                                                                   |



**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design             | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                         | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                   | Comments                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------|-------------------------|------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Hall et al. (2021)</a><br>Denmark<br>1968–2016<br>Case–control | <i>Cases:</i> 178 germ cell tumours (50 yolk sac tumours, 65 teratomas) in children (aged < 16 yr), identified through the Danish Cancer Registry.<br><br><i>Controls:</i> 4355; alive at the time of case diagnosis, frequency-matched (25:1) on birth year and sex. Drawn from Central Population Registry.<br><br><i>Exposure assessment method:</i> Job title and industry were taken from the SPF, a mandatory supplement to the state pension in which all employed workers participate, apart from the self-employed. Exposure assessment by a JEM that had been previously created for the Nordic Occupational Cancer Study, based on air samples. | Germ cell tumours                                    | Paternal occupational exposure to gasoline from 3 mo preconception to birth (OR):<br>Never<br>Ever | NR<br>6                 | 1<br>0.57 (0.25–1.32)  | Birth year, sex, birth place (urban vs rural/ small town), paternal age | <i>Strengths:</i> Population-based, objective exposure, all germ cell tumours in children aged < 16 yr are included who were born in Denmark.<br><br><i>Limitations:</i> It was not possible to ascertain exposures of self-employed parents, because they do not participate in the SPF. |
|                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Germ cell tumours                                    | Paternal occupational exposure to gasoline from birth to diagnosis (OR):<br>Never<br>Ever          | NR<br>13                | 1<br>0.72 (0.40–1.28)  |                                                                         |                                                                                                                                                                                                                                                                                           |
|                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Germ cell tumours                                    | Maternal occupational exposure to gasoline from birth to diagnosis (OR):<br>Never<br>Ever          | NR<br>7                 | 1<br>2.14 (0.96–4.76)  | Birth year, sex, birth place (urban vs rural/ small town), maternal age |                                                                                                                                                                                                                                                                                           |

**Table 2.2 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                    | Cancer type (histopathology), incidence or mortality | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments |
|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------|-------------------------|------------------------|-----------------------|----------|
| <a href="#">Hall et al. (2021)</a><br>(cont.)                  | The gasoline estimates were given as “parts per million of benzene originating from gasoline in the workroom air”, which meant that “gasoline exposure” was given as the benzene concentration in the work environment air where gasoline has been used. Industries were classified via ISIC. |                                                      |                            |                         |                        |                       |          |

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ANLL, acute non-lymphoblastic leukaemia; Aus-ALL, Australian Study of Causes of Acute Lymphoblastic Leukaemia; CA, California; CI, confidence interval; CNS, central nervous system; CT, Connecticut; ELF-MF, extremely low-frequency magnetic field; ESCALE, Etude Sur les Cancers et les Leucémies de l'Enfant; FL, Florida; GIS, geographic information system; HIV, human immunodeficiency virus; HL, Hodgkin lymphoma; HOPE, Hepatoblastoma Origins and Pediatric Epidemiology; HR, hazard ratio; ISIC, International Standard Industrial Classification of All Economic Activities; JEM, job-exposure matrix; MI, Michigan; mo, month(s); MoBa, Norwegian Mother and Child Cohort; NC, not calculated; NHL, non-Hodgkin lymphoma; NJ, New Jersey; NO<sub>2</sub>, nitrogen dioxide; NR, not reported; OR, odds ratio; PA, Pennsylvania; PM<sub>10</sub>, particulate matter with diameter < 10 µm; RR, relative risk; SES, socioeconomic status; SPF, Supplementary Pension Fund; UK, United Kingdom; UKCCS, United Kingdom Childhood Cancer Study; USA, United States of America; vs, versus; wk, week(s); yr, year(s).

In eight studies, data were collected from parental interview and investigated parental occupational exposure to gasoline as assessed via self-report ([Kantor et al., 1979](#); [Shu et al., 1988](#); [De Roos et al., 2001](#); [Infante-Rivard et al., 2005](#); [McKinney et al., 2008](#); [Shi et al., 2013](#); [Jiang et al., 2016](#); [Janitz et al., 2017](#)). In four of these studies, industrial hygienist assessment based on job titles or tasks was additionally used ([De Roos et al., 2001](#); [Infante-Rivard et al., 2005](#); [McKinney et al., 2008](#); [Janitz et al., 2017](#)). In two other studies, parental occupations were obtained from state or national records, and in one of these studies, a JEM was applied to assign exposure ([Kantor et al., 1979](#); [Hall et al., 2021](#)). In three interview-based studies, both occupational and non-occupational (e.g. personal refuelling) exposures were assessed ([Tsai et al., 2006](#); [Bailey et al., 2011](#); [Kirkeleit et al., 2018](#)).

All studies reported on dichotomous exposures (ever versus never). Duration of exposure (number of days) was considered only in one study, and intensity of exposure was not investigated in any. The time period most often assessed was pregnancy. In several studies, information was also collected about preconception exposures, but few studies considered exposures that occurred during the child's life before cancer diagnosis.

In a separate group of studies, cancer risk was examined in relation to residential proximity to petrol stations, and these studies reported on tumours of the central nervous system (CNS) and haematopoietic cancers ([Harrison et al., 1999](#); [Brosselin et al., 2009](#); [Mazzei et al., 2022](#); [Malavolti et al., 2023](#); [Norzaee et al., 2024](#)). Two meta-analyses examined risk of leukaemia in relation to residential proximity to petrol stations ([Steinmaus and Smith, 2017](#); [Mazzei et al., 2022](#)).

The Working Group considered as uninformative those studies in which parental employment as a mechanic was examined or in which diverse parental occupations were grouped ([Kwa and Fine, 1980](#); [Zack et al., 1980](#); [van Steensel-Moll](#)

[et al., 1985](#); [Johnson et al., 1987](#); [Olshan et al., 1990](#); [Hum et al., 1998](#)). Also considered to be uninformative were those studies in which broader categories of exposures were grouped, for example, exposures to petroleum or petroleum products, unspecified fuels, refineries, repair garages, or oil products ([Spitz and Johnson, 1985](#); [Nasca et al., 1988](#); [Wilkins and Sinks, 1990](#); [Shu et al., 1995, 1999](#); [Buckley et al., 1997](#); [Knox and Gilman, 1997](#); [Smulevich et al., 1999](#); [Cordier et al., 2001](#); [Schüz et al., 2001](#); [Moore et al., 2005](#); [Reid et al., 2011](#); [Slater et al., 2011](#)). Additionally, the Working Group excluded studies in which risk estimates could not be derived ([Fabia and Thuy, 1974](#)) and ecological studies ([Weng et al., 2009](#)). With regard to studies on residential proximity to petrol stations, the Working Group excluded one article in which the distance between the residence and the petrol station was greater than 150 m (see Section 1.4.1), because exposure to gasoline was considered to be lower for longer distances ([Abdul Rahman et al., 2008](#)) and a second article in which there was no differentiation between proximity to gasoline stations and proximity to repair garages ([Steffen et al., 2004](#)). The Working Group also excluded three meta-analyses on residential proximity in which analyses of petrol stations were grouped with analyses that encompassed proximity to repair garages ([Filippini et al., 2015](#); [Carlos-Wallace et al., 2016](#); [Malavolti et al., 2023](#)).

Studies on occupational exposure during pregnancy and cancer risk in the offspring may be affected by live birth bias, a type of selection bias caused by restriction of the analysed sample to live births. Although there were few studies examining whether exposure to gasoline in pregnancy is related to spontaneous abortion or still-birth ([Xu et al., 1998](#); [Ooka et al., 2021](#)), a large, well-conducted study that included review of the exposure by an industrial hygienist reported that gasoline exposure in pregnancy increases the risk of spontaneous abortion ([Xu et al., 1998](#)). If gasoline did induce spontaneous abortion,

it is possible that some of the studies reviewed below were affected by live birth bias, especially those in which maternal occupational exposures occurring during pregnancy were explored ([Shu et al., 1988](#); [De Roos et al., 2001](#); [Infante-Rivard et al., 2005](#); [Tsai et al., 2006](#); [McKinney et al., 2008](#); [Janitz et al., 2017](#); [Kirkeleit et al., 2018](#); [Hall et al., 2021](#)). When live birth bias occurs, it biases exposure–outcome associations downward ([Leung et al., 2021](#)).

### 2.2.2 Childhood haematopoietic cancers

In the MoBa cohort (described in Section 2.1.9), crude associations were estimated between parental exposure and childhood ALL ([Kirkeleit et al., 2018](#)). A positive but imprecise association was estimated for ever maternal exposure to gasoline or exhaust and ALL (HR, 2.71; 95% CI, 0.97–7.58). In comparison to no exposure, maternal exposure of 30–180 days (suggested by the authors as being representative of occupational exposure) increased the risk of ALL (HR, 4.03; 95% CI, 1.25–13.0). With regard to paternal exposure, exposure of 1–29 days was associated with ALL risk, but 30–180 days was not. Maternal smoking and birth weight were taken into account in a sensitivity analysis (excluding mothers who smoked or with unknown smoking status at week 17 of pregnancy) and did not change results. [The Working Group noted the cohort design as a strength but noted as limitations the small number of events (the study being intended as a pilot), self-reported exposure, and lack of differentiation between exposure to gasoline and exhaust. Benzene exposure, modelled or measured as a marker of traffic exhaust, may be associated with childhood ALL ([Filippini et al., 2019](#)).]

A population-based case–control study ascertained cases from the Shanghai Cancer Institute, China, and controls from the general population in the same geographical area using neighbourhood population lists ([Shu et al.,](#)

[1988](#)). Participation rates were 99.7% for cases and 98.4% for controls. Information on parental occupation was collected by in-person interview, mostly with mothers. Parents were queried about their employment and occupational exposures, which were then classified according to the system of the Chinese Third National Population Census. Results showed that maternal occupational exposure to gasoline during pregnancy was associated with both ALL (OR, 1.7; 95% CI, 1.0–3.0) and ANLL (OR, 2.1; 95% CI, 1.1–4.3). [The Working Group noted that the strengths of the study included the population-based design and high participation rate. A limitation of the study was the lack of detailed information on the exposure assessment; it was not clear whether the questionnaire contained questions about gasoline exposure specifically or how exposure was assigned.]

A case–control study ascertained cases from all hospitals treating childhood leukaemia in Quebec, Canada, and controls from public records who were matched on sex and age ([Infante-Rivard et al., 2005](#)). Participation rates were 93.1% for cases and 86.2% for controls. Mothers were interviewed regarding all jobs held for 2 years before the index pregnancy until the child's birth, and were queried about specific exposures, tasks and the time spent on them, and the work environment. Data were collected on job tasks; materials and machines used; machine maintenance responsibilities; presence of gases, dusts, fumes, solvents, oils, and other agents; use of protective equipment; and an open-ended description of the mother's job activities. Additional specialized questionnaires were administered for jobs with a higher potential for occupational exposures. Industrial hygienists classified all jobs by the likelihood of each exposure, assigning jobs to standard Canadian job titles and classifying solvent exposures using subject matter knowledge for men's jobs in the region. On the basis of industrial hygienist review, the results showed a positive but imprecise increase in the risk of

childhood ALL after maternal occupational exposure during pregnancy to leaded gasoline (OR, 4.14; 95% CI, 0.46–37.16), but no increase with unleaded gasoline (OR, 0.83; 95% CI, 0.22–3.10) although the results were imprecise. [The Working Group noted that a strength of this study was the careful and detailed exposure assessment. There was potential for exposure misclassification if women were more or less likely to be occupationally exposed than were men with the same job titles. The Working Group also noted that, although the study had a large sample size, there was a small number of exposed mothers, leading to wide confidence intervals.]

In a population-based study in the UK, children with cancer and matched controls were recruited from service lists of the Family Health Services Authority (England and Wales) or Health Board (Scotland) ([McKinney et al., 2008](#)). Eligible cases were notified by paediatric oncologists. Controls were matched on age and sex and were randomly selected from the same health services list as the corresponding case. General practitioners were asked for their consent to approach the families of the controls ([UK Childhood Cancer Study Investigators, 2000](#)). Mothers and fathers were interviewed using separate but similar questionnaires and self-reported their occupational exposures. [McKinney et al. \(2008\)](#) focused on maternal exposure. In the exposure assessment, undertaken by experts who were blind to case–control status, exposure was assigned on the basis of exposure probability and exposure level. In a validation study, strong agreement between reviewers was found for exposure status and moderate agreement for exposure probability. On the basis of self-report, maternal exposure to petrol during pregnancy was related to an increase in risk of ALL (OR, 2.1; 95% CI, 1.2–3.6), with similar risk estimates for preconception and postnatal exposures. In contrast, expert assessment yielded a null risk for maternal exposure during pregnancy (OR, 0.9; 95% CI, 0.2–3.3), but an elevated risk for

maternal postnatal occupational exposures (OR, 2.4; 95% CI, 0.8–6.9). NHL was associated with self-reported maternal exposure to petrol during preconception (OR, 3.7; 95% CI, 1.8–7.5) and during pregnancy (OR, 4.4; 95% CI, 1.9–10.4). In contrast, when maternal exposure to petrol was determined by expert assessment, NHL was not associated with preconception exposure (OR, 1.1; 95% CI, 0.4–3.2), but an elevated although imprecise estimate was reported for postnatal exposure (OR, 3.0; 95% CI, 0.6–14.5) for NHL. Hodgkin lymphoma was not associated with maternal exposure to petrol in the preconception period, and statistical power was limited for the pregnancy and postnatal time periods. [The Working Group considered that petrol is an equivalent term for automotive gasoline. For only 33% of cases and 36% of controls was exposure rated as “certain”. The exposure agreement in the validation study was not reported. There was a moderate participation rate (64%) among controls. Latency was not taken into account in analyses of postnatal exposures; although some parents probably held the same job across the study period, this was not reported. The industrial hygienist analysis included only exposed parents, and those with partial exposure were not included, hindering the direct comparison of self-reported and expert-reviewed analyses to assess recall bias.]

In a case–control study in Shenzhen Children’s Hospital, China, 71 cases of ALL were ascertained from the Department of Hematology and Oncology. Controls ( $n = 142$ ), matched on gender and age, were selected from hospital patients treated for trauma caused by accidents ([Jiang et al., 2016](#)). Parents completed a questionnaire that collected information on their exposure to chemical factors, including occupational exposures, before and during pregnancy. Exposure was defined as exposure to a chemical substance for  $\geq 30$  days per year. Analyses co-adjusted for chemical exposures in the home. Paternal exposure to gasoline or diesel was

related to highly positive but imprecise associations with ALL in children (adjusted OR, 8.15; 95% CI, 2.68–24.83). Maternal pre-pregnancy exposure to gasoline or diesel was related to increases in ALL risk in the child (crude OR, 4.22; 95% CI, 1.08–16.54); exposure during pregnancy was not reported. [The Working Group noted that this study lacked detailed information on the exposure assessment, such as whether parents were specifically queried about gasoline exposure. Participation rates were not reported. Information was not provided on possible occupational co-exposures. The Working Group noted that the sample size was small leading to imprecise estimates, and results may have been subject to recall bias.]

In a case–control study in Shanghai, China, [Shi et al. \(2013\)](#) enrolled from three children's hospitals all children aged  $\leq 15$  years with acute leukaemia; children with Down syndrome or other genetic conditions were excluded ( $n = 201$ ). Controls were matched 1:1 on gender and age and selected from among children attending clinics for health or orthopaedic conditions in the same hospitals. Parents completed a questionnaire and underwent interviews. Mothers and fathers were asked about exposures to chemical substances in the 3 months before the start of pregnancy. Maternal exposure to gasoline or diesel before pregnancy was related to imprecise increases in the child's risk of leukaemia (OR, 2.8; 95% CI, 0.5–15.5), whereas paternal exposure to gasoline or diesel was not associated (OR, 0.9; 95% CI, 0.4–1.7). [The Working Group noted that the strengths of this study included the comprehensive adjustment for confounders, including by rural versus town residence, which may partially adjust for exposure to traffic pollution. Limitations included the potential for recall bias, lack of reporting of possible occupational co-exposures, and no reporting of participation rates. Limited information was available on exposure ascertainment. The pre-pregnancy period may not be the most relevant exposure

period, although women often hold the same jobs throughout preconception and pregnancy. Gasoline and diesel were combined in the analyses, which reduced the informativeness of the results for the present evaluation.]

In a national, population-based study of ALL in Australia, 416 cases were recruited among patients who had reached remission from all 10 paediatric oncology centres ([Bailey et al., 2011](#)). Controls were matched on sex, state of residence, and age, and recruited via random-digit dialling. In the questionnaire, mothers were queried about the number of times they had refuelled their vehicle for non-occupational use in the year before or during the pregnancy, as well as exposure (refuelling) for work purposes. Occupational and non-occupational refuelling were combined in the analyses. For petrol refuelling, high exposure was defined as four times or more per month for mothers and six times or more for fathers. The results showed no increase in ALL risk with any petrol refuelling by mothers (OR, 1.00; 95% CI, 0.71–1.41) or with a high frequency of petrol refuelling (OR, 1.10; 95% CI, 0.76–1.58). Any petrol refuelling by fathers (OR, 0.90; 95% CI, 0.54–1.51) and high frequency of petrol refuelling (OR, 0.99; 95% CI, 0.56–1.73) in the year before conception were also not associated with increases in ALL risk. [The Working Group noted that refuelling of one's personal vehicle probably represents a far lower exposure than that seen in other studies, which primarily focused on occupational exposures. Restricting the sample to children who had reached remission introduces some selection bias, but this bias was expected to be small because the survival rate for ALL is relatively high.]

Five studies were considered in which residential distance from gasoline stations was examined ([Harrison et al., 1999](#); [Brosselin et al., 2009](#); [Mazzei et al., 2022](#); [Malavolti et al., 2023](#); [Norzaee et al., 2024](#)).



Residential proximity to petrol stations was investigated in a study in the UK ([Harrison et al., 1999](#)). Cases were ascertained from district health authorities in the West Midlands. The cases' postcodes were taken from the cancer registration database and allocated to a 100 m<sup>2</sup> grid. The postal addresses of petrol stations were obtained from telephone directory records and similarly allocated to a grid reference. Cases of solid tumours were designated as controls for cases of leukaemia. Geographic information system (GIS) analysis determined the number of cases and controls within 100 m of a petrol station. The analyses among people living within 100 m of a petrol station showed an elevated risk of leukaemia, compared with risk of solid tumours (OR, 1.99; 95% CI, 0.73–5.43). [The Working Group noted that the strengths of this study included the population-based design and inclusion of all cancers registered in the region. The use of solid tumours as controls, which could bias results downward, limited the informativeness of the results for the present evaluation. There was probably some misclassification of exposure because of the reliance on grid reference rather than exact address to determine exposure.]

A study in France examined risk of acute leukaemia in families residing next to a petrol station ([Brosselin et al., 2009](#)). In this study, cases were recruited among children < 15 years, excluding cases who had died or those receiving palliative care, from all paediatric oncology departments in France. Controls were randomly selected from the French population, using a quota sampling method to make the controls similar to cases in age and sex, and also geographically similar to the overall French population. Participation rates were 91% for cases and 71.2% for controls. Controls were younger than cases and had moved less often. Telephone interviews were conducted with biological mothers of participants (765 cases and 1681 controls). Residential proximity was defined as at least one residence by a petrol station between the

date of conception and the date of diagnosis (cases) or interview (controls). “Next to a business” indicated that there was no building or road between the residence and the business of interest. Residential proximity to a petrol station, between conception and diagnosis, was associated with an elevated risk of acute leukaemia (OR, 2.1; 95% CI, 1.1–4.0) and of ALL (OR, 2.0; 95% 1.0–4.0). [The Working Group noted that the strengths of this study included the large sample size, the nationwide study design, and that the questionnaire included specific questions about petrol stations close to the residence, although the self-reported ascertainment may have introduced recall bias. The Working Group noted that the actual distance between the home and the petrol station was not ascertained; thus, it was not clear what proportion fell within 150 m, which reduced the informativeness of the study for the present evaluation.]

In a nationwide study in Switzerland, residential distance to petrol stations was examined ([Mazzei et al., 2022](#)). Researchers identified cases of paediatric cancer from the Swiss Childhood Cancer Registry from 1985 to 2015 (1880 cases of leukaemia, 18 800 controls). Addresses at birth and diagnosis were obtained from the cancer registry. Successful geocoding was possible for 93% of cases. Controls were obtained from Swiss national censuses (1990, 2000, and annually from 2010 to 2015) and matched 10:1 on age, sex, and census year of diagnosis. Petrol station location was obtained from business censuses (1995, 1998, 2001, 2005, 2008, and annually starting in 2011). The authors calculated the minimum distance from the case and control residences to the nearest petrol station. Cases diagnosed between census years were linked to the nearest station in the year closest to the case's year of diagnosis. No associations with leukaemia (all leukaemia types combined) were observed for residence within 0–50 m of a petrol station, either at birth (OR, 0.52; 95% CI, 0.16–1.67) or at diagnosis (OR, 1.08; 95% CI, 0.46–2.51), or for

residence within >50–100 m of a petrol station. [The Working Group noted that the strengths of the study included the population-based design and the reliance on GIS-based measures of petrol station proximity. The Working Group noted the small sample size for participants living within 50 m of a petrol station. Probably because of the small sample size, the authors did not stratify by leukaemia subtype. There was a risk of misclassification of residences if participants had moved between the census years, which would have biased results to the null.]

In a study of two provinces in Italy, [Malavolti et al. \(2023\)](#) examined distance to the nearest petrol station in relation to childhood leukaemia. Researchers identified cases through the Italian hospital-based registry of childhood malignancies (182 cases of leukaemia and 148 cases of ALL). Controls ( $n=726$ ) were matched on sex, year of birth, and province of residence at the time of diagnosis, and were selected at random from children enrolled in the National Health Service directory, in which all residents are compulsorily registered. Residential address at diagnosis was drawn from the cancer registry and, for controls, in the corresponding year from the National Health Service. Information on petrol stations was obtained from the Trade Observatory. The results showed that, compared with residence 1000 m or further, residence < 50 m from a petrol station was associated with imprecise increases in risk of leukaemia (OR, 2.2; 95% CI, 0.5–9.4) and ALL (OR, 2.9; 95% CI, 0.6–13.4). [The Working Group noted the adjustment for particle matter of diameter < 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ), the population-based design, use of an objective assessment of residential location, and reporting of childhood ALL as important strengths that increased the informativeness of this study for the present evaluation.]

In a hospital-based study in Iran, [Norzaee et al. \(2024\)](#) examined residential distance to a petrol station. Cases were ascertained from three children's hospitals in Tehran (428 cases of leukaemia, including 328 cases of ALL and

100 cases of AML; and 428 cases of lymphoma, including 97 cases of NHL and 331 cases of Hodgkin lymphoma). Controls ( $n=428$ ) were selected from hospital records of children visiting the hospital for check-ups or for non-blood disease-related reasons (fractures, osteoarticular disorders, trauma, injuries, gastrointestinal diseases, infections, poisoning). Eligibility was limited to those who had resided in their home for  $\geq 1$  year before the diagnosis date. Residential addresses were obtained from hospital records. Investigators used GIS to identify land uses within the 100 m buffer around the home. Positive associations were observed between residence within 100 m of a petrol station and risk of leukaemia (OR, 2.12; 95% CI, 0.98–4.58), but not of lymphoma (OR, 1.05; 95% CI, 0.46–2.43). [The Working Group noted that a strength of this study was that the investigators included all eligible children, without relying on willingness to participate. Exposure assessment was based on objective assessment rather than self-report. These strengths increased the informativeness of the study for the present evaluation.]

Three meta-analyses in which residential proximity to either a petrol station or repair garage was examined were therefore considered uninformative for the present evaluation ([Filippini et al., 2015](#); [Carlos-Wallace et al., 2016](#); [Malavolti et al., 2023](#)). A meta-analysis was conducted to examine residential proximity to petrol stations only, in relation to risk of acute leukaemia ([Steinmaus and Smith, 2017](#)). The meta-analysis was reported in three publications ([Harrison et al., 1999](#); [Steffen et al., 2004](#); [Brosselin et al., 2009](#)). Although effect estimates unique to gasoline stations were available in the articles by [Harrison et al. \(1999\)](#) and [Brosselin et al. \(2009\)](#), the article by [Steffen et al. \(2004\)](#) solely provided risk estimates for gasoline stations and repair garages, combined. The 2023 meta-analysis ([Malavolti et al., 2023](#)) included the three studies above as well as the results of the Swiss study ([Mazzei et al., 2022](#)). The authors

reported that the meta-OR for residential proximity to petrol stations and leukaemia was 2.01 (1.25–3.22). [The Working Group noted the small number of studies that contributed to meta-analyses, the inclusion of one study that examined repair garage proximity, and the inclusion of one study that used other cancer types as controls ([Harrison et al., 1999](#)). A Working Group meta-analysis was therefore deemed to be warranted (see [Figs 2.1–S2.5](#) in Section 2.7 and Annex 3, Supplementary figures for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>).]

### 2.2.3 Other childhood cancers

#### (a) Tumours of the central nervous system

In a study in Switzerland (see description above), [Mazzei et al. \(2022\)](#) examined associations between residence within 50 m of a petrol station and CNS tumours ( $n = 1290$ ). Address at diagnosis was weakly and imprecisely associated with risk of CNS tumours during the entire study period (OR, 1.30; 95% CI, 0.51–3.35). [The Working Group noted that the strengths of the study included the population-based design and the reliance on GIS-based measures of proximity to a petrol station. The Working Group noted the small sample size of participants who resided within 50 m of a petrol station. There was a risk of misclassification of residence if participants had moved home between the census years, which would have biased results to the null.]

#### (b) Neuroblastoma

Cases of neuroblastoma ( $n = 538$ ) were recruited from Children's Oncology Group hospitals in the USA and Canada in 1992–1996, with a response rate of 73% ([Olshan et al., 1999](#)). An update of this study limited occupations to those within 2 years of the child's birth, and an industrial hygienist conducted a review of self-reported exposures, focusing on increasing specificity ([De Roos et al., 2001](#)). This latest analysis was

considered more informative by the Working Group. Controls ( $n = 504$ ) were identified by random-digit dialling, with a response rate of 74%. Investigators interviewed every participating mother and also the father when available (88% of cases; 88% of controls). Some paternal interviews were obtained by proxy interviews with the mother (14.2% of case fathers and 31.7% of control fathers). Parents were prompted to recall all occupations since age 18 years, using an interview guide. Parents were asked about specific chemicals, including the form and routes of exposure, the work activities being performed, and the dates of exposure. The industrial hygienist, blind to case status and parent's gender, indicated that the parent was occupationally exposed if exposure was probable in that occupation. Maternal self-reported exposure to gasoline was positively but imprecisely associated with neuroblastoma (OR, 1.6; 95% CI, 0.6–3.8), whereas classification of gasoline exposure by the industrial hygienist did not show an association (OR, 0.8; 95% CI, 0.2–4.2). Paternal occupational exposure to gasoline was not related to neuroblastoma, either when exposure was self-reported or assigned by the industrial hygienist. Because the review by the industrial hygienist had focused on decreasing the number of false positives, the authors described the possibility of false negatives in the results. [The Working Group noted that a strength of this analysis was the large sample size and the review by an industrial hygienist. The conservative approach taken by the industrial hygienist may have biased effect estimates downward.]

#### (c) Wilms tumour

In a study comparing paternal occupation among cases of Wilms tumour and controls, [Kantor et al. \(1979\)](#) ascertained all cases of Wilms tumour reported to the Connecticut Tumor Registry in 1935–1973 and who were born in Connecticut, USA. Cases ( $n = 149$ ) were matched to birth certificate records. Controls ( $n = 149$ ),

matched on sex, race, and year of birth, were selected from birth certificate records. The occupation of the father was obtained from the birth certificate. Classification of occupations followed the design of earlier studies in which a specialist in industrial hygiene, blind to case status, identified occupations with potential exposure to hydrocarbons ([Fabia and Thuy, 1974](#); [Hakulinen et al., 1976](#)). [Kantor et al. \(1979\)](#) reported in the text, but did not show in the tables, that cases and controls were similar with regard to birth weight, maternal pregnancy history, gestational age, and parental ages and places of birth. Results showed that three case fathers and no control fathers had been employed as service station attendants. [The Working Group noted the study's strengths, which were the population-based study design and the reliance on prospectively collected records of occupations, which lessens recall and selection biases. The completeness of the tumour registry was not reported. The study design did not allow ascertainment of the duration of employment of the fathers in the given occupations, thus there was the possibility of misclassification of the occupation in the periconception period, which would bias the results towards the null. It was not possible to assess the comparability of the cases and controls, but the statement that their birth weights were comparable may suggest that the matching was not optimal, because Wilms tumour is typically associated with high birth weight.]

A study on Wilms tumour was conducted in six states where 303 cases were identified through state cancer registries in the USA ([Tsai et al., 2006](#)). Controls ( $n=575$ ) were recruited through random-digit dialling. In a standard questionnaire given to mothers, they were asked about occupational history. Exposure to gasoline during pregnancy was not related to the odds of Wilms tumour (OR, 0.76; [95% CI, 0.26–2.17]). [The Working Group noted the lack of information provided about gasoline exposure

assessment, making it difficult to assess the validity of the study.]

#### (d) *Hepatoblastoma*

[Janitz et al. \(2017\)](#) carried out a study in 383 children with hepatoblastoma recruited from hospitals that were members of the Children's Oncology Group (a nationwide clinical trials group) in the USA. Controls ( $n=387$ ) were selected from birth certificates from 32 states and frequency-matched to cases on year of birth, region, sex, and birth weight. Participation rates were 60.7% among case families and 51.3% among control families ([Puumala et al., 2012](#)). Cases and controls differed by maternal educational attainment, maternal race/ethnicity, and by birth weight. In the interview, mothers were asked about jobs held between 1 year before the index pregnancy and the child's birth, including job titles, duties, and exposures. On the basis of job classification, an industrial hygienist classified exposures as likely, possible, or unlikely. Likely exposure to gasoline/diesel was not associated with hepatoblastoma either for maternal (OR, 1.03; 95% CI, 0.36–2.97) or paternal exposures (OR, 0.87; 95% CI, 0.55–1.36). [The Working Group noted the low participation rates, and demographic differences between cases and controls. The inability to successfully match on birth weight was not surprising given the low birth weights commonly seen in hepatoblastoma ([Spector and Birch, 2012](#)). This was a large sample size for a study on hepatoblastoma. The inclusion of industrial hygienist review was a strength.]

#### (e) *Germ cell tumours*

In a population-based study, [Hall et al. \(2021\)](#) ascertained 178 cases of germ cell tumour from the cancer registry and 4355 controls matched 25:1 on sex and age from national records in Denmark. Parental employment was taken from national pension records. The authors employed a JEM developed for the NOCCA that was based upon on air samples collected at thousands of



workplaces. Applying this JEM to parental occupational history, the authors estimated that there was no increased risk of germ cell tumour associated with paternal exposure before conception (3 months before pregnancy) or with paternal exposure in the child's life before diagnosis. With regard to mothers, maternal occupational exposure during the child's life (from birth to cancer diagnosis) was related to an increase in the odds of germ cell tumours (OR, 2.14; 95% CI, 0.96–4.76), but exposure to gasoline during pregnancy was too rare to be informative. [Because the number of case mothers exposed during pregnancy was <5, effect estimates were not reported for that time period. Latency was not considered in the analyses of postnatal exposure. The Working Group considered it plausible that maternal occupational gasoline exposure would affect child exposure in the postnatal period.]

### 2.3 Adult lymphatic and haematopoietic malignancies

See [Table 2.3](#).

The association between exposure to automotive gasoline and risk of lymphatic and haematopoietic malignancies in adults has been assessed in quite heterogeneous settings. Prospective results for gas station workers were obtained from an occupational cohort study ([Lagorio et al., 1994a](#)), a pooled analysis of occupational cohort studies ([Lyngé et al., 1997](#)), and a cohort study in the general population ([Neasham et al., 2011a](#)). Other cohort or nested case-control studies involved an environmentally exposed population ([Patel et al., 2004](#); [Talbot et al., 2011](#)), refinery distribution workers ([Schnatter et al., 1993, 1996, 2012](#); [Rushton and Romaniuk, 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#); [Sorahan and Mohammed, 2016](#)), or people with occupational exposure to gasoline ([Heineman et al., 1992](#)). There were 11 case-control studies on exposure to gasoline alone ([Siemiatycki et al., 1987a](#); [Holly](#)

[et al., 1997](#); [Persson and Fredrikson, 1999](#); [Nisse et al., 2001](#); [Orsi et al., 2007](#); [Cocco et al., 2010](#); [Lv et al., 2011](#); [Morales-Suárez-Varela et al., 2013](#); [Poynter et al., 2017](#)) or together with diesel exposure ([West et al., 1995](#); [Björk et al., 2001](#)), and two case-control studies on both exposure to gasoline (one study on gasoline and diesel together) and employment as a service station attendant ([Clavel et al., 1995, 1996](#); [Dryver et al., 2004](#)).

The cohort studies by [Grandjean and Andersen \(1991b\)](#) and by [Jakobsson et al. \(1993\)](#) were excluded because cohort members were also included in the pooled analysis of the studies in Nordic countries by [Lyngé et al. \(1997\)](#). The cohort study by [Wong et al. \(1993\)](#) was superseded by the nested case-control study by [Wong et al. \(1999\)](#). The case-control studies by [Farrow et al. \(1989\)](#), [Nisse et al. \(1995\)](#), and [Lv et al. \(2007\)](#) were updated by [West et al. \(1995\)](#), [Nisse et al. \(2001\)](#), and [Lv et al. \(2011\)](#). The two case-control studies by [Persson et al. \(1989\)](#) and [Persson et al. \(1993\)](#) were pooled in [Persson and Fredrikson \(1999\)](#). The study by [Perrotta et al. \(2012\)](#) overlapped with that by [Cocco et al. \(2010\)](#) and was therefore excluded. Cohort studies and nested case-control studies of seamen in Nordic countries (including [Andersen et al., 1999](#) and [Nilsson et al., 1998](#)) were not included, because they were uninformative regarding exposure to automotive gasoline. The case-control studies by [Karunanayake et al. \(2008\)](#) and by [Nadon et al. \(1995\)](#), identified as being included in the meta-analysis by [Kane and Newton \(2010\)](#) described below, were excluded from the present evaluation, because the exposures assessed were to crude petroleum in the former study and gasoline emissions in the latter. The case-control study on AML by [Park et al. \(2023\)](#) was excluded because cases included only patients referred to the hospital occupational health department (57% of all patients), and thus participation in the study was strongly driven by the type and nature of occupational exposure: jobs entailing exposure to carcinogens suspected or known

**Table 2.3 Epidemiological studies on exposure to automotive gasoline and adult lymphatic and haematopoietic malignancies**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                                                                      | Population size, description<br>Exposure assessment method                                                                                                                                                                                                             | Cancer type (histopathology), incidence or mortality                                                                                                                                             | Exposure category or level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Exposed cases or deaths                                                                         | Risk estimate (95% CI)                                                                                                                                                                                                                | Covariates controlled       | Comments                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Lyng et al. (1997)</a><br>Service station attendant cohort, Denmark, Norway, Sweden, and Finland<br>1970 census/ follow-up ending in 1987 (Denmark), 1991 (Norway), 1989 (Sweden), 1985 (Finland) (incidence)<br>Cohort | 16 524 men and 2445 women; workers in the service station industry identified via the 1970 census in Denmark, Norway, Sweden, Finland. Incidence (ICD-7) through the national cancer registries.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Lymphatic and haematopoietic (ICD-7, 200–204)<br><br>NHL (ICD-7, 200, 202)<br><br>NHL (CLL)<br><br>Multiple myeloma (ICD-7, 203)<br><br>HL (ICD-7, 201)<br><br>Leukaemia (ICD-7, 204)<br><br>AML | Employment in the service station industry (national referent) (SIR):<br>Men<br>Women<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women | 82<br>4<br><br>37<br>2<br><br>8<br>2<br><br>9<br>0<br><br>10<br>0<br><br>26<br>2<br><br>13<br>0 | [0.9 (0.7–1.1)]<br>[0.4 (0.1–1.1)]<br><br>1.1 (0.8–1.5)<br>0.6 (0.0–2.0)<br><br>0.8 (0.3–1.6)<br>2.7 (0.3–9.6)<br><br>0.6 (0.3–1.1)<br>NC<br><br>1.0 (0.5–1.8)<br>NC<br><br>0.9 (0.6–1.3)<br>0.7 (0.1–2.4)<br><br>1.4 (0.8–2.4)<br>NC | Country, age, calendar time | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Study of the entire population; large sample size; incident data.<br><br><i>Limitations:</i> Exposure assessment on the basis of having worked in the gasoline service station industry rather than as an attendant with refuelling activities. |



**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality                                                    | Exposure category or level                                                                                                                                                                                                                                                                                                                                              | Exposed cases or deaths                                                    | Risk estimate (95% CI)                                                                                                                              | Covariates controlled                                               | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Lynge et al. (1997)</a><br>(cont.)                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Leukaemia (all other leukaemia)                                                                         | Employment in the service station industry (national referent) (SIR):<br>Men<br>Women                                                                                                                                                                                                                                                                                   | 5<br>0                                                                     | 0.5 (0.2–1.2)<br>NC                                                                                                                                 | Country, age, calendar time                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <a href="#">Heineman et al. (1992)</a><br>Denmark<br>1970–1984<br>Nested case–control | <i>Source cohort:</i><br>Individuals aged > 18 yr or born after 1897 included in the Danish SPF.<br><i>Cases:</i> 791 men diagnosed with multiple myeloma and reported to the Danish Cancer Registry.<br><i>Controls:</i> 3070 population controls (from Danish Central Population Registry), matched on sex and year of birth (4:1). Participants with a diagnosis of malignancy > 91 days before matched case diagnosis date were excluded as controls.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Multiple myeloma (ICD-8, 203)<br><br>Multiple myeloma (ICD-8, 203)<br><br>Multiple myeloma (ICD-8, 203) | Wholesale trade fuel/oil/gas (OR):<br>Never<br>Ever<br><br>Occupational exposure to gasoline (OR):<br>Never<br>Possible<br>Probable<br><br>Possible occupational exposure to gasoline (10-yr lag period) (OR):<br>Never<br>> 1 mo to < 5 yr<br>≥ 5 yr<br><br>Probably occupational exposure to gasoline (10-yr lag period) (OR):<br>Never<br>> 1 mo to < 5 yr<br>≥ 5 yr | NR<br>12<br><br>NR<br>146<br>41<br><br>NR<br>51<br>36<br><br>NR<br>18<br>6 | 1<br>0.8 (0.4–1.6)<br><br>1<br>1.2 (1.0–1.5)<br>1.4 (0.9–2.1)<br><br>1<br>0.9 (0.6–1.3)<br>1.3 (0.7–2.2)<br><br>1<br>1.6 (0.8–3.2)<br>0.7 (0.2–1.9) | Age<br><br><br>Age, engine exhausts, phthalates, and vinyl chloride | <i>Strengths:</i> Assessment of gasoline exposure by occupational hygienists on the basis of occupational histories obtained from a registry; large sample size; focus on a specific lymphatohaematopoietic malignancy; 10-yr exposure latency; adjustment for exposure to other occupational agents.<br><br><i>Limitations:</i> Occupational history was not available before 1964 and was more incomplete for occupations, implying that earlier jobs could not be considered. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                            | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                            | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                     | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                         |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Schnatter et al. (1993)</a><br>Update of <a href="#">Schnatter et al. (1992)</a><br>Distribution workers, Canada 1964–1983 (mortality) Cohort | 6672; all active male employees and living retirees as of 1 January 1964 or new male employees (1964–1983) with ≥ 1 yr of employment in marketing and distribution for petroleum industries in Canada. Mortality data from Statistics Canada, National Death Index, or other sources (regional registries). There were 4889 marketing or distribution workers ever exposed to hydrocarbons.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | NHL                                                  | Distribution workers exposed to hydrocarbons, (national referent) (SMR):<br>Any exposure level | 4                       | 0.78 (0.21–2.00)       | Age, calendar year    | <i>Strengths:</i> Analyses restricted to workers ever exposed to hydrocarbons.<br><br><i>Limitations:</i> Small sample size; co-exposure to other potential occupational carcinogens was likely. |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | NHL                                                  | Distribution workers exposed to hydrocarbons (national referent) (SMR):<br>Less than daily     | 4                       | [1.7 (0.5–4.4)]        |                       |                                                                                                                                                                                                  |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Multiple myeloma                                     | Distribution workers exposed to hydrocarbons (national referent) (SMR):<br>Any exposure level  | 5                       | 1.99 (0.64–4.63)       |                       |                                                                                                                                                                                                  |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Multiple myeloma                                     | Distribution workers exposed to hydrocarbons (national referent) (SMR):<br>Less than daily     | 2                       | [1.67 (0.2–6.8)]       |                       |                                                                                                                                                                                                  |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                      | Daily                                                                                          | 3                       | [2.28 (0.5–6.7)]       |                       |                                                                                                                                                                                                  |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                      | Exposed to hydrocarbons (national referent) (SMR):<br>Any level                                | 7                       | 1.01 (0.40–2.08)       |                       |                                                                                                                                                                                                  |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Leukaemia                                            | Exposed to hydrocarbons (national referent) (SMR):<br>Any level                                | 4                       | [0.84 (0.2–2.2)]       |                       |                                                                                                                                                                                                  |
|                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Leukaemia (myeloid/ lymphatic)                       | Exposed to hydrocarbons (national referent) (SMR):<br>Any level                                | 4                       | [0.84 (0.2–2.2)]       |                       |                                                                                                                                                                                                  |

**Table 2.3 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                                             | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                              | Cancer type (histopathology), incidence or mortality                                                                                                                                       | Exposure category or level                                                                                                                                                                                                                                                                                                       | Exposed cases or deaths | Risk estimate (95% CI)                                                               | Covariates controlled             | Comments                                                                                                                                                                                                                                                                                                                                                    |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------------------------------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Schnatter et al. (1996)</a><br>Update of <a href="#">Schnatter et al. (1992)</a><br>Distribution workers, Canada 1964–1983 (mortality)<br>Nested case–control                                 | <i>Source cohort:</i> See <a href="#">Schnatter et al. (1993)</a><br><br><i>Cases:</i> 14; leukaemia recorded as cause of death in death certificate.<br><br><i>Controls:</i> 55; 4 controls per case, frequency-matched on decade of birth, alive at the time of case death.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Leukaemia                                                                                                                                                                                  | Per year of employment (OR):<br>Per year of employment                                                                                                                                                                                                                                                                           | 14                      | 1.061 (0.985–1.144)                                                                  | Age, calendar year                | <i>Strengths:</i> Analysis restricted to participants exposed to hydrocarbons.<br><br><i>Limitations:</i> Small sample size; co-exposure to other potential occupational carcinogens was likely. No analysis by leukaemia subtypes.                                                                                                                         |
| <a href="#">Sorahan (2007)</a><br>Update of <a href="#">Rushton (1993a)</a><br>UK<br>Enrolment, 1946–1975/<br>follow-up, 1951–2003 (mortality), 1971–2003 (incidence) (incidence and mortality)<br>Cohort | 16 477 male workers in petroleum distribution centres first employed in 1946–1974 with ≥ 1 yr employment and some employment after 1951.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> .                                                                                                                                      | NHL (lymphosarcoma, ICD-9, 200, 202), incidence<br>NHL (lymphosarcoma, ICD-9, 200, 202), mortality<br>Multiple myeloma (ICD-9, 203), incidence<br>Multiple myeloma (ICD-9: 203), mortality | Distribution workers employment (national referent) (SRR):<br>Ever employment<br>Distribution workers employment (national referent) (SMR):<br>Ever employment<br>Distribution workers employment (national referent) (SRR):<br>Ever employment<br>Distribution workers employment (national referent) (SMR):<br>Ever employment | 84<br>54<br>38<br>29    | [0.96 (0.77–1.19)]<br>[1.00 (0.75–1.31)]<br>[0.96 (0.68–1.32)]<br>[0.96 (0.65–1.38)] | Age, calendar year, country in UK | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Relatively large sample size; analysis by lymphatic and haematopoietic malignancies.<br><br><i>Limitations:</i> Possible co-exposure with other occupational carcinogens and lack of control for occupational confounders. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                         | Population size, description<br>Exposure assessment method | Cancer type (histopathology), incidence or mortality | Exposure category or level                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled             | Comments |
|----------------------------------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------|-------------------------|------------------------|-----------------------------------|----------|
| <a href="#">Sorahan (2007)</a><br>Update of <a href="#">Rushton (1993a)</a><br>(cont.) |                                                            | HL (ICD-9, 201), incidence                           | Distribution workers employment (national referent) (SRR):  |                         |                        | Age, calendar year, country in UK |          |
|                                                                                        |                                                            |                                                      | Ever employment                                             | 11                      | [0.79 (0.40–1.42)]     |                                   |          |
|                                                                                        |                                                            | HL (ICD-9, 201), mortality                           | Distribution workers employment (SMR):                      |                         |                        |                                   |          |
|                                                                                        |                                                            |                                                      | Hodgkin disease                                             | 15                      | [1.24 (0.69–2.04)]     |                                   |          |
|                                                                                        |                                                            | Leukaemia (ICD-9, 204–208), incidence                | Distribution workers employment (national referent) (SRR):  |                         |                        |                                   |          |
|                                                                                        |                                                            |                                                      | Ever employment                                             | 81                      | [1.18 (0.94–1.47)]     |                                   |          |
|                                                                                        |                                                            | Leukaemia (ICD-9, 204–208), mortality                | Job title (ever employment) (national referent) (SMR):      |                         |                        |                                   |          |
|                                                                                        |                                                            |                                                      | Any job title                                               | 67                      | [1.24 (0.96–1.57)]     |                                   |          |
|                                                                                        |                                                            |                                                      | Operator                                                    | 19                      | [1.48 (0.89–2.32)]     |                                   |          |
|                                                                                        |                                                            |                                                      | Driver                                                      | 26                      | [1.10 (0.72–1.61)]     |                                   |          |
|                                                                                        |                                                            | Administrative and clerical                          | 8                                                           | [1.70 (0.73–3.35)]      |                        |                                   |          |
|                                                                                        |                                                            | Supervisors                                          | 5                                                           | [1.82 (0.59–4.24)]      |                        |                                   |          |
|                                                                                        |                                                            | Leukaemia (monocytic, ICD-9, 206), incidence         | Distribution workers employment (national referent) (SRR):  |                         |                        |                                   |          |
|                                                                                        |                                                            |                                                      | Ever employment                                             | 3                       | [2.73 (0.56–7.97)]     |                                   |          |
|                                                                                        |                                                            | Leukaemia (monocytic, ICD-9, 206), mortality         | Distribution workers employment, (national referent) (SMR): |                         |                        |                                   |          |
|                                                                                        |                                                            |                                                      | Ever employment                                             | 1                       | [0.71 (0.02–3.98)]     |                                   |          |
|                                                                                        |                                                            | Leukaemia (lymphoid, ICD-9, 204), incidence          | Distribution workers employment (national referent) (SRR):  |                         |                        |                                   |          |
|                                                                                        |                                                            |                                                      | Ever employment                                             | 32                      | [1.01 (0.69–1.42)]     |                                   |          |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                            | Population size, description<br>Exposure assessment method | Cancer type (histopathology), incidence or mortality           | Exposure category or level                                                    | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled             | Comments |
|-------------------------------------------------------------------------------------------|------------------------------------------------------------|----------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------------|----------|
| <a href="#">Sorahan (2007)</a><br>Update of<br><a href="#">Rushton (1993a)</a><br>(cont.) |                                                            | Leukaemia (lymphoid, ICD-9, 204), mortality                    | Distribution workers employment (SMR):<br>Ever employment                     | 23                      | [1.42 (0.90–2.13)]     | Age, calendar year, country in UK |          |
|                                                                                           |                                                            | Leukaemia (myeloid, ICD-9, 205, which includes AML), incidence | Distribution workers employment (national referent) (SRR):<br>Ever employment | 42                      | [1.33 (0.96–1.80)]     |                                   |          |
|                                                                                           |                                                            | Leukaemia (myeloid), ICD-9, 205 which includes AML), mortality | Distribution workers employment (national referent) (SMR):<br>Ever employment | 36                      | [1.17 (0.82–1.62)]     |                                   |          |
|                                                                                           |                                                            | Leukaemia (subtypes), mortality                                | Only for the period 1968–2003 (SMR):                                          |                         |                        |                                   |          |
|                                                                                           |                                                            |                                                                | CLL                                                                           | 18                      | [1.50 (0.89–2.37)]     |                                   |          |
|                                                                                           |                                                            |                                                                | AML                                                                           | 29                      | [1.39 (0.93–2.00)]     |                                   |          |
|                                                                                           |                                                            |                                                                | ANLL                                                                          | 30                      | [1.27 (0.86–1.81)]     |                                   |          |

**Table 2.3 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                              | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Cancer type (histopathology), incidence or mortality | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled             | Comments                                                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------|-------------------------|------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Rushton and Romaniuk (1997)</a><br>UK<br>(distribution workers cohort)<br>Enrolment, not specified to 1975/follow-up, 1993<br>(incidence and mortality)<br>Nested case-control | <i>Source cohort:</i> Male workers in petroleum distribution centres.<br><br><i>Cases:</i> 90; cases were men who died from leukaemia or were registered with a diagnosis of leukaemia in the cancer register, which was available since 1971.<br><br><i>Controls:</i> 354; 4 controls for each case were randomly selected from the cohort, within an interval of 3 yr from the case's date of birth.<br><br><i>Exposure assessment method:</i> See <a href="#">Sorahan (2007)</a> in <a href="#">Table 2.1</a> . | NHL (CLL)                                            | Duration (yr) (OR):        |                         |                        | Age, calendar year, country in UK | <i>Exposure assessment critique:</i> See <a href="#">Sorahan (2007)</a> in <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Relatively large sample size; analysis by lymphatic and haematopoietic malignancies.<br><br><i>Limitations:</i> Possible co-exposure with other occupational carcinogens and lack of control for occupational confounders. |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Leukaemia                                            | Per year                   | 31                      | 1.06 (1.01–1.11)       |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                      | Duration (yr) (OR):        |                         |                        |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                      | Per year                   | 31                      | 1.03 (1.00–1.05)       |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | AML and monocytic leukaemia                          | Duration (yr) (OR):        |                         |                        |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                      | Per year                   | 31                      | 1.03 (0.99–1.07)       |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | CML                                                  | Duration (yr) (OR):        |                         |                        |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                      | Per year                   | 11                      | 1.04 (0.96–1.12)       |                                   |                                                                                                                                                                                                                                                                                                                                                                                           |



Table 2.3 (continued)

| Reference, location, enrolment/ follow-up period, study design                                                                                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                        | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                               | Exposed cases or deaths | Risk estimate (95% CI)                                                               | Covariates controlled             | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|---------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------------------------------------------------------------------------------|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Sorahan and Mohammed (2016)</a><br>UK<br>1995–2011 (incidence)<br>Cohort                                                              | 16 467; workers in petroleum distribution centres first employed in 1946–1974 with ≥ 1 yr employment and some employment after 1951. Incidence data on MDS were available from 1995 to 2011.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> .                                                                                            | MDS                                                  | Duration of employment (national referent) (SRR):<br>Ever employment<br>1–9 yr<br>10–19 yr<br>≥ 20 yr<br>Trend-test <i>P</i> value, 0.99 | 21<br>7<br>6<br>8       | [0.77 (0.48–1.18)]<br>[0.80 (0.32–1.65)]<br>[0.72 (0.26–1.56)]<br>[0.79 (0.34–1.56)] | Age, calendar year, country in UK | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><i>Design and analysis:</i><br><i>Strengths:</i> Relatively large sample size; analysis focused on a specific lymphatic and haematopoietic malignancy.<br><i>Limitations:</i> Possible co-exposure with other occupational carcinogens and lack of control for occupational confounders.                                                                                                                                                                         |
| <a href="#">Schnatter et al. (2012)</a><br>UK, Australia, Canada<br>Canada, 1964–1983; UK, 1950–1997; Australia, 1981–1999<br>Nested case–control | <i>Source cohort:</i><br>Male petroleum workers cohort: 1-yr employment inclusion criteria for UK and Canada, 5 yr for Australia.<br><br><i>Cases:</i> 370; cases of haematolymphoid malignancy (via mortality or cancer incidence registries). AML ( <i>n</i> = 60), CLL ( <i>n</i> = 80), CML ( <i>n</i> = 28), MDS ( <i>n</i> = 29), and MPD ( <i>n</i> = 30). | NHL (CLL)<br><br>AML                                 | OR:<br>Other workers<br>Ever tanker driver (≥ 1 yr)<br>OR:<br>Other workers<br>Ever tanker driver (≥ 1 yr)                               | NR<br>NR<br>NR<br>NR    | 1<br>0.64 (0.36–1.14)<br>1<br>2.02 (1.08–3.78)                                       | Matching factors                  | <i>Comments:</i> Only the analysis for “tanker drivers” was considered relevant for gasoline evaluation as they were thought to represent a petroleum distribution population with probable exposure to gasoline. Overlap with the UK and Canadian distribution cohorts ( <a href="#">Schnatter et al., 1996</a> ; <a href="#">Sorahan, 2007</a> ; <a href="#">Sorahan and Mohammed, 2016</a> ). Not included in the meta-analysis because of overlap and because this analysis was interpreted in the context of dose–response relation |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------|-------------------------|------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Schnatter et al. (2012)</a><br>(cont.)             | <i>Controls:</i> 1587; cancer-free at case diagnosis, five age-matched (Australian study) or four age- and company-matched (Canadian and UK studies).<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | MDS                                                  | OR:                         |                         |                        |                       | (see Limitations) and comparison with a group of exposed participants, and preference in the meta-analysis was given when the comparison was done with the never exposed, therefore the single cohort results were preferred.<br><i>Design and analysis:</i><br><i>Strengths:</i> Lymphatic and haematopoietic cancer subtypes and a large sample size.<br><br><i>Limitations:</i> As the analysis was conducted internally in the cohort, workers in the reference category were also exposed to gasoline to a certain extent, with a potential bias towards the null of the OR estimates for tanker drivers. The analyses on tanker drivers were not adjusted for potential confounders, including smoking and other occupational co-exposures, introducing potential bias in an unpredictable direction. |
|                                                                |                                                                                                                                                                                                                                 |                                                      | Other workers               | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 |                                                      | Ever tanker driver (≥ 1 yr) | NR                      | 2.16 (0.79–5.88)       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 | CML                                                  | OR:                         |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 |                                                      | Other workers               | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 |                                                      | Ever tanker driver (≥ 1 yr) | NR                      | 1.26 (0.51–3.11)       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 | MPD                                                  | OR:                         |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 |                                                      | Other workers               | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                                                |                                                                                                                                                                                                                                 |                                                      | Ever tanker driver (≥ 1yr)  | NR                      | 1.38 (0.60–3.21)       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

| <b>Reference,<br/>location,<br/>enrolment/<br/>follow-up<br/>period, study<br/>design</b>                                                                     | <b>Population size,<br/>description<br/>Exposure assessment<br/>method</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <b>Cancer type<br/>(histopathology),<br/>incidence or<br/>mortality</b> | <b>Exposure<br/>category or<br/>level</b>                                                                                                                                                                                                                                                                                                                          | <b>Exposed<br/>cases or<br/>deaths</b>                                                                      | <b>Risk estimate<br/>(95% CI)</b>                                                                                                                                                                                                                                | <b>Covariates<br/>controlled</b>                                                                              | <b>Comments</b>                                                                                                                                                                                                                                                                                                                                                                                            |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Wong et al.<br/>(1999)</a><br><br>USA<br><br>Enrolment,<br>1946–1985/<br>follow-up not<br>specified<br>(mortality)<br><br>Nested case–<br>control | <i>Source cohort:</i> SMR estimates were based on a follow-up through June 1989 as described in <a href="#">Wong et al. (1993)</a> ; 9026 land-based; 9109 marine; gasoline distribution employees ( $\geq 1$ yr) at land-based terminals or on marine vessels, between 1946 and 1985. Outcome data from: Social Security Administration, Death Master File, National Death Index, participating companies.<br><br><i>Cases:</i> 35 leukaemia, 13 AML, 11 multiple myeloma, 12 kidney; the nested case–control study was restricted to land-based workers; cases identified through underlying and contributory causes of death listed on death certificates (ICD-8). SMR estimates for the land-based workers were from <a href="#">Wong et al. (1993)</a> with follow-up through June 1989. | NHL<br><br>HL<br><br>Leukaemia<br><br>Leukaemia (ICD-8, 204–207)        | Petroleum distribution (land and marine) workers exposed to gasoline (SMR):<br><br>Petroleum distribution (land and marine) workers exposed to gasoline (SMR):<br><br>Job category (ever vs never) (RR):<br><br>Plantmen<br><br>Warehousemen<br><br>Labourers<br><br>Mechanics<br><br>Clerks or office<br><br>Foremen or supervisors<br><br>Drivers<br><br>Loaders | Exposed<br><br>Exposed<br><br>Exposed<br><br>NR<br><br>NR<br><br>NR<br><br>NR<br><br>NR<br><br>NR<br><br>NR | 0.42 (0.25–0.65)<br><br>0.48 (0.18–1.05)<br><br>0.80 (0.56–1.07)<br><br>0.83 (0.40–1.72)<br><br>0.54 (0.12–2.40)<br><br>0.96 (0.26–3.51)<br><br>0.83 (0.30–2.34)<br><br>0.53 (0.18–1.58)<br><br>1.36 (0.59–3.18)<br><br>0.77 (0.35–1.71)<br><br>0.79 (0.09–6.82) | NR<br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br>Date of birth, sex, company | <i>Strengths:</i> The study had an improved exposure assessment compared with previous cohorts and reports results specific for cancer subtypes (AML and multiple myeloma).<br><br><i>Limitations:</i> Mortality data; co-exposure to other potential carcinogens is likely, and no occupational or lifestyle-related confounders were considered in the analysis. HWSB because of 1-yr exposure criteria. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                               | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments |
|----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------|
| <a href="#">Wong et al. (1999)</a><br>(cont.)                  | <i>Controls:</i> 5 controls per case; Individuals alive at the time of case death and either alive at the end of follow-up or dead for cause other than the cancers of interest (leukaemia, AML, multiple myeloma, kidney cancer).<br>Matching on company, date of birth, sex.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Multiple myeloma                                     | Petroleum distribution (land and marine) workers exposed to gasoline (SMR): |                         |                        | NR                          |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Exposed                                                                     | 17                      | 0.79 (0.46–1.24)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          | Multiple myeloma (ICD-8, 203.0)                      | Land-based workers (per 1-unit increase) (RR):                              |                         |                        | Date of birth, sex, company |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Duration of employment (yr)                                                 | NR                      | [1.00 (0.93–1.08)]     |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Cumulative total hydrocarbon exposure (ppm-years)                           | NR                      | [1.00 (0.998–1.001)]   |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          | Multiple myeloma (ICD-8, 203.0)                      | Job category (ever vs never) (RR):                                          |                         |                        |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Plantmen                                                                    | NR                      | 0.55 (0.15–2.10)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Warehousemen                                                                | NR                      | 1.82 (0.32–10.4)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Labourers                                                                   | NR                      | 0                      |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Mechanics                                                                   | NR                      | 0.45 (0.05–3.81)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Clerks or office                                                            | NR                      | 0.29 (0.04–2.28)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Foremen or supervisors                                                      | NR                      | 1.92 (0.43–8.59)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Drivers                                                                     | NR                      | 0.91 (0.21–3.96)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Loaders                                                                     | NR                      | 1.00 (0.11–9.51)       |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          | Leukaemia (ICD-8, 204–207)                           | Land-based workers (per 1-unit increase) (RR):                              |                         |                        |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Duration of employment (yr)                                                 | NR                      | [0.97 (0.94–1.01)]     |                             |          |
|                                                                |                                                                                                                                                                                                                                                                                                                                                          |                                                      | Cumulative total hydrocarbon exposure (ppm-years)                           | NR                      | [1.00 (1.00–1.00)]     |                             |          |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                    |
|----------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|-----------------------------|
| <a href="#">Wong et al. (1999)</a><br>(cont.)                  |                                                            | AML                                                  | Petroleum distribution (land and marine) workers exposed to gasoline (SMR): |                         |                        | NR                    | Date of birth, sex, company |
|                                                                |                                                            |                                                      | Exposed                                                                     | 18                      | 1.17 (0.69–1.85)       |                       |                             |
|                                                                |                                                            | AML (ICD-8, 205.0)                                   | Job category (ever vs never) (RR):                                          |                         |                        |                       |                             |
|                                                                |                                                            | Plantmen                                             | NR                                                                          | 1.17 (0.35–3.91)        |                        |                       |                             |
|                                                                |                                                            | Warehousemen                                         | NR                                                                          | 0                       |                        |                       |                             |
|                                                                |                                                            | Labourers                                            | NR                                                                          | 2.42 (0.21–27.4)        |                        |                       |                             |
|                                                                |                                                            | Mechanics                                            | NR                                                                          | 0.91 (0.17–4.80)        |                        |                       |                             |
|                                                                |                                                            | Clerks or office                                     | NR                                                                          | 0.33 (0.04–2.62)        |                        |                       |                             |
|                                                                |                                                            | Foremen or supervisors                               | NR                                                                          | 1.12 (0.30–4.18)        |                        |                       |                             |
|                                                                |                                                            | Drivers                                              | NR                                                                          | 0.42 (0.13–1.44)        |                        |                       |                             |
|                                                                |                                                            | Loaders                                              | NR                                                                          | 0                       |                        |                       |                             |
|                                                                |                                                            | AML (ICD-8, 205.0)                                   | Land-based workers (per 1-unit increase) (RR):                              |                         |                        |                       |                             |
|                                                                |                                                            | Duration of employment (yr)                          | NR                                                                          | [0.97 (0.91–1.04)]      |                        |                       |                             |
|                                                                |                                                            | Cumulative total hydrocarbon exposure (ppm-years)    | NR                                                                          | [1.00 (0.999–1.001)]    |                        |                       |                             |

**Table 2.3 (continued)**

| Reference, location, enrolment/follow-up period, study design                                                                                                                                                                     | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality                                                         | Exposure category or level                                                                                                                                                                                 | Exposed cases or deaths | Risk estimate (95% CI)                                                 | Covariates controlled     | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------------------------------------------------------|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Lagorio et al. (1994a)</a><br>Service station attendant cohort, Latium, Italy<br>Enrolment 1980/follow-up, 1981 to July 1991 (residents in Rome), June 1992 (residents in other municipalities) (mortality)<br>Cohort | 2665; service gas station managers in Latium region identified through a nationwide survey in 1980, who were alive as of 1 January 1981.<br>Vital status through municipality registry of residence and cause of death (ICD-9) through linkage with national or regional mortality registry, or through review of death certificates.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Lymphatic and haematopoietic (ICD-9, 200–208)<br><br>NHL (ICD-9, 200, 202)<br><br>Leukaemia (ICD-9, 204–208) | SMR (Latium region as referent):<br>Service station attendants<br><br>SMR (Latium region as referent):<br>Service station attendants<br><br>SMR (Latium region as referent):<br>Service station attendants | 5<br><br>3<br><br>2     | [0.68 (0.22–1.60)]<br><br>[1.58 (0.33–4.61)]<br><br>[0.56 (0.07–2.01)] | Age, sex, calendar period | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Cohort members were probably exposed to refuelling activities, because self-service stations were still rare in the study area when the study was carried out and most of the gas station workforce included self-employed workers.<br><br><i>Limitations:</i> Small sample size; short duration of follow-up; broad outcome categories; mortality data. |



**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                         | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Patel et al. (2004)</a><br>Area of a gasoline spill, Pennsylvania, USA<br>1990–2000 (incidence)<br>Cohort | 663; people living ≥ 6 mo (1990–2000) in the US EPA remediation area affected by the Tranguch gasoline spill. First household in the area were identified, then, within each household, all individuals aged > 18 yr were interviewed. Data collected through questionnaire and medical record review between 2001 and 2002 (retrospective design). Cancer identified through self-reporting in the questionnaire, later on validated through medical records and Pennsylvania Cancer Registry. Participation rate, 63% (full questionnaire completion).<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | NHL                                                  | (Pennsylvania referent rates) (SIR):<br>Individuals affected by the gasoline spill | 2                       | 1.23 (0.15–4.46)       | Age                   | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Specific source of exposure different from other studies on gasoline.<br><br><i>Limitations:</i> Small sample size; short duration of follow-up; possible incomplete ascertainment of the outcome; latency was not considered in the analysis.<br><br><i>Other comments:</i> Later follow-up for leukaemia only in <a href="#">Talbott et al. (2011)</a> , hence results for leukaemia were reported only from <a href="#">Talbott et al. (2011)</a> . |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Cancer type (histopathology), incidence or mortality | Exposure category or level                          | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------|-------------------------|------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Talbot et al. (2011)</a><br>Area of a gasoline spill, PA, USA<br>1990–2000 stratified on 1990–1994 (insufficient latency) and 1995–2001 (risk period) (incidence)<br>Cohort | 663; previous update in <a href="#">Patel et al. (2004)</a> , see <a href="#">Patel et al. (2004)</a> for inclusion criteria.<br>Data collected through questionnaire and medical record review between 2001 and 2002 (retrospective design).<br>Cancer identified through self-reporting in the questionnaire, later on validated through medical records and PA cancer registry. Data from the cancer registry for 2001 were added in this follow-up.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Leukaemia (ICD-9, 204–208)                           | Time interval (PA referent rates) (SIR):            |                         |                        | Age, sex              | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><i>Design and analysis:</i><br><i>Strengths:</i> Specific source of exposure, different from other studies on gasoline; latency considered in the analysis, using the non-risk period as negative outcome control.<br><i>Limitations:</i> Short duration of follow-up and small sample size.<br><i>Other comments:</i> Later follow-up for leukaemia only in <a href="#">Talbot et al. (2011)</a> , hence results for leukaemia were reported in <a href="#">Talbot et al. (2011)</a> , and results for NHL were reported in <a href="#">Patel et al. (2004)</a> . |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | 1990–1994                                           | 0                       | NC                     |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | 1995–2001                                           | 5                       | 6.49 (2.10–15.13)      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Leukaemia (ICD-9, 204–208)                           | Household exposure level (PA referent rates) (SIR): |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | Low                                                 | 1                       | 2.51 (0.08–13.99)      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | Medium                                              | 2                       | 4.88 (0.59–17.61)      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | High                                                | 2                       | 16.81 (2.02–60.67)     |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | AML (ICD-9, 205)                                     | Time interval (PA referent rates) (SIR):            |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | 1990–1994                                           | 0                       | NC                     |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                      | 1995–2001                                           | 3                       | 11.54 (2.38–33.69)     |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                | Cancer type (histopathology), incidence or mortality | Exposure category or level                          | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                             | Comments                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------|-------------------------|------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Neasham et al. (2011a)</a><br>EPIC cohort, Europe<br>Enrolment 1992–2000/ follow-up until 2006 (incidence)<br>Cohort | 218 968; EPIC cohort: men and women aged 35–70 yr at recruitment; restricted to centres with information on occupational history (Denmark, Greece, Germany, Italy – four centres; Spain, and the UK – one centre). Follow-up through cancer registries, vital statistics (mortality), in Germany and Greece: a combination of methods including health insurance records, cancer and pathology registries, and active follow-up.<br><br><i>Exposure assessment method: See <a href="#">Table 2.1</a>.</i> | NHL                                                  | Employment as a gasoline station worker (HR):<br>No | 652                     | 1                      | Centre, sex, education level, alcohol consumption, smoking status | <i>Exposure assessment critique: See <a href="#">Table 2.1</a>.</i><br><br><i>Design and analysis: Strengths:</i> Large general population cohort; adjustment for educational level, alcohol, and smoking.<br><br><i>Limitations:</i> Relatively short follow-up and relatively small number of events; lack of consideration of latency in the analyses. |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Yes                                                 | 11                      | 1.32 (0.73–2.41)       |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | NHL (excluding multiple myeloma/ plasmacytoma)       | Employment as a gasoline station worker (HR):<br>No | 514                     | 1                      |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Yes                                                 | 6                       | 1.19 (0.64–2.72)       |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Multiple myeloma (including plasmacytoma)            | Employment as a gasoline station worker (HR):<br>No | 138                     | 1                      |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Yes                                                 | 5                       | 2.61 (1.06–6.43)       |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | HL                                                   | Employment as a gasoline station worker (HR):<br>No | 37                      | 1                      |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Yes                                                 | 2                       | 4.59 (1.08–19.6)       |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      |                                                     |                         |                        |                                                                   |                                                                                                                                                                                                                                                                                                                                                           |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                             | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                          | Cancer type (histopathology), incidence or mortality | Exposure category or level                             | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                             | Comments                                                                                                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Siemiatycki et al. (1987a)</a><br>Montreal, Canada<br>1979–1985<br>(incidence)<br>Case–control | <i>Cases:</i> 206 patients (aged 35–70 yr) with NHL diagnosed at one of the 19 participating hospitals. Response rate, 82%.<br><br><i>Controls:</i> 2599 other cancers (excluding lung and HL); controls were selected among the other cancer sites (excluding lung and HL). No matching.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | NHL<br>(ICD-8, 200, 202)                             | Exposure to automotive gasoline (OR):<br>Never<br>Ever | NR<br>20                | 1<br>[0.8 (0.5–1.3)]   | Age, SES, ethnic group, cigarette smoking, and blue- and white-collar job history | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Automotive gasoline exposure assessment; control for potential confounders, including SES and smoking.<br><br><i>Limitations:</i> Use of other cancer sites as controls. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                                       | Exposed cases or deaths  | Risk estimate (95% CI)                                                  | Covariates controlled                                 | Comments                                                                                                                                                                                                                                                                                                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|-------------------------------------------------------------------------|-------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">West et al. (1995)</a><br>Wales, UK<br>NR, but previous publication ( <a href="#">Farrow et al., 1989</a> ) reported 1985–1986 (incidence)<br>Case–control | <i>Cases:</i> 400 patients (aged > 15 yr) with MDS diagnosed in the Haematology Department, University Hospital of Wales. Response rate, 62%.<br><br><i>Controls:</i> NR but probably 400; controls matched (1:1) on age, sex, area of residence, calendar year, and hospital; chosen among individuals receiving inpatient or outpatient care for medicine, otorhinolaryngology, orthopaedics, and geriatrics, excluding cancer patients.<br><br><i>Exposure assessment method:</i> Self-reported occupational history and exposure (at work, through hobbies or at home) of a list of 70 pre-specified chemicals. | MDS                                                  | Lifetime occupational exposure to petrol or diesel (OR):<br>Never<br>Low exposure threshold: ≥ 10 h of any intensity<br>Medium: ≥ 50 h at medium intensity<br>High: ≥ 2500 h of medium intensity | 92<br>31<br><br>47<br>33 | 1<br>[1.01 (0.72–1.44)]<br><br>[1.02 (0.67–1.56)]<br>[1.09 (0.66–1.81)] | Age, sex, area of residence, reference year, hospital | <i>Strengths:</i> Focus on a specific subtype of lymphatic and haematopoietic malignancy.<br><br><i>Limitations:</i> Response rate among controls was unknown; combined assessment of diesel and gasoline exposure; lack of adjustment for other occupational exposures and lifestyle-related confounders. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                       | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality         | Exposure category or level                                                                                                                                                           | Exposed cases or deaths | Risk estimate (95% CI)                        | Covariates controlled                       | Comments                                                                                                                                                    |
|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-----------------------------------------------|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Clavel et al. (1995)</a><br>Multicentre, France<br>1990–1992 (incidence)<br>Case-control | <i>Cases:</i> 291 (229 men, 62 women); cases diagnosed between January 1980 and January 1990 (including deceased) were identified in 18 hospitals throughout France. Only living cases in 1990–1992 (75% of all cases) were included. Response rate, 61%.<br><br><i>Controls:</i> 541 (425 men, 116 women); matched on sex, date of birth, date of admission and residence (2:1). Identified from patients hospitalized (1980–1990) in the same hospitals for other conditions (mainly orthopaedics/ rheumatology, excluding those admitted for cancer). Alive controls, as for the cases, were contacted in 1990–1992. Response rate, 58%.<br><br><i>Exposure assessment method:</i> Self-reported occupational history and occupational exposure to solvents. | NHL (hairy cell leukaemia)<br><br>NHL (hairy cell leukaemia) | Occupational exposure to petrol or diesel (self-reported), men (OR):<br>Never<br>Ever<br><br>Occupational exposure to petrol or diesel (self-reported), women (OR):<br>Never<br>Ever | NR<br>79<br><br>NR<br>3 | 1<br>1.5 (1.0–2.1)<br><br>1<br>2.1 (0.4–10.6) | Date of birth, date of admission, residence | <i>Strengths:</i> Focus on a specific type of NHL.<br><br><i>Limitations:</i> Restricted to survivors; combined assessment of diesel and gasoline exposure. |



**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                           | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Cancer type (histopathology), incidence or mortality | Exposure category or level                                         | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                         | Comments                                                                                                                                                                         |
|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------|-------------------------|------------------------|---------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Clavel et al. (1996)</a><br>Multicentre, France<br>1990–1992<br>Case–control | <p><i>Cases:</i> 226 men, diagnosed between January 1980 and January 1990 (including deceased), were identified in 18 hospitals throughout France. Only cases alive in 1990–1992 (75% of all cases) were included. Response rate, 61%.</p> <p><i>Controls:</i> 425 men; matched on date of birth, date of admission and residence (2:1). Identified from patients hospitalized (1980–1990) in the same hospitals for other conditions (mainly orthopaedics/ rheumatology, excluding those admitted for cancer). Living controls, as for the cases, were contacted in 1990–1992. Response rate, 58%.</p> <p><i>Exposure assessment method:</i> Self-reported occupational history.</p> | NHL (hairy cell leukaemia)                           | Employment as petrol station attendant, men (OR):<br>Never<br>Ever | NR<br>2                 | 1<br>1.9 (0.3–13.9)    | Date of birth, date of admission, residence, smoking, farming | <p><i>Strengths:</i> Focus on a specific type of NHL.</p> <p><i>Limitations:</i> Restricted to survivors; combined assessment of diesel and gasoline exposure; only 2 cases.</p> |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                            | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                           | Exposed cases or deaths  | Risk estimate (95% CI)                          | Covariates controlled         | Comments                                                                                                                                               |
|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|-------------------------------------------------|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Holly et al. (1997)</a><br>San Francisco Bay Area, USA<br>1988–1995<br>(incidence)<br>Case-control | <i>Cases:</i> 312 homosexual men with NHL; response rate estimated to be about 45% in a later publication.<br><i>Controls:</i> 420 homosexual men, frequency-matched on sex, county of residence, age. Identified through random-digit dialling; response rate not reported.<br><br><i>Exposure assessment method:</i> Self-reported (no proxy interviews) history of chemical and occupational exposures during in-person interview. | NHL<br><br><br>NHL                                   | Hours of gasoline exposure (self-reported), HIV-positive men (OR):<br>< 10 h<br>≥ 10 h<br><br>Hours of gasoline exposure (self-reported), HIV-negative men (OR):<br>< 10 h<br>≥ 10 h | 221<br>42<br><br>40<br>8 | 1<br>0.65 (0.36–1.2)<br><br>1<br>1.7 (0.72–4.0) | Sex, county of residence, age | <i>Strengths:</i> Study in a specific population.<br><i>Limitations:</i> Gasoline exposure assessment based on self-report; low/unknown response rate. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                     | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Persson and Fredrikson (1999)</a><br>Sweden<br>Study 1: 1964–1986; study 2: 1975–1984 (incidence)<br>Case–control | <i>Cases:</i> 106 (study 1), 93 (study 2); individuals (aged 20 to < 80 yr) with NHL identified either in Orebro Medical Centre Hospital (study 1, response rate, 96%) or in the Regional Cancer Registry at University Hospital in Linköping (study 2, response rate, 90%).<br><br><i>Controls:</i> 479; matching not mentioned. Controls drawn randomly from population registries covering same geographical area as cases (study 1, response rate, 75%; study 2, response rate not reported).<br><br><i>Exposure assessment method:</i> Self-reported occupational exposure through mailed questionnaires. | NHL                                                  | Occupational exposure to gasoline (self-reported, time window 5–45 yr before diagnosis) (OR):<br>Never<br>Ever | NR<br>20                | 1<br>1.9 (0.9–3.8)     | Age, sex              | <i>Strengths:</i> 5-yr exposure latency was considered.<br><br><i>Limitations:</i> Relatively small sample size; occupational exposure to gasoline was based on self-report; the response rate was lower among controls than cases; results for ever exposure to gasoline were not adjusted for potential confounders. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                   | Cancer type (histopathology), incidence or mortality | Exposure category or level                          | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------|-------------------------|------------------------|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Nisse et al. (2001)</a><br>Hospital-based, Lille, France<br>September 1991 to February 1996<br>(incidence)<br>Case-control | <i>Cases:</i> 204; MDS cases diagnosed at the Department of Haematology, University Hospital of Lille. Exclusion: MDS secondary to previous chemotherapy.<br><br><i>Controls:</i> 204; matched on age, sex (1:1). Drawn from electoral list of the same area as cases.<br><br><i>Exposure assessment method:</i> Questionnaire information was further reviewed by occupational experts who could get in contact with the study participants or the occupational physician at the workplace. | MDS                                                  | Occupational petrol exposure (OR):<br>Never<br>Ever | NR<br>15                | 1<br>2.5 (0.9–7.7)     | Age, sex, residence   | <i>Strengths:</i> Focus on a specific haematolymphoid malignancy.<br><br><i>Limitations:</i> Response rate among cases and controls was not reported; relatively small sample size; results adjusted for potential confounders were not reported.<br><br><i>Other comments:</i> In <a href="#">Nisse et al. (1995)</a> , it was written that controls were selected from patients' list of the same general practitioner as the cases. Also, exclusion criteria only mentioned in <a href="#">Nisse et al. (1995)</a> : previous cancer or blood or dermatological disorder. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                               | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled             | Comments                                                                                                                                                                                                                                                                                                                                       |
|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Björk et al. (2001)</a><br>Southern Sweden<br>Recruitment, 1995–1997<br>Case–control | <p><i>Cases:</i> 226; patients with Ph+ CML, cytogenetically analysed at the Department of Clinical Genetics in Lund (1976–1993), which was routine in the catchment area of Lund hospital during the study period. Participation rate, 89% (next-of-kin interviews, 81%).</p> <p><i>Controls:</i> 251; matched on sex, age, and county of residence at the calendar year of cases diagnosis. Identified through the Swedish national bureau of statistics. Participation rate, 72% (next-of-kin interviews, 14%).</p> <p><i>Exposure assessment method:</i> Individual exposure assessment was carried out by industrial hygienists.</p> | CML (Ph+)                                            | Occupational or hobby-related exposure to gasoline or diesel fuel (OR):<br>Never<br>Ever | 185<br>35               | 1<br>1.0 (0.59–1.7)    | Sex, age, and county of residence | <p><i>Strengths:</i> Focus on a specific subtype of haematolymphoid malignancy.</p> <p><i>Limitations:</i> Considerable difference between cases and controls in the proportion of next-of-kin interviews; combined assessment of diesel and gasoline exposure; no adjustment for potential occupational or lifestyle-related confounders.</p> |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                             | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Cancer type (histopathology), incidence or mortality | Exposure category or level                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------|-------------------------|------------------------|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Dryver et al. (2004)</a><br>Sweden<br>1990–1998<br>(incidence)<br>Case-control | Cases: 859; NHL cases identified through regional Swedish tumour registry. Participation rate, 74%.<br>Controls: 1310; matched on sex, age and parish, population-based. Participation rate, 69%.<br><br><i>Exposure assessment method:</i> Semiquantitative assessment on the basis of JEMs. Information on occupational history and exposures to specific group of substances was collected through self-reported questionnaire. FINJEM was used to estimate group exposure for each occupation by multiplying the proportion of individuals in a given profession exposed to a given agent by the mean level of exposure. | NHL (ICD-7, 200, 202)                                | Employment as gas station attendant (OR):        |                         |                        | Age, sex, parish      | <i>Exposure assessment critique:</i> The analyses of qualitative and categorical measures on the basis of self-reported exposure to gasoline were more specific to gasoline than the analysis of JEM-based measures of exposures. In the JEM-based measure, gasoline was included with aliphatic and alicyclic hydrocarbon solvents, and risk measures associated with this exposure variable could not be attributed solely to gasoline.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Relatively large sample size.<br><i>Limitations:</i> Lack of adjustment for occupational confounders. |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                      | Never                                            | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | NHL (ICD-7, 200, 202)                                | Ever                                             | 27                      | 1.62 (0.92–2.83)       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                      | Occupational gasoline exposure (OR):             |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | NHL (ICD-7, 200, 202)                                | Never                                            | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                      | Ever                                             | 78                      | 1.46 (1.04–2.05)       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                      | Duration of occupational gasoline exposure (OR): |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                      | < 5 yr                                           | NR                      | 1                      |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                      | ≥ 5 yr                                           | 45                      | 1.92 (1.20–3.08)       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |



**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Cancer type (histopathology), incidence or mortality                                                                                     | Exposure category or level                                                                                                                                                                                                                                                               | Exposed cases or deaths                                   | Risk estimate (95% CI)                                                                                     | Covariates controlled                                                | Comments                                                                                                                                                                                                                                          |
|-----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Orsi et al. (2007)</a><br>Multicentre hospital-based, France<br>September 2000 to December 2004 (incidence)<br>Case-control | <i>Cases:</i> 824 cases with incident lymphoid malignancy (aged 18–75 yr), residing in hospital catchment areas. Patients under immunosuppression or with AIDS were excluded. Recruited < 6 mo after diagnosis.<br><i>Controls:</i> 752; individually matched on centre, age, sex. Patients admitted to same hospital as cases (mainly in orthopaedics/ rheumatology) with no prior history of haematological malignancy or occupation-/alcohol-/ smoking-related diseases.<br><i>Exposure assessment method:</i> Self-reported occupational history and ever exposure to a predefined list of occupational hazards. | Lymphatic and haematopoietic<br><br>NHL<br><br>Lymphoproliferative syndrome (CLL and hairy cell leukaemia)<br>Multiple myeloma<br><br>HL | Occupational petrol exposure (self-reported) (OR):<br>Never<br>Ever<br>Occupational petrol exposure (self-reported) (OR):<br>Never<br>Ever<br>Occupational petrol exposure (self-reported) (OR):<br>Never<br>Ever<br>Occupational petrol exposure (self-reported) (OR):<br>Never<br>Ever | NR<br>129<br>NR<br>62<br>NR<br>32<br>NR<br>17<br>NR<br>18 | 1<br>1.0 (0.7–1.4)<br>1<br>0.9 (0.6–1.4)<br>1<br>0.9 (0.5–1.4)<br>1<br>1.2 (0.6–2.4)<br>1<br>1.0 (0.5–2.0) | Age, sex, centre, socioeconomic category (white collar, blue collar) | <i>Strengths:</i> Relatively large sample size; high response rate; haematolymphoid cancer subtypes were analysed separately.<br><i>Limitations:</i> The exposure was self-reported and only ever exposure was analysed with no exposure lagging. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                  | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Cocco et al. (2010)</a><br>Epilymph study, Czechia, France, Germany, Ireland, Italy, Spain<br>1998–2004 (incidence)<br>Case–control | Cases: 2348 adults with a first lymphoma diagnosis, resident in the referral area of the study recruitment centres. Participation rate, 88%.<br><br>Controls: 2462; matched on sex, 5-yr age group, and residence area. General population (random sampling, participation rate, 52%) in Germany and Italy, hospital controls (with diagnoses other than cancer, infectious diseases, and immunodeficiency diseases) in the other centres (participation rate, 81%).<br><br><i>Exposure assessment method:</i> Quantitative assessment on the basis of occupational history. Industrial hygienists applied several metrics on the basis of the detailed data collected through the questionnaire. Cumulative exposure was also estimated. | NHL (B-cell lymphoma)                                | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        | Age, sex, education, centre | <i>Exposure assessment critique:</i> The reliability of the cumulative exposure score of gasoline exposure was uncertain given the somewhat arbitrary cut-points for the different ranks. No results were presented for duration of exposure.<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Large sample size; specific lymphoma subtypes were analysed separately; thorough exposure assessment; trend analyses on different exposure metrics.<br><br><i>Limitations:</i> Relatively low participation rate among population controls; results unadjusted for potential occupational and lifestyle-related confounders. |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Never exposed to solvents                                                                                                                                                   | 1061                    | 1                      |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Ever                                                                                                                                                                        | 353                     | 1.1 (0.9–1.3)          |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Trend-test <i>P</i> value, 0.000405 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | NHL (B-cell lymphoma)                                | Occupational gasoline exposure (high confidence) (OR):                                                                                                                      |                         |                        |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Never exposed to solvents                                                                                                                                                   | 1061                    | 1                      |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | All exposed                                                                                                                                                                 | 248                     | 1.2 (1.0–1.5)          |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Low cumulative                                                                                                                                                              | 55                      | 0.9                    |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Medium cumulative                                                                                                                                                           | 77                      | 1.2                    |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | High cumulative                                                                                                                                                             | 113                     | 1.5                    |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Trend-test <i>P</i> value, 0.01 (for trend in ORs for cumulative exposure)                                                                                                  |                         |                        |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | NHL (DLBCL)                                          | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Never exposed to solvents                                                                                                                                                   | 325                     | 1                      |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Ever                                                                                                                                                                        | 91                      | 0.9 (0.7–1.2)          |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                      | Trend-test <i>P</i> value, 0.354000 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description Exposure assessment method | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments |
|----------------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------|
| <a href="#">Cocco et al. (2010)</a><br>(cont.)                 |                                                         | NHL (DLBCL)                                          | Occupational gasoline exposure (high confidence) (OR):                                                                                                                      |                         |                        | Age, sex, education, centre |          |
|                                                                |                                                         |                                                      | Never exposed to solvents                                                                                                                                                   | 325                     | 1                      |                             |          |
|                                                                |                                                         |                                                      | All exposed                                                                                                                                                                 | 63                      | 0.9 (0.7–1.3)          |                             |          |
|                                                                |                                                         |                                                      | Low cumulative                                                                                                                                                              | 17                      | 0.7                    |                             |          |
|                                                                |                                                         |                                                      | Medium cumulative                                                                                                                                                           | 22                      | 1.0                    |                             |          |
|                                                                |                                                         |                                                      | High cumulative                                                                                                                                                             | 24                      | 1.1                    |                             |          |
|                                                                |                                                         |                                                      | Trend-test <i>P</i> value, 0.81 (for trend in ORs for cumulative exposure)                                                                                                  |                         |                        |                             |          |
|                                                                |                                                         | NHL (follicular)                                     | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        |                             |          |
|                                                                |                                                         |                                                      | Never exposed to solvents                                                                                                                                                   | 140                     | 1                      |                             |          |
|                                                                |                                                         |                                                      | Ever                                                                                                                                                                        | 33                      | 1.0 (0.6–1.6)          |                             |          |
|                                                                |                                                         |                                                      | Trend-test <i>P</i> value, 0.177660 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |          |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments |
|----------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------|
| <a href="#">Cocco et al. (2010)</a><br>(cont.)                 |                                                            | NHL (follicular)                                     | Occupational gasoline exposure (high confidence) (OR):                                                                                                                      |                         |                        | Age, sex, education, centre |          |
|                                                                |                                                            |                                                      | Never exposed to solvents                                                                                                                                                   | 140                     | 1                      |                             |          |
|                                                                |                                                            |                                                      | All exposed                                                                                                                                                                 | 18                      | 0.8 (0.5–1.5)          |                             |          |
|                                                                |                                                            |                                                      | Low cumulative                                                                                                                                                              | 9                       | 1.3                    |                             |          |
|                                                                |                                                            |                                                      | Medium cumulative                                                                                                                                                           | 4                       | 0.6                    |                             |          |
|                                                                |                                                            |                                                      | High cumulative                                                                                                                                                             | 5                       | 0.7                    |                             |          |
|                                                                |                                                            |                                                      | Trend-test <i>P</i> value, 0.30 (for trend in ORs for cumulative exposure)                                                                                                  |                         |                        |                             |          |
|                                                                |                                                            | NHL (CLL)                                            | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        |                             |          |
|                                                                |                                                            |                                                      | Never exposed to solvents                                                                                                                                                   | 217                     | 1                      |                             |          |
|                                                                |                                                            |                                                      | Ever                                                                                                                                                                        | 84                      | 1.3 (0.9–1.8)          |                             |          |
|                                                                |                                                            |                                                      | Trend-test <i>P</i> value, 0.000084 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |          |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description Exposure assessment method | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments |
|----------------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------|
| <a href="#">Cocco et al. (2010)</a><br>(cont.)                 |                                                         | NHL (CLL)                                            | Occupational gasoline exposure (high confidence) (OR):                                                                                                                      |                         |                        | Age, sex, education, centre |          |
|                                                                |                                                         |                                                      | Never exposed to solvents                                                                                                                                                   | 217                     | 1                      |                             |          |
|                                                                |                                                         |                                                      | All exposed                                                                                                                                                                 | 67                      | 1.7 (1.2–2.3)          |                             |          |
|                                                                |                                                         |                                                      | Low cumulative                                                                                                                                                              | 14                      | 1.3                    |                             |          |
|                                                                |                                                         |                                                      | Medium cumulative                                                                                                                                                           | 24                      | 1.8                    |                             |          |
|                                                                |                                                         |                                                      | High cumulative                                                                                                                                                             | 29                      | 1.8                    |                             |          |
|                                                                |                                                         |                                                      | Trend-test <i>P</i> value, 0.001 (for trend in ORs for cumulative exposure)                                                                                                 |                         |                        |                             |          |
|                                                                |                                                         | Multiple myeloma                                     | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        |                             |          |
|                                                                |                                                         |                                                      | Never exposed to solvents                                                                                                                                                   | 160                     | 1                      |                             |          |
|                                                                |                                                         |                                                      | Ever                                                                                                                                                                        | 60                      | 1.2 (0.8–1.7)          |                             |          |
|                                                                |                                                         |                                                      | Trend-test <i>P</i> value, 0.002070 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |          |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments |
|----------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------|
| <a href="#">Cocco et al. (2010)</a><br>(cont.)                 |                                                            | Multiple myeloma                                     | Occupational gasoline exposure (high confidence) (OR):                                                                                                                      |                         |                        | Age, sex, education, centre |          |
|                                                                |                                                            |                                                      | Never exposed to solvents                                                                                                                                                   | 160                     | 1                      |                             |          |
|                                                                |                                                            |                                                      | All exposed                                                                                                                                                                 | 41                      | 1.3 (0.9–1.9)          |                             |          |
|                                                                |                                                            |                                                      | Low cumulative                                                                                                                                                              | 8                       | 0.9                    |                             |          |
|                                                                |                                                            |                                                      | Medium cumulative                                                                                                                                                           | 9                       | 0.9                    |                             |          |
|                                                                |                                                            |                                                      | High cumulative                                                                                                                                                             | 24                      | 2.0                    |                             |          |
|                                                                |                                                            |                                                      | Trend-test <i>P</i> value, 0.02 (for trend in ORs for cumulative exposure)                                                                                                  |                         |                        |                             |          |
|                                                                |                                                            | NHL (T-cell lymphoma)                                | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        |                             |          |
|                                                                |                                                            |                                                      | Never exposed to solvents                                                                                                                                                   | 78                      | 1                      |                             |          |
|                                                                |                                                            |                                                      | Ever                                                                                                                                                                        | 24                      | 1.1 (0.6–1.8)          |                             |          |
|                                                                |                                                            |                                                      | Trend-test <i>P</i> value, 0.079680 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |          |
|                                                                |                                                            | HL                                                   | Occupational gasoline exposure (any confidence) (OR):                                                                                                                       |                         |                        |                             |          |
|                                                                |                                                            |                                                      | Never exposed to solvents                                                                                                                                                   | 195                     | 1                      |                             |          |
|                                                                |                                                            |                                                      | Ever                                                                                                                                                                        | 46                      | 0.7 (0.5–1.1)          |                             |          |
|                                                                |                                                            |                                                      | Trend-test <i>P</i> value, 0.027000 (product of <i>P</i> values for trends in intensity, frequency and duration; <i>P</i> = 0.000125 if all three <i>P</i> values are 0.05) |                         |                        |                             |          |



**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Cancer type (histopathology), incidence or mortality | Exposure category or level                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                                                                                                                                              | Comments                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------|-------------------------|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lv et al. (2011)<br>Shanghai, China<br>June 2003 to November 2006<br>(incidence)<br>Case-control | Cases: 403 adults (aged ≥ 18 yr) with a diagnosis of primary MDS from 27 hospitals in Shanghai, with sample sent to the Sino-US Joint Laboratory for comprehensive diagnosis.<br><br>Controls: 806; matched on sex, age, and hospital (2:1). Patients admitted for different conditions unrelated to haematological diseases.<br><br>Exposure assessment method: The questionnaire collected the occupational history that was assessed by a panel of experts with regard to exposure to selected occupational hazards. | MDS                                                  | Occupational exposure to gasoline (OR):<br>Never | NR                      | 1                      | Age, sex, hospital                                                                                                                                                                                                                                 | <i>Strengths:</i> Focus on a specific haematolymphoid malignancy; adjustment for potential occupational, and socioeconomic confounders and for smoking.<br><br><i>Limitations:</i> The number of exposed cases was relatively small; response rate was not reported (but it was likely to be high, being a hospital-based study). |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | Ever                                             | 10                      | 2.35 (0.92–5.99)       |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | MDS                                                  | Occupational exposure to gasoline (OR):<br>Never | NR                      | 1                      | Age, sex, hospital, anti-tuberculosis drugs, D860 for diabetes, Chinese medicines, exposed to high-voltage power lines, new building/ renovation, benzene, pesticides, herbicides, glues, tobacco smoking, alcohol intake, hair dye use, education |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | Ever                                             | 10                      | 2.34 (0.70–7.77)       |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | RCMD                                                 | Occupational exposure to gasoline (OR):<br>Never | NR                      | 1                      | Age, sex, hospital                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | Ever                                             | 9                       | 5.54 (1.49–20.65)      |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | RAEB                                                 | Occupational exposure to gasoline (OR):<br>Never | NR                      | 1                      |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | Ever                                             | NR                      | 1.00 (0.09–11.03)      |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                   |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Cancer type (histopathology), incidence or mortality                                                 | Exposure category or level                                                  | Exposed cases or deaths | Risk estimate (95% CI)                    | Covariates controlled                                                    | Comments                                                                                                                                                                                                          |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------|-------------------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Morales-Suárez-Varela et al. (2013)</a><br>Denmark, France, Germany, Italy, Spain, Sweden<br>January 1995 to June 1997<br>(incidence)<br>Case-control | <i>Cases:</i> 100; incident mycosis fungoides (aged 35–69 yr) cases identified through hospitals or pathology departments, regional or national registries. Participation rate, 84.75%.<br><br><i>Controls:</i> 2846; population controls from same area as cases, frequency-matched on sex, age drawn from population registries, except for Spain where colon cancer patients were used as controls. Participation rate, 68.2%.<br><br><i>Exposure assessment method:</i> A JEM was applied using self-reported occupational history information. | NHL (T-cell lymphoma) (mycosis fungoides, ICD-O, morphology 9700/3–97 001/3, topography 173.0–173.9) | Cumulative gasoline exposure (OR):<br>Never exposed<br>< Median<br>≥ Median | 91<br>5<br>4            | 1<br>0.99 (0.22–4.38)<br>0.90 (0.20–4.07) | Age, sex, country, smoking habit, alcohol intake, BMI, educational level | <i>Strengths:</i> Focus on a specific haematolymphoid cancer subtype; use of a JEM; adjustment for educational level and lifestyle-related potential confounders.<br><br><i>Limitations:</i> Limited sample size. |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                      | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                 | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                                                 | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Poynter et al. (2017)</a><br>Minnesota, USA<br>AML study, June 2005 to November 2009; MDS study, April 2010 to October 2014 (incidence)<br>Case-control | Cases: 420 AML; 265 MDS; cases identified through Minnesota Cancer Surveillance System. Participation rates, 58% and 59%, respectively.<br><br>Controls: 1388 (701 from the AML study, 698 from the MDS study); individuals without myeloid leukaemia frequency-matched on decile of age. Participation rates of 64% and 49%, respectively. Identified through Minnesota State driver's license/ identification card list. | AML                                                  | Occupational and/or residential gasoline exposure (self-reported) (adjusted OR): |                         |                        | Age, sex, household income, smoking, exposure to radiation, and having lived on a farm or in a rural area $\geq 1$ yr | <i>Exposure assessment critique:</i> Limitations included potential recall bias, and reliance on self-reported exposures.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Relatively large sample size; with focus on focusing on a specific haematolymphoid malignancy; adjustment for SES, tobacco smoking and radiation.<br><br><i>Limitations:</i> Low response rate in both cases and controls; exposure assessment on the basis of self-report.<br><br><i>Other comments:</i> Crude OR for $\geq 5$ yr gasoline exposure for AML, [1.30]. Residential exposure in this study was meant to indicate gasoline exposure in general population (e.g. when self-refuelling own cars). |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | None                                                                             | 343                     | 1                      |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | < 5 yr                                                                           | 14                      | 0.96 (0.50–1.83)       |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | $\geq 5$ yr                                                                      | 46                      | 1.40 (0.95–2.06)       |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Trend-test <i>P</i> value, 0.22                                                  |                         |                        |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            | MDS                                                  | Occupational and/or residential gasoline exposure (self-report) (OR):            |                         |                        | Age, sex, exposure to chemotherapy, and having lived on a farm or in a rural area $\geq 1$ yr                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | None                                                                             | 225                     | 1                      |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | < 5 yr                                                                           | 4                       | NC                     |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | $\geq 5$ yr                                                                      | 33                      | 1.11 (0.71–1.74)       |                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

**Table 2.3 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                  | Cancer type (histopathology), incidence or mortality | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments |
|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------|-------------------------|------------------------|-----------------------|----------|
| <a href="#">Poynter et al. (2017)</a> (cont.)                  | <i>Exposure assessment method:</i> Exposure was assessed through a self-administered questionnaire that collected data on demographics, lifestyle factors, medical history, reproductive history, and detailed occupational and residential chemical exposures. Participants reported exposure with information on the duration of exposure. Job titles were also recorded. |                                                      |                            |                         |                        |                       |          |

AIDS, acquired immunodeficiency syndrome; AML, acute myeloid leukaemia; ANLL, acute non-lymphoblastic leukaemia; BMI, body mass index; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; DLBCL, diffuse large B-cell lymphoma; EPIC, European Prospective Investigation into Cancer and Nutrition; FINJEM, Finnish Job-Exposure Matrix; h, hour(s); HIV, human immunodeficiency virus; HL, Hodgkin lymphoma; HR, hazard ratio; HWSB, healthy-worker survivor bias; ICD, International Classification of Diseases; ICD-O, International Classification of Diseases – Oncology; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; mo, month(s); MPD, myeloproliferative disorder; NC, not calculated; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; PA, Pennsylvania; Ph+, Philadelphia chromosome-positive; ppm, parts per million; RAEB, refractory anaemia with excess of blasts; RCMD, refractory cytopenia with multiple dysplasia; RR, relative risk; SES, socioeconomic status; SIR, standardized incidence ratio; SMR, standardized mortality ratio; SPF, Supplementary Pension Fund; SRR, standardized registration ratio; UK, United Kingdom; USA, United States of America; US EPA, United States Environmental Protection Agency; yr, year(s).

to cause AML may have been overrepresented and other jobs may be underrepresented. The study by [Micheli et al. \(2014\)](#), a matched population-based case-control study on mortality from haematolymphoid cancer, was excluded because its focus was on residence close to a petrochemical refinery, and the authors reported only crude distributions of residence for > 10 years or < 10 years in proximity (< 200 m) to a gasoline station. A correlation study on leukaemia occurrence and average annual gasoline consumption in 19 European countries ([Swaen and Slangen, 1995](#)) and a study on a cluster of 7 cases of Hodgkin lymphoma detected in the population living within 1 mile [1.6 km] from a refinery in the USA with a history of a gasoline leak ([Dahlgren et al., 2008](#)) were excluded because of their ecological nature.

Different studies used different classifications and subcategorizations for lymphatic and haematopoietic malignancies, depending on the period when the study was conducted and the sample size of the study. The Working Group did not attempt to standardize the lymphatic and haematopoietic cancer subtypes by translating the study's classifications to the present version of ICD, because classification systems for these malignancies have changed considerably over time. In [Table 2.3](#), the cancer subtypes described in the original publication are listed, specifying the classification system that was used in the original study and including, when available, the codes for the corresponding ICD version if reported.

### 2.3.1 Cohort and nested case-control studies

The cohort studies contributing evidence on automotive gasoline exposure and risk of lymphatic and haematopoietic malignancies are described in chronological order below, according to how these studies are reported in Section 2.1; detailed information on each study is available in Section 2.1.

A large study on gasoline station workers was conducted by [Lynge et al. \(1997\)](#), who pooled cohort studies in the Nordic countries on the basis of record linkage between the 1970 censuses and the countries' national cancer registries (see Section 2.1.1). Information on occupation and industry was collected at the time of the census. The pooled analysis included 16 524 men and 2445 women employed in the service station industry who were followed for cancer incidence for a period of 15–20 years. Among men, there was no evidence of an increased risk of haematolymphoid cancer on the basis of a total of 82 cases of incident cancer. The SIR estimate was close to 1 for NHL (1.1; 95% CI, 0.8–1.5), Hodgkin lymphoma (1.0; 95% CI, 0.5–1.8), and leukaemia (0.9; 95% CI, 0.6–1.3), and was 0.6 (95% CI, 0.3–1.1) for multiple myeloma, and 1.4 (95% CI, 0.8–2.4) for AML. There were only 4 cases of any incident haematolymphoid cancer among women, corresponding to an SIR of [0.4] (95% CI, [0.1–1.1]).

[The Working Group noted that a strength of the study was the relatively large number of cases of incident cancer and population coverage of four different countries. However, the authors could not distinguish whether having worked in the gasoline service station industry implied involvement in refuelling activities, resulting in nondifferential misclassification of exposure to automotive gasoline and average low exposure levels. Both mechanisms may have diluted the effect estimates. The analyses did not consider issues of latency, which may result in further dilution. There was also left truncation because workers who were not active in the gasoline service station industry at the time of the 1970 census could not be included in the cohort. As individuals with a shorter duration of employment were more likely to have left before 1970, healthy-worker survivor effect and potential higher exposure levels in short-term workers may have led to downward bias.]

[Heineman et al. \(1992\)](#) conducted a nested case-control study in 791 patients with multiple myeloma diagnosed between 1970 and 1984 and 3080 controls. The authors used linkage of the Danish Cancer Registry and the Danish Supplementary Pension Fund (see Section 2.1.1), which has since 1964 registered information on type of industry and, when available, job title for wage-earners aged 16–66 years. This information was used by industrial hygienists to assess exposure to gasoline for cases and controls. Employment in wholesale trade in the fuel/oil/gas industry was reported for 12 cases and 56 controls and was not associated with risk of multiple myeloma. The total number of cases and controls for which information was available on whether they were exposed to gasoline was not explicitly reported in the article. In age-adjusted analyses, possible exposure to gasoline was positively associated with a 20% increased risk of multiple myeloma, which moved to a 40% increased risk for probable exposure. Using a 10-year exposure lag, stratification of analyses by probability and duration of exposure, and adjustment of models for age and occupational exposure to engine exhaust, phthalates, and vinyl chloride, most OR estimates were below or close to unity, except for an OR of 1.6 (95% CI, 0.8–3.2) for probable exposure with a duration of < 5 years.

[The Working Group noted the use of record linkage as a strength. The study also had other strengths: the focus was on a specific haematolymphoid malignancy, namely multiple myeloma, a 10-year latency was considered in the analysis, and the sample size was large. Nondifferential misclassification of the exposure was possible given the incomplete job history.]

[Schnatter et al. \(1993\)](#) (in an update of the study by [Schnatter et al., 1992](#)) led an industry-conducted cohort study in 4889 active or living retired male workers who worked for  $\geq 1$  year between 1964 and 1983 in the marketing or distribution operating sector of a petroleum

company in Canada and were also classified as being ever exposed to hydrocarbons (75% of the full cohort of 6672 workers) (see Section 2.1.3). The SMR for workers ever exposed to hydrocarbons was close to unity for NHL and leukaemia, whereas it was 1.99 (95% CI, 0.64–4.63) for multiple myeloma. [The Working Group noted the estimated 2.4% underascertainment of deaths and a HWE for the SMR analyses.]

[Schnatter et al. \(1996\)](#) published the results of a case-control study on haematolymphoid malignancies nested in the same cohort (as described by [Schnatter et al., 1993](#)), considering the full cohort without restriction to workers exposed to hydrocarbons. Cases were men who died from leukaemia, as recorded on the death certificate. Four controls for each case were extracted from the cohort, matching on decade of birth. For 14 cases of leukaemia and the corresponding 55 controls, the study reported an OR of 1.061 (95% CI, 0.985–1.144) for years of employment, considered as a continuous variable.

[The Working Group noted that the study was quite small, and the duration of follow-up was relatively short. The primary focus on benzene meant that only some results were deemed relevant for the present evaluation. Even with restriction of the analyses to workers ever exposed to hydrocarbons, which was a strength of the study, co-exposure to other potential occupational carcinogens was likely for petroleum distribution workers, and this was noted as a strong limitation because of probable upward bias in the estimates. The nested case-control study was less informative as it was based on the full cohort and reported only the results for leukaemia, without distinguishing between subtypes, although this analysis may have reduced the HWE.]

[Sorahan \(2007\)](#) (in an update of the study by [Rushton, 1993a](#)) conducted an industry-sponsored cohort study of 16 477 male petroleum distribution workers in the UK who were first employed between 1946 and 1974 for  $\geq 12$  months and had some employment after 1950 (see Section 2.1.3).



Follow-up was conducted for cancer mortality between 1951 and 2003 and for cancer incidence between 1971 and 2003. [The Working Group considered that the results on cancer incidence were more informative for the present evaluation, except for the results stratified by job title, which were presented in the article for mortality data only.] The SRR estimates were not far from unity, being [0.79] for Hodgkin lymphoma, [0.96] for NHL, [0.96] for multiple myeloma, and [1.18] for all leukaemia. The SRR for myeloid leukaemia (which included AML) was elevated [1.33 (95% CI, 0.96–1.80)]. Results by more defined leukaemia subtypes, only possible for mortality in the period 1968–2003, showed elevated SMRs for CLL ([1.50 (95% CI, 0.89–2.37)]), AML ([1.39 (95% CI, 0.93–2.00)]), and ANLL ([1.27 (95% CI, 0.86–1.81)]).

In 1997, [Rushton and Romaniuk \(1997\)](#) published the results of an industry-funded case-control study on leukaemia that was nested in the same cohort as the study by [Sorahan \(2007\)](#). Cases were men who had died from leukaemia or who were registered with a diagnosis of leukaemia in the cancer register, which included data available since 1971. Four controls for each case were randomly selected from the cohort, matched on birth date ( $\pm 3$  years). The study focused on exposure to benzene, and most of the results were therefore not considered in the present evaluation. However, the Working Group did consider the results on duration of employment, which was reported for 90 cases of leukaemia and 354 controls. Results were also reported for CLL (31 cases), acute myeloid and monocytic leukaemia (31 cases), and CML (11 cases). When duration of employment in years was considered as a continuous variable, the ORs were 1.03 (95% CI, 1.00–1.05) for all leukaemia, 1.06 (95% CI, 1.01–1.11) for CLL, 1.03 (95% CI, 0.99–1.07) for acute myeloid and monocytic leukaemia, and 1.04 (95% CI, 0.96–1.12) for CML.

The same cohort was used to study the incidence of MDS with an extended follow-up, funded by the Energy Institute of the UK ([Sorahan and Mohammed, 2016](#)) (see Section 2.1.3). Because national comparisons of incidence data for MDS were possible from 1995 onwards (1997 in Scotland), the follow-up period for this analysis was from 1995 (or 1997) to 2011. There was no evidence of an increased incidence of MDS either overall or by duration of employment.

[The Working Group noted that the cohort studies ([Schnatter et al., 1993](#); [Rushton and Romaniuk, 1997](#); [Sorahan and Mohammed, 2016](#)) were relatively large and had long follow-up periods, but worker exposure to gasoline was not assessed (see Section 1.6.1 for a discussion on exposure assessment), and co-exposure to other potential occupational carcinogens was likely for petroleum distribution workers, with a potential bias upwards that limited the informativeness of the study for the present evaluation. The Working Group considered that the estimates of SMR and SRR from [Schnatter et al. \(1993\)](#) and [Sorahan and Mohammed \(2016\)](#) were prone to downward bias from the HWE and to some bias from the healthy-worker survivor effect because of restriction to employees with 1 year of employment.]

[Schnatter et al. \(2012\)](#) carried out a pooled analysis (with updated mortality and incidence data) of three case-control studies on lymphatic and haematopoietic malignancies nested in cohorts of petroleum distribution workers from Canada ([Schnatter et al., 1996](#)) the UK ([Rushton and Romaniuk, 1997](#)), and Australia ([Glass et al., 2003](#)). [The Working Group noted that the study by Glass et al. included workers involved in refinement and upstream work.] The pooled study included 60 cases of AML and 241 controls, 28 cases of CML and 122 controls, 80 cases of CLL and 345 controls, 29 cases of MDS and 129 controls, as well as 30 cases of MPD and 124 controls. [The Working Group noted that because this study focused on exposure to benzene, most

of the results were not considered informative for the present evaluation. This included the results on duration of employment, because the Australian cohort was not restricted to distribution workers (and study-specific results were not presented).] The study reported the results for ever-versus-never working as a tanker driver for  $\geq 1$  year. [The Working Group considered this comparison as a proxy for an exposure–response analysis in the cohort, because tanker drivers are expected to have higher levels of exposure to gasoline than are other distribution workers, as discussed in Section 1 for exposure to gasoline.)] The ORs for having ever versus never worked as a tanker driver were 2.02 (95% CI, 1.08–3.78) for AML, 2.16 (95% CI, 0.79–5.88) for MDS, 0.64 (95% CI, 0.36–1.14) for CLL, 1.26 (95% CI, 0.51–3.11) for CML, and 1.38 (95% CI, 0.60–3.21) for myeloproliferative disorders.

[The Working Group noted as strengths that the study had a clear definition of lymphatic and haematopoietic cancer subtypes and a large sample size. The study was informative only regarding the exposure–response relation. As the analysis was conducted internally in the cohort, workers in the reference category were also exposed to gasoline to a certain extent, with a potential bias towards the null of the OR estimates for tanker drivers. The analyses for tanker drivers were not adjusted for potential confounders, including smoking and other occupational co-exposures, introducing potential bias in an unpredictable direction. However, the authors reported that information on smoking was available from interview and workplace records for 51% of the cohort, and the *P* value associated with the positive dose–response relation between exposure to benzene and MDS became stronger after adjustment for smoking, suggesting that smoking was not a strong confounder.]

An industry-funded case–control study nested in the mortality cohort of gasoline-exposed land-based distribution workers in the

USA was conducted by [Wong et al. \(1999\)](#) (see Section 2.1.3). Cohort analyses were originally conducted by [Wong et al. \(1993\)](#) for marine-based and land-based workers separately. Cases were deceased individuals for leukaemia, including AML, or multiple myeloma. For each case, up to 5 controls were selected from the cohort, matched to cases on company, date of birth, and sex. No job category was associated with the risk of leukaemia, AML, or multiple myeloma. There was no evidence of an increased risk of any of the three cancer groups with increasing duration of employment or estimated yearly cumulative exposure expressed in terms of unit increase in parts per million-years (ppm-years) of total cumulative exposure to hydrocarbons.

[The Working Group noted as strengths that the study reported detailed exposure information and the focus was on haematolymphoid cancer subtypes, namely AML and multiple myeloma, although it was based only on mortality data. Downward bias from the HWE was noted as a strong limitation, as was co-exposure to other potential occupational carcinogens (which is likely for petroleum distribution workers), and the lack of adjustment for occupational or life-style-related confounders in the analysis. This might have biased the estimates in an unpredictable direction, including a downward bias if workers with high levels of exposure to gasoline had a lower level of exposure to potential occupational carcinogens.]

[Lagorio et al. \(1994a\)](#) conducted a cohort study in 2665 service station managers in the Latium region, Italy (see Section 2.1.4). Workers were enrolled in 1980 through a survey of service stations and were followed for mortality from 1981 until mid-1992. SMRs were calculated on the basis of the regional rates, standardizing on age, gender, and calendar time. [The 90% confidence intervals reported in the original study were converted to 95% confidence intervals by the Working Group.] There was no evidence of

an increased risk of haematolymphoid malignancies in this study with very few cases.

[The Working Group noted that the cohort members were probably exposed to refuelling activities, because self-service stations were still rare in the study area when the study was carried out, and most of the gas station workforce included self-employed workers. As discussed in Section 1.4.2, this was a strength of the study because self-service stations are very common in most European countries and North America. However, the cohort was quite small, had a relatively short duration of follow-up, and was based on mortality data. Moreover, the analyses were affected by left truncation, because workers who were not active at the time of the survey in 1980 could not be included in the cohort. As individuals with a shorter duration of employment were more likely to have left employment before 1980, the healthy-worker survivor effect and potential higher levels of exposure in short-term workers may have led to downward bias.]

[Patel et al. \(2004\)](#) conducted a historical cohort study in individuals resident in the area affected by a gasoline spill between 1990 and 2000 in Pennsylvania, USA (see Section 2.1.6). Among the 663 cohort members, 4 cases of leukaemia and 2 cases of NHL were observed. The SIR for NHL was 1.23 (95% CI, 0.15–4.46).

For leukaemia, the Working Group considered that the results reported in [Talbot et al. \(2011\)](#), which were based on an extended follow-up, were more informative. The authors extended the follow-up through the Pennsylvania Cancer Registry up to 2001 and calculated SIRs separately for the period 1990–1994, in which exposure to the gasoline spill was expected to have little impact on leukaemia risk when considering latency, and the period 1995–2001, which is the actual risk period during which effects caused by the spill might have been observed (see Section 2.1.6). They also conducted a spatial analysis based on the distance between the participant's residence and the projected underground

gasoline plume, categorizing the exposure as high, medium, and low. With 5 observed cases, there was an increased SIR for leukaemia and for AML, specifically for the period 1995–2001. Spatial analyses revealed evidence of increasing SIRs for leukaemia with increasing exposure levels.

[The Working Group considered that these studies were informative for the present evaluation, because the source of exposure was different from that investigated in the other studies and the results could complement other evidence, despite the small sample size and short follow-up. It was also possible that the retrospective study design, based on self-reported incident cancers, resulted in incomplete ascertainment of cancer cases, resulting in a downward bias in the SIR. Finally, latency was not considered in the analysis by [Patel et al. \(2004\)](#), possibly diluting the estimates. The analysis by [Talbot et al. \(2011\)](#) improved the initial analyses, because it was stratified into two periods on the basis of considerations regarding latency; the period with insufficient latency was used as a type of negative outcome control for the risk period; and spatial analyses with three estimated exposure levels were conducted.]

[Neasham et al. \(2011a\)](#) studied occupational risk factors for lymphoma within the EPIC cohort, a large European prospective cohort into which participants were recruited from the general population between 1992 and 2000 (see Section 2.1.7). The study included 218 968 participants who were followed for cancer incidence until 2006. Information on ever employment in 52 jobs (selected a priori) was collected through questionnaires at the time of enrolment. Analyses were carried out internally in the cohort, and adjustment for educational level, alcohol and smoking was possible. HRs were increased for NHL, driven by the estimates for multiple myeloma and plasmacytoma, and for Hodgkin lymphoma, for ever employment as a gasoline station worker.

[The Working Group noted that the study had important strengths, mainly the large sample size, the use of prospectively collected information, and the possibility to adjust for potential confounders. Limitations were that data were not analysed by time since beginning of employment, latency, or duration of employment. Considering the relatively short duration of the follow-up, the lack of consideration of latency in the analysis might have diluted the risk estimates.]

### 2.3.2 Case-control studies

The case-control studies contributing evidence on gasoline exposure and risk of lymphatic and haematopoietic malignancies are described in chronological order below.

[Siemiatycki et al. \(1987a\)](#) conducted a population-based case-control study on 20 cancer types, including NHL and Hodgkin lymphoma, among men aged 35–70 years between 1979 and 1985 in Montreal, Canada (see Section 2.1.10). For each case, controls were selected from among the patients recruited with other cancers, excluding individuals with lung cancer. For each case and control, the job history and potential confounders were collected through interviews, and 300 potential exposures were assessed on the basis of job histories. Analyses were restricted to cancer types for which at least 150 cases were recorded, thus results were not reported for Hodgkin lymphoma. As there was no evidence of a positive association between exposure to automotive gasoline and NHL, further analyses by exposure level and duration were not carried out.

[The Working Group noted that the study involved the assessment of exposure to automotive gasoline using expert judgement, which was a strength when compared with studies based on self-report. The ORs were adjusted for potential confounders. A limitation of the study was the use of cancer controls, which could potentially bias the OR estimates downward if exposure to automotive gasoline, or other co-occurring

occupational exposures in the same jobs, were risk factors for at least one of the cancer types. It was likely that this was the case, leading to a potentially large bias, although the use of patients with many other cancer types as controls diluted this source of selection bias.]

[West et al. \(1995\)](#) conducted a population-based case-control study on all newly diagnosed cases of MDS in south-east Wales and west Yorkshire, UK. Controls were selected from among individuals receiving inpatient or outpatient care in medicine, otorhinolaryngology, orthopaedics, or geriatrics hospital departments, excluding cancer patients, and were matched to cases on age, sex, area of residence, calendar year, and hospital. Cases and controls were asked to report their occupational history and main hobbies, and whether they had ever been exposed to any of 70 specific chemicals, providing details of the nature of the exposure. Intensity of exposure was estimated using information reported by the participants, in consultation with industrial chemists and occupational hygienists. Three thresholds were used to classify participants as exposed on the basis of lifetime duration of exposure and level of intensity:  $\geq 10$  hours of any intensity, defined as, for example, being in the same room as the chemical, material or hazard;  $\geq 50$  hours of medium intensity, defined as, for example, working with the chemical;  $\geq 2500$  hours of medium intensity. Petrol and diesel were grouped together and included both occupational exposure and exposure during transportation, i.e. self-service refuelling. The response rate was 62% for cases and was not reported for controls. The authors did not find evidence for an association between exposure to petrol or diesel, considered jointly, and the risk of MDS.

[The Working Group noted that the study had the strength of focusing on a specific subtype of haematolymphoid malignancy, but also had important limitations, including the combined assessment of diesel and gasoline exposure, the



fact that the exposure assessment was based on self-report, and the lack of information on response rate among controls. These limitations could lead to exposure misclassification, co-exposure to other occupational carcinogens, and selection bias, which together would bias the study estimates in an unpredictable direction. Analyses were not adjusted for other occupational exposures and lifestyle-related confounders; however, smoking was not a clear risk factor for MDS in this study.]

[Clavel et al. \(1995\)](#) conducted a multicentre hospital-based case-control study on hairy cell leukaemia in 18 French hospitals. Case and control patients were admitted to the hospitals between January 1980 and January 1990 and were interviewed using a postal questionnaire in 1990–1992. The response rate was 61% for cases and 58% for controls. The OR for ever exposure to petrol or diesel was increased in men (OR, 1.5; 95% CI, 1.0–2.1) and in women (OR, 2.1; 95% CI, 0.4–10.6), although the confidence intervals for women were very imprecise. [Clavel et al. \(1996\)](#) reported on an analysis of ever employment as a petrol station attendant that was from the same case-control study but was restricted to men.

[The Working Group noted that this study was less informative for the present evaluation as it was based on prevalent cases of hairy cell leukaemia with up to 10 years of survival, with a high risk of selection bias in an unpredictable direction. Moreover, exposure to gasoline and diesel were considered jointly, and the analysis on petrol station attendants was based on only two exposed patients.]

[Holly et al. \(1997\)](#) conducted a population-based case-control study on patients diagnosed with NHL in the hospitals of the San Francisco Bay Area, USA, between 1988 and 1995. Controls were sampled from the general population using random-digit dialling and were matched to cases on sex, county of residence, and age. The study population was restricted to homosexual men. Information on chemical exposures, including

gasoline, during work, leisure, or school was obtained by interview. The response rate was estimated to be 45% for cases in a later publication ([Holly et al., 2002](#)) and was not reported for controls. The OR for patients with NHL and gasoline exposure of  $\geq 10$  hours compared with gasoline exposure of  $< 10$  hours was 0.65 (95% CI, 0.36–1.2) in individuals who were positive for human immunodeficiency virus (HIV) and 1.7 (95% CI, 0.72–4.0) for individuals who were HIV-negative.

[The Working Group noted that this study was less informative for the present evaluation, as the exposure assessment was based on self-report and the response rate was low for cases and unknown for controls, resulting in a high potential for exposure misclassification, and information and selection bias.]

[Persson and Fredrikson \(1999\)](#) pooled two case-control studies that were conducted between 1964 and 1986 at the Orebro Medical Centre Hospital and between 1975 and 1984 at the University Hospital in Linköping, both in Sweden. The participants were Swedish-born individuals aged between 20 and  $< 80$  years who were residents in the catchment areas of the hospitals. The two studies recruited cases of incident Hodgkin lymphoma and NHL, but the pooled analysis focused on NHL; this diagnosis was confirmed by a physician in the first study and centrally reviewed by pathologists in the second study. Controls were randomly sampled from the same geographical areas and using the same inclusion criteria as for the cases. Case and control participants completed a questionnaire on potential risk factors for NHL, including information on occupational exposures with a lag time of 5 years. The response rate for cases was 96% and 90%, respectively, in the first and second study. The response rate for controls was 75% in the first study (see [Persson et al., 1989](#)), but it was not reported in the second study (see [Persson et al., 1993](#)). The OR for NHL in relation

to ever occupational exposure to gasoline was 1.9 (95% CI, 0.9–3.8).

[The Working Group noted that the exposure assessment in this study was based on self-report and that the response rate was lower (when reported) for controls than for cases. Differential misclassification and selection of controls by occupational status was possible, also considering that the analyses were not adjusted for potential confounders. An upward bias would be expected if occupational exposures tended to be more frequently reported among cases than controls and control individuals with a lower socioeconomic position were less likely to participate in the study.]

[Nisse et al. \(2001\)](#) conducted a case-control study in patients with MDS diagnosed between September 1991 and February 1996 at Lille University Hospital, France. Control individuals were randomly sampled from the general population, matching to cases on sex, age, and geographical department of residence. Cases and controls were interviewed to collect information on potential risk factors for MDS, including occupational history and information on past occupational exposure to 70 specific chemical or physical hazards. This information was reviewed by occupational experts who were able to contact the study participants or the occupational physician at the workplace. The study included 204 cases and 204 controls, and the response rate was not reported. There was some evidence of an association between ever exposure to petrol and risk of MDS (OR, 2.5; 95% CI, 0.9–7.7), and weak evidence of a difference in lifetime cumulative exposure between cases and controls ( $P = 0.8$ ).

[The Working Group noted that this was one of the studies that were specifically about MDS. The sample size was relatively small. The response rate for cases and controls was not reported, and selection bias was possible, especially if participation were driven by socioeconomic status or occupational characteristics.

The results adjusted for socioeconomic status or potential confounders were not reported.]

[Björk et al. \(2001\)](#) conducted a case-control study in 255 patients with Philadelphia chromosome-positive CML (Ph+ CML) that was analysed cytogenetically between 1976 and 1993 at Lund University Hospital, Sweden. Controls were sampled from the general population, matching to cases on sex, age, and county of residence. Cases and controls, or their next of kin, were contacted in 1995–1997 to participate in the study interview. The response rate was 89% for cases and 72% for controls, but next of kin were interviewed far more often for cases (81%) than for controls (14%). Case and control participants were asked to report occupational history and hobby activities. Individual exposure assessment was carried out by industrial hygienists, and participants who could not be classified as exposed or unexposed with high confidence were excluded from the analyses. The authors found no evidence of an association between exposure to gasoline or diesel fuel and the risk of Ph+ CML.

[The Working Group noted that this study had the strength of focusing on a specific subtype of haematolymphoid malignancy but also had important limitations that reduced its informativeness for the present evaluation. The proportion of interviews with next of kin differed considerably between cases and controls, possibly leading to underestimation of the frequency of exposure among cases and biasing the OR estimates downward. Diesel and gasoline fuels were assessed jointly. Analyses were not adjusted for other occupational exposures or lifestyle-related confounders. Smoking, however, was not a risk factor for Ph+ CML in this study, and thus it was unlikely to act as a confounder.]

[Dryver et al. \(2004\)](#) conducted a population-based case-control study on cases of pathologically confirmed NHL listed in the regional Swedish tumour registry between 1990 and 1998 and controls sampled from the general



population, matching to cases on sex, age, and parish. Case patients whose primary physician gave consent and control individuals were sent a postal questionnaire, which was returned by 74% of cases and 69% of controls. The questionnaire collected information on whether the person had ever been employed in any of 25 specific occupations, including employment as gas station attendant, and had ever been exposed to a list of 16 specific exposures, including gasoline. With 859 cases and 1310 controls, the study found evidence of an association with risk of NHL for ever employment as a gas station attendant (OR, 1.62; 95% CI, 0.92–2.83) and for exposure to gasoline (OR, 1.46; 95% CI, 1.04–2.05). The risk associated with gasoline exposure increased with duration of exposure (OR for > 5 years of gasoline exposure versus < 5 years, 1.92; 95% CI, 1.20–3.08).

[The Working Group noted that the main strength of this study was its large sample size. The response rate was relatively low but was similar for cases and controls. The use of self-reported information may have led to differential exposure misclassification through recall bias, which would have shifted the OR estimates upwards if cases were more likely to report or overreport occupational exposures than were controls, and to nondifferential exposure misclassification, which would have probably biased the estimates towards the null. The results for exposure to aliphatic and alicyclic hydrocarbon solvents assessed by JEM were not considered directly for the present evaluation but were considered to be consistent with the self-reported results. No exposure lagging was considered in the analysis, leading to potential dilution of the exposure estimates. Analyses were not adjusted for other occupational exposures or socioeconomic position.]

[Orsi et al. \(2007\)](#) conducted a hospital-based case-control study on haematolymphoid malignancies diagnosed between September 2000 and December 2004 among individuals aged 18–75 years in the main hospitals of six cities

in France. Diagnoses were centrally reviewed. Case patients treated with immunosuppressant drugs or with acquired immunodeficiency syndrome (AIDS) were excluded. Controls were patients admitted to the same hospitals as the cases, mainly in orthopaedics and rheumatology departments, for reasons other than cancer and diseases directly related to occupation, smoking, or alcohol consumption. Cases and controls were asked to report a detailed occupational history and whether they had ever been exposed to a predefined list of occupational hazards, including petrol. The use of ad hoc occupational questionnaires specific to potential exposures and a review by industrial hygienists were mentioned in the article. The refusal rate was 5.5% for cases and 11.7% for controls. There was no evidence of an association between self-reported ever occupational exposure to petrol and risk of lymphoproliferative syndromes (including CLL and hairy cell leukaemia), NHL, Hodgkin lymphoma, and multiple myeloma, which were analysed both jointly and separately.

[The Working Group noted that this was a relatively large study with a high response rate and that haematolymphoid cancer subtypes were analysed separately. Because gasoline exposure was self-reported, there was potential for recall bias, which would probably bias the estimates upwards, and exposure misclassification in cases and controls, which would bias the estimates towards the null. The results were adjusted for socioeconomic status but were not adjusted for other potential occupational hazards or smoking.]

[Cocco et al. \(2010\)](#) conducted a multicentre case-control study on lymphoid malignancies diagnosed between 1998 and 2004 in six countries in Europe. Case patients were adult residents in the referral areas of the participating centres who had a histologically or cytologically confirmed diagnosis of lymphoma during the study period. A centralized panel of pathologists reviewed about 20% of the diagnostic

slides: as reported by [Besson et al. \(2006\)](#), 87% of the initial diagnoses were confirmed, 10% were changed, and for 2% the review was inconclusive. In Italy and Germany, controls were randomly sampled from the general population, whereas in the other countries controls were individuals hospitalized for reasons other than cancer or infectious and immunodeficiency-related diseases. Case and control participants were interviewed to obtain information on risk factors for lymphoma, including occupational history and potential exposure to selected hazards. Further information was obtained using 14 questionnaire modules for specific occupations. Industrial hygienists reviewed the information obtained through the interviews to assess exposure to 43 agents, including gasoline. They reported their confidence in the assessment, and assessed intensity, frequency, and cumulative exposure. The response rate was 88% for cases, 81% for hospital controls, and 52% for population controls. The study included 2348 cases of lymphoma and 2462 controls, and separate analyses were conducted for the following entities: B-cell NHL; its major subtypes, namely diffuse large B-cell lymphoma (DLBCL), CLL, follicular lymphoma and multiple myeloma; T-cell lymphoma; and Hodgkin lymphoma. “Never exposure” to the solvents assessed in the study was used as the reference category, and analyses were adjusted for age, sex, education, and centre. High confidence in the assessment of gasoline exposure was reported for 70.8% of exposed cases and 63.9% of exposed controls. Ever exposure to gasoline with any confidence was associated with an OR for CLL of 1.3 (95% CI, 0.9–1.8), the OR for multiple myeloma was 1.2 (95% CI, 0.8–1.7), and for all other subtypes the OR estimates were close to or below unity. When analyses were restricted to individuals for whom exposure was assessed with a high degree of confidence, there was an excess risk of 70% for CLL, 30% for multiple myeloma, and 20% for all B-NHL. For these three subtypes, the risk

increased with increasing cumulative exposure, categorized in tertiles based on the distribution among controls.

[The Working Group noted that this study was highly informative for the evaluation of the risk of lymphoma for several reasons. First, the study had rather large sample size. Second, the major lymphoma subtypes were analysed separately. Third, there was a thorough assessment of occupational exposure to gasoline. Fourth, trend analyses were conducted for different exposure metrics. The response rate was relatively high for cases and hospital controls, but lower for population controls. The fact that there was an association specifically for some lymphoma subtypes and not for all argued for a limited role of selection bias and other biases, including recall bias, as it was unlikely that these sources of bias affected only certain cancer subtypes. The results were adjusted for education but were not adjusted for potential occupational confounders and lifestyle-related factors. As reported by [Besson et al. \(2006\)](#), however, smoking and alcohol consumption were not associated with risk of NHL in this study and are not expected to be confounders for NHL.]

[Lv et al. \(2011\)](#) conducted a hospital-based case-control study on MDS in 27 major hospitals in Shanghai, China, between June 2003 and November 2006. Controls, matched to cases 2:1 on age, sex, and hospital, were recruited among patients admitted for various conditions unrelated to haematological diseases, including respiratory infections, acute gastrointestinal infections, genitourinary diseases, cardiovascular diseases, and trauma. Case and control participants were interviewed to obtain information about potential risk factors for MDS. They were asked to report their occupational history, on the basis of which a panel of experts assessed occupational exposure to selected hazards, including gasoline. The study included 403 patients with MDS and 806 controls. The response rate was not reported. Analyses were conducted for all cases

of MDS and for refractory anaemia with excess of blasts (RAEB) and refractory cytopenia with multiple dysplasia (RCMD) separately. There was some evidence of an association between ever-versus-never exposure to gasoline and the risk of MDS (OR, 2.35; 95% CI, 0.92–5.99), which was particularly strong for RCMD.

[The Working Group considered that the analyses by MDS subtype were a strength of this study. This was also the only study on haematolymphoid malignancies that was not conducted in Europe or North America. Response rate was not reported, but it was expected to be high and similar for cases and controls, being a hospital-based study. Analyses were adjusted for potential occupational and socioeconomic confounders, and smoking. The analysis was also adjusted for benzene exposure, which may be considered to be an inappropriate adjustment because exposure to benzene can result from exposure to automotive gasoline.]

Within the context of a European multi-centre population-based case-control study on seven rare cancers, [Morales-Suárez-Varela et al. \(2013\)](#) analysed the association between gasoline exposure and risk of mycosis fungoides. Cases aged between 35 and 69 years were recruited between January 1995 and June 1997 in 25 areas in six countries in Europe. Diagnoses were confirmed by a reference pathologist. Controls were selected from the general population or, in one country, among patients with colon cancer and were frequency-matched to cases (all seven cancer sites) on sex, age, and study area. Case and control participants were interviewed to obtain information on potential risk factors for mycosis fungoides and on occupational history. Exposure to gasoline was assessed using a JEM, calculating a cumulative exposure score based on the probability, frequency, and intensity of exposure and the duration of each job. The participation rate was 84.75% among cases and 68.2% among controls, including next-of-kin interviews in 3–4% of the participants for both

groups. Sensitivity analyses were conducting using different lag times for the exposure and excluding jobs with a low exposure probability; however, only results for ever exposure with any probability and no lag were shown, because OR estimates were reported to remain unchanged in the sensitivity analyses. On the basis of a relatively low number of exposed cases, there was no evidence of an association between exposure to gasoline and risk of mycosis fungoides.

[The Working Group noted as strengths of this study the focus on a specific subtype of haematolymphoid malignancy and the use of a JEM-based exposure assessment. Estimates were adjusted for educational level and lifestyle-related potential confounders. However, the sample size was inherently small, given the rarity of mycosis fungoides, which limited the informativeness of the study for the present evaluation.]

[Poynter et al. \(2017\)](#) conducted a population-based case-control study on MDS and AML in Minnesota, USA. Cases were identified through the Minnesota Cancer Surveillance System among residents aged 20–85 years (20–79 years for AML) between June 2005 and November 2009 for AML and between April 2010 and October 2014 for MDS. The diagnosis of MDS was centrally reviewed and confirmed by an expert panel. Randomly selected population controls, with no prior diagnosis of myeloid leukaemia, were frequency-matched to cases on decile of age. The controls in the AML and MDS studies were then combined and used jointly for both cancer types. Information on exposure to several risk factors, including occupational and/or residential exposure to gasoline for  $\geq 8$  hours per week for  $\geq 1$  year, was collected by a self-administered questionnaire. Information on duration of exposure was also obtained. The response rate was 58% for AML cases, 59% for MDS cases, 64% for AML controls, and 49% for MDS controls). The OR for  $\geq 5$  years of exposure to gasoline compared with no exposure was 1.11

(95% CI, 0.71–1.74) for MDS and 1.40 (95% CI, 0.95–2.06) for AML [crude OR for AML, 1.30].

[The Working Group noted that this was a relatively large study focusing on a specific haematolymphoid malignancy. A strength was that the analysis was adjusted for the main potential confounders, including socioeconomic position, smoking, and exposure to radiation. Information on exposure was obtained by questionnaire, and there was the potential for misclassification and information bias if exposure to gasoline was reported differently (possibly more often, with an upwards bias) by case participants than by controls. The response rate was quite low for both cases and controls, which could lead to considerable bias in an unpredictable direction, although adjustment for socioeconomic status may have reduced these concerns.]

### 2.3.3 Meta-analyses

[Kane and Newton \(2010\)](#) conducted a systematic review and meta-analysis to study the relation between working in the production, distribution, and use of gasoline and risk of NHL. The review included studies on refinery workers, petroleum distribution workers, and also motor mechanics, who were not considered in the present evaluation. The authors also included three studies on filling station attendants for which the pooled OR was 1.22 (95% CI, 0.93–1.60); all three studies were also included in the present evaluation ([Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Dryver et al., 2004](#)). [Kane and Newton \(2010\)](#) also included five studies on self-reported exposure to gasoline, of which three were included in the present evaluation ([Holly et al., 1997](#); [Persson and Fredrikson, 1999](#); [Orsi et al., 2007](#)) and two were excluded ([Nadon et al., 1995](#); [Karunanayake et al., 2008](#)). [The Working Group considered this meta-analysis to be less informative for the present evaluation because it used different study inclusion criteria, and it could not focus on NHL subtypes. The Working Group considered that a

new meta-analysis carried out by the Working Group was warranted.]

## 2.4 Cancers of the urinary tract

See [Table 2.4](#).

This section contains a review of the epidemiological literature on automotive gasoline exposure and cancers of the urinary tract. In all the studies, information on job history was used to assess the study participants' exposure to automotive gasoline on the basis of job or industry, JEMs, or by relying on the judgement of industrial hygienists. As in many occupational environments, exposure to gasoline cannot be isolated from exposures to a variety of other chemicals, such as exhaust gases or petroleum chemicals, the Working Group selected the results in occupational groups in which exposure to gasoline was considered predominant, such as land-based petroleum distribution workers ([Schnatter et al., 1993](#); [Wong et al., 1993, 1999](#); [Gun et al., 2006](#); [Sorahan, 2007](#)) or gas station attendants ([Howe and Lindsay, 1983](#); [Silverman et al., 1983](#); [McLaughlin et al., 1984, 1985](#); [Schoenberg et al., 1984](#); [Malker et al., 1987](#); [McCredie and Stewart, 1993](#); [Lagorio et al., 1994a](#); [Mandel et al., 1995](#); [Lynge et al., 1997](#); [Teschke et al., 1997](#); [Colt et al., 2004, 2011](#); [Band et al., 2005](#)), or studies in which exposure to gasoline was assessed specifically ([Siemiatycki et al., 1987a](#); [Sharpe et al., 1989](#); [Partanen et al., 1991](#); [Kogevinas et al., 2003](#); [Lohi et al., 2008](#)).

Bladder cancer risk was also described in a small community-based cohort of individuals who were environmentally exposed to gasoline ([Patel et al., 2004](#)). A case-control study on bladder cancer and exposure to “oil and gasoline” at work was also considered ([Mommensen and Aagard, 1984](#)).

**Table 2.4 Epidemiological studies on exposure to automotive gasoline and cancers of the kidney, bladder, and other organs of the urinary tract**

| Reference, location, enrolment/follow-up period, study design                                                                   | Population size, description<br>Exposure assessment method                                                                                                                                                                                 | Cancer type (histopathology), incidence or mortality               | Exposure category or level                                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                  | Comments                                                                                                                                                                                                                                                                                                                                         |
|---------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|------------------------------------------------------------------|-------------------------|------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Howe and Lindsay (1983)</a><br>Record linkage study, Canada 1965–1969/follow-up, through 1973 (mortality)<br>Cohort | 415 201 (men); individuals with occupation recorded in 1965–1969, representing 10% of Canadian labour. Mortality data through linkage with Statistics Canada.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> .        | Urinary bladder (bladder plus other urinary organs, ICD-8 188–189) | SMR ( $P \leq 0.001$ ):<br>Service station attendants            | 7                       | [5.29 (2.1–10.9)]      | Age                                    | <i>Strengths:</i> Large study population.<br><i>Limitations:</i> No distinction between different urinary organs.                                                                                                                                                                                                                                |
| <a href="#">McLaughlin et al. (1987)</a><br>Sweden 1960 census/follow-up, 1961–1979 (incidence)<br>Cohort                       | Number NR; employed men in the 1960 census in Sweden. Cancer-Environment Registry of entire population employed in 1960, using employment data from the 1960 census.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney                                                             | Occupation recorded in 1960 census (SIR):<br>Gas station workers | 9                       | [0.59 (0.27–1.12)]     | Sex, birth cohort, region of residence | <i>Strengths:</i> High-quality population-based exposure and outcome registries with near-complete national follow-up.<br><i>Limitations:</i> Cross-sectional exposure assessment at single time point, 1960.<br><i>Other comments:</i> Results were adjusted for sex, and 5-yr birth cohort, compared with the Swedish population in 1961–1979. |



**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                                                                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                     | Cancer type (histopathology), incidence or mortality                                         | Exposure category or level                                                                                                                                                                                                                                                                                                                                                                                            | Exposed cases or deaths                                          | Risk estimate (95% CI)                                                                                                                                                          | Covariates controlled                  | Comments                                                                                                                                                                                                                                                                                                                        |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Malker et al. (1987)</a><br>Sweden<br>1960 census/ follow-up, 1961–1979 (incidence)<br>Cohort                                                                                                                             | Number NR; employed men in the 1960 census in Sweden. Cancer-Environment Registry of entire population employed in 1960, using employment data from the 1960 census. Observed numbers were compared with the Swedish population in 1961–1979.<br><i>Exposure assessment method:</i> See <a href="#">McLaughlin et al. (1987)</a> , <a href="#">Table 2.1</a> . | Urinary bladder                                                                              | Occupation recorded in 1960 census (SIR):<br>Gas station workers                                                                                                                                                                                                                                                                                                                                                      | 29                                                               | [1.34 (0.90–1.92)]                                                                                                                                                              | Sex, birth cohort, region of residence | <i>Strengths:</i> High-quality population-based exposure and outcome registries with near-complete national follow-up.<br><br><i>Limitations:</i> Cross-sectional exposure assessment at single time point, 1960.                                                                                                               |
| <a href="#">Lyng et al. (1997)</a><br>Service station attendant cohort<br>Denmark, Norway, Sweden, and Finland<br>1970 census/ Follow-up ending in 1987 (Denmark), 1991 (Norway), 1989 (Sweden), 1985 (Finland) (incidence)<br>Cohort | 16 524 men; 2445 women; workers (male and female) in the service station industry identified through 1970 census in Denmark, Norway, Sweden, Finland.<br>Incidence (ICD-7) through the national cancer registries.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> .                                                                       | Kidney<br><br>Kidney<br><br>Kidney (urinary pelvis/UUT), renal pelvis<br><br>Urinary bladder | Employment in the service station industry (SIR): (national referent):<br>Men<br>Women<br><br>Country: employment in the service station industry (national referent), men (SIR):<br>Denmark<br>Norway<br>Sweden<br>Finland<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women<br><br>Employment in the service station industry (national referent) (SIR):<br>Men<br>Women | 53<br>4<br><br>10<br>17<br>22<br>4<br><br>10<br>0<br><br>86<br>2 | 1.3 (1.0–1.7)<br>1.2 (0.3–3.0)<br><br>[1.5 (0.7–2.8)]<br>[1.5 (0.8–2.3)]<br>[1.1 (0.7–1.7)]<br>[1.4 (0.4–3.4)]<br><br>2.0 (1.0–3.7)<br>NC<br><br>1.1 (0.9–1.3)<br>0.5 (0.0–1.9) | Country, age, sex                      | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Large cohort of service station attendants at the time where fuelling operations were common.<br><br><i>Limitations:</i> Lack of adjustment for smoking, lack of data on duration and latency. |



**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                           | Population size, description<br>Exposure assessment method                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level     | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                      | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------|-------------------------|------------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Lohi et al. (2008)</a><br>Record linkage, Finland<br>1970 census/ follow-up, 1971–1995 (incidence)<br>Cohort | 1.6 million; economically active people (born in 1906–1945) identified in the 1970 census. Follow-up through Finnish Cancer Registry.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney (RCC)                                         | Gasoline exposure, men (RR):   |                         |                        | Age, calendar period, cigarette smoking, BMI, social class | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Strengths:</i> Large size, quantitative exposure (JEM). Adjustment for non-occupational risk factors is also a strength, although it was conducted at an occupational group level, possibly resulting in overadjustment.<br><br><i>Limitations:</i> Since only the occupation held at entry in the cohort in 1970 was known, no analysis by duration of exposure, cumulative exposure or by latency since first exposure was possible, possibly resulting in RR estimates biased towards the null. |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Not exposed                    | NR                      | 1                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Low                            | 332                     | 1.13 (0.99–1.28)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Middle                         | 62                      | 1.05 (0.81–1.35)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | High                           | 12                      | 0.83 (0.49–1.40)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 | Kidney (RCC)                                         | Gasoline exposure, women (RR): |                         |                        |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Not exposed                    | NR                      | 1                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Low                            | 4                       | 1.21 (0.45–3.24)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Middle                         | 2                       | 2.74 (0.69–11.00)      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | High                           | 0                       | 0                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 | Urinary bladder                                      | Gasoline exposure, men (RR):   |                         |                        |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Not exposed                    | NR                      | 1                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Low                            | 366                     | 1.00 (0.89–1.13)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Middle                         | 87                      | 1.05 (0.85–1.30)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | High                           | 35                      | 1.11 (0.79–1.54)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 | Urinary bladder                                      | Gasoline exposure, women (RR): |                         |                        |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Not exposed                    | NR                      | 1                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Low                            | 3                       | 1.55 (0.50–4.86)       |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | Middle                         | 0                       | 0                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                                                                                                          |                                                                                                                                                                                                                 |                                                      | High                           | 0                       | 0                      |                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                             | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                             | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                    | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                     | Comments                                                                                                                                                                                                                                                                                                                                                                                                                        |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------|------------------------|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Schnatter et al. (1993)</a><br>Update of <a href="#">Schnatter et al. (1992)</a><br>Canadian distribution workers 1964–1983 (mortality) Cohort | 6672; all active male employees and living retirees as of 1 January 1964 or new male employees (1964–1983) with ≥ 1 yr of employment in marketing and distribution for petroleum industries in Canada. Mortality data from Statistics Canada, National Death Index, or other sources (regional registries). 4889 marketing or distribution workers ever exposed to hydrocarbons.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney                                               | Exposed to hydrocarbons (national referent) (SMR):                                            |                         |                        | Age, calendar year                        | <i>Strengths:</i> Assessment of hydrocarbon exposure frequency. Available information on duration of employment, years of latency after first exposure. Internal analysis to eliminate the healthy-worker effect.<br><br><i>Limitations:</i> No information on exposure intensity. No information on potential confounders. Left truncation of the cohort with potential downward bias. HWSB because of 1-yr exposure criteria. |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Any job                                                                                       | 7                       | 1.58 (0.63–3.25)       |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Tank truck drivers                                                                            | 2                       | [2.10 (0.3–7.6)]       |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Kidney                                               | Frequency of hydrocarbon exposure in distribution workers (national referent) (SMR):          |                         |                        |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Less than daily exposure                                                                      | 2                       | [0.99 (0.1–3.6)]       |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Daily exposure                                                                                | 5                       | 2.08 (0.67–4.85)       |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Kidney                                               | Years employed for marketing/distribution workers exposed to hydrocarbons (SMR):              |                         |                        |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | < 10 yr                                                                                       | 0                       | 0                      |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 10–19 yr                                                                                      | 1                       | 1.02                   |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 20–29 yr                                                                                      | 4                       | 2.60                   |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | ≥ 30 yr                                                                                       | 1                       | 0.75                   |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Kidney                                               | Latency for marketing/distribution workers exposed to hydrocarbons (national referent) (SMR): |                         |                        |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | > 10 yr                                                                                       | 6                       | 1.46                   |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | > 20 yr                                                                                       | 6                       | 1.81                   |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Kidney                                               | Frequency of hydrocarbon exposure (RR):                                                       |                         |                        | Age, SES, year hired, death pre/post 1974 |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Non-exposed                                                                                   | 2                       | 1                      |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Less than daily exposure                                                                      | 2                       | 0.84 (0.06–12.17)      |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Daily exposure                                                                                | 5                       | 3.80 (0.45–32.29)      |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                 |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                                               | Population size, description<br>Exposure assessment method                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                         | Exposed cases or deaths | Risk estimate (95% CI)               | Covariates controlled       | Comments                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Schnatter et al. (1993)</a><br>(cont.)                                                                                                                                                           |                                                                                                                                                                                                                 | Urinary bladder                                      | Exposed to hydrocarbons (national referent) (SMR):<br>Any job<br>Tank truck drivers                                                | 3<br>2                  | 0.49 (0.09–1.43)<br>[1.92 (0.2–6.9)] | Age, calendar year          |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                                              |                                                                                                                                                                                                                 | Urinary bladder                                      | Frequency of hydrocarbon exposure in distribution workers (national referent) (SMR):<br>Less than daily exposure<br>Daily exposure | 0<br>3                  | NC<br>[1.07 (0.2–3.1)]               |                             |                                                                                                                                                                                                                                                                                                                                                           |
| <a href="#">Sorahan (2007)</a><br>Update of <a href="#">Rushton (1993a)</a><br>UK<br>Enrolment, 1946–1975/<br>follow-up, 1951–2003 (mortality), 1971–2003 (incidence)<br>(incidence and mortality)<br>Cohort | 16 477; male workers in petroleum distribution centres first employed in 1946–1974 with ≥ 1 yr employment and some employment after 1951.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney, mortality                                    | Distribution workers employment (national referent) (SMR):<br>Ever employment                                                      | 54                      | [1.14 (0.86–1.49)]                   | Age, calendar year, country | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><i>Design and analysis:</i><br><i>Strengths:</i> Large study size. Long follow-up. Mortality and cancer morbidity data.<br><i>Limitations:</i> Analysis by duration of employment, latency, or occupation not available for most cancers. HWSB because of 1-yr exposure criteria. |
|                                                                                                                                                                                                              |                                                                                                                                                                                                                 | Kidney and other urinary, incidence                  | Distribution workers employment (national referent) (SRR):<br>Ever employment                                                      | 75                      | [0.95 (0.75–1.19)]                   |                             |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                                              |                                                                                                                                                                                                                 | Urinary bladder, mortality                           | Distribution workers employment (national referent) (SMR):<br>Ever employment                                                      | 84                      | [1.01 (0.81–1.25)]                   |                             |                                                                                                                                                                                                                                                                                                                                                           |
|                                                                                                                                                                                                              |                                                                                                                                                                                                                 | Urinary bladder, incidence                           | Distribution workers employment (national referent) (SRR):<br>Ever employment                                                      | 257                     | [1.19 (1.05–1.35)]                   |                             |                                                                                                                                                                                                                                                                                                                                                           |

Table 2.4 (continued)

| Reference, location, enrolment/ follow-up period, study design                                                                                                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                       | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled       | Comments                                                                                                                                                                                                                                                                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Wong et al. (1999)</a><br>(supersedes <a href="#">Wong et al., 1993</a> )<br>USA<br>Enrolment 1946–1985/follow-up not specified (mortality)<br>Nested case–control | <i>Source cohort:</i> Marine vessel and land-based terminal petroleum distributors (See <a href="#">Wong et al., 1993</a> ).<br><br><i>Cases:</i> 35 leukaemia, 13 AML, 11 multiple myeloma, 12 kidney. The nested case–control study was restricted to land-based workers; cases identified through underlying and contributory causes of death listed on death certificates (ICD-8).<br><br><i>Controls:</i> 5 controls per case; Individuals alive at the time of case death and either alive at the end of follow-up or dead for cause other than the cancers of interest (leukaemia, AML, multiple myeloma, kidney cancer). Matching on company, date of birth, sex.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney                                               | Petroleum distribution workers (land and marine) exposed to gasoline (national referent) (SMR):  |                         |                        | NR                          | <i>Strengths:</i> Quantitative exposure assessment, including dose–response analysis.<br><br><i>Limitations:</i> Use of mortality rather than morbidity data, the very small number of kidney cancer cases in the cohort, and the lack of information on smoking and other risk factors for kidney cancer. HWSB because of 1-yr exposure criteria. |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Kidney                                               | Petroleum distribution workers (land-based only) exposed to gasoline (per 1-unit increase) (RR): | Exposed 24              | 0.73 (0.47–1.09)       | Company, date of birth, sex |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Duration of employment (yr)                                                                      | NR                      | [0.97 (0.90–1.05)]     |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Duration of exposure (yr)                                                                        | NR                      | [1.00 (0.93–1.07)]     |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Cumulative total hydrocarbon exposure (ppm-years)                                                | NR                      | [1.00 (0.99–1.01)]     |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Frequency of peak exposure                                                                       | NR                      | [1.00 (1.00–1.00)]     |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Year of first exposure (≤ 1948 vs ≥ 1949)                                                        | NR                      | [0.33 (0.07–1.62)]     |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Kidney                                               | Petroleum distribution workers (land-based only) exposed to gasoline, job (RR):                  |                         |                        |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Drivers                                                                                          | NR                      | 1.15 (0.32–4.19)       |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Loaders                                                                                          | NR                      | 5.73 (0.88–37.3)       |                             |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Urinary bladder                                      | Petroleum distribution workers (land and marine) exposed to gasoline (national referent) (SMR):  |                         |                        | NR                          |                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Exposed                                                                                          | 33                      | 0.72 (0.49–1.01)       |                             |                                                                                                                                                                                                                                                                                                                                                    |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                               | Cancer type (histopathology), incidence or mortality | Exposure category or level                          | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled     | Comments                                                                                                                                                                                                                               |
|-----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------|-------------------------|------------------------|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Gun et al. (2006)</a><br>Petroleum distribution (Health Watch), Australia<br>1980–2000 (deaths)<br>Cohort | 16 547 men and 1356 women; employees of Australian Institute of Petroleum companies enrolled through a survey interview in 1980–1983, 1985, and/ or 2000. Mortality from National Death Index and incidence from National Cancer Statistics Clearing House (include cancer registries in various states except Victoria and South Australia, which were separately used for additional cancer identification).<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney                                               | Tanker drivers (national referent) (SIR):<br>Kidney | 12                      | 2.05 (1.06–3.59)       | Sex, age, calendar period | <i>Strengths:</i> High participation rates in the early surveys.<br><br><i>Limitations:</i> Smoking was not taken into account. Only for tanker drivers exposure to gasoline can be inferred, but those results are limited to kidney. |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                                                                 | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level              | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled     | Comments                                                                                                                                                                                                         |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------|-------------------------|------------------------|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Lagorio et al. (1994a)</a><br>Gas station attendant cohort, Latium, Italy<br>Enrolment 1980/ follow-up, 1981 to July 1991 (residents in Rome), June 1992 (residents in other municipalities) (mortality)<br>Cohort | 2665 service gas station managers in Latium region identified through a nationwide survey in 1980, who were alive as of 1 January 1981. Vital status through municipality registry of residence and cause of death through linkage with national or regional mortality registry, or through review of death certificates.<br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney                                               | No. of deaths:                          |                         |                        | None                      | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><i>Design and analysis:</i> <i>Strengths:</i> See <a href="#">Table 2.1</a> .<br><i>Limitations:</i> Small sample size, short follow-up. |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Service station attendants              | 0                       | NC                     |                           |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 | Urinary bladder                                      | SMR (Latium region as referent):        |                         |                        | Sex, age, calendar period |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Service station attendants              | 6                       | [1.20 (0.44–2.61)]     |                           |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 | Urinary bladder                                      | SMR, women (Latium region as referent): |                         |                        | .                         |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Service station attendants              | 0                       | NC                     |                           |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 | Urinary bladder                                      | SMR, men (Latium region as referent):   |                         |                        | Age, calendar period      |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | All service station attendants          | 6                       | [1.22 (0.45–2.67)]     |                           |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Small service stations                  | 4                       | [1.16 (0.32–2.97)]     |                           |                                                                                                                                                                                                                  |
|                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                 |                                                      | Large service stations                  | 2                       | [1.39 (0.17–5.02)]     |                           |                                                                                                                                                                                                                  |



**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                        | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------|-------------------------|------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Patel et al. (2004)</a><br>Area of a gasoline spill, PA, USA 1990–2000 (incidence) Cohort | 663 people living $\geq 6$ mo (1990–2000) in the US EPA remediation area affected by Tranguh gasoline spill. First household in the area were identified, then, within each household, all individuals aged $> 18$ yr were interviewed. Data collected through questionnaire and medical records review between 2001 and 2002 (retrospective design). Cancer identified through self-reporting in the questionnaire, later on validated through medical records. Participation rate, 63% (full questionnaire completion).<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Urinary bladder                                      | SIR (state of PA referent):<br>Individuals affected by the gasoline spill | 3                       | 1.28 (0.26–3.73)       | Age                   | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> This was the only available study on gasoline environmental exposure.<br><i>Limitations:</i> Small sample size, indirect assessment of gasoline exposure. Although this study collected the information on potential confounders such as smoking and chronic diseases, this information was not used in the analysis.<br><br><i>Other comments:</i> Later follow-up for leukaemia only in <a href="#">Talbot et al. (2011)</a> , hence results for leukaemia are reported only from <a href="#">Talbot et al. (2011)</a> . |

Table 2.4 (continued)

| Reference, location, enrolment/follow-up period, study design                                                                                                                                              | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Cancer type (histopathology), incidence or mortality | Exposure category or level                                             | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled  | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------|-------------------------|------------------------|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">McLaughlin et al. (1985)</a><br>(supersedes <a href="#">McLaughlin et al., 1984</a> )<br>Minneapolis St Paul, Minnesota, USA<br>January 1974 to June 1979 (interviews in 1980)<br>Case-control | <i>Cases:</i> 495 (313 men); RCC cases from records of all hospitals of Minneapolis St Paul area. Next of kin interviewed if patients deceased ( $n = 237$ ) or too ill to participate ( $n = 14$ ).<br><br><i>Controls:</i> 695 (428 men); Controls identified through random-digit dialling (aged < 65 yr) or through Health Care Financing Administration for Medicare (> 65 yr). Next of kin interviewed for 493 deceased controls matched to the deceased cases on age at death, year of death and sex.<br><br><i>Exposure assessment method:</i> Self-reported occupational history. | Kidney (RCC)                                         | Duration of employment as a gasoline station attendant, men (yr) (OR): |                         |                        | Age, cigarette smoking | <i>Strengths:</i> Ascertainment of incident cases during the study period virtually complete. Histologically or X-ray-confirmed cases of RCC. Very high response rate among cases and controls (98%). Particular attention paid to gasoline station attendants among all petroleum-related professions. Adjustment for smoking.<br><br><i>Other comments:</i> A supplementary control group of deceased frequency-matched controls was constituted, but this control group did not appear to have been used in the analyses presented.<br><br><i>Limitations:</i> High proportion of RCC patients were deceased (237) or were too ill to be interviewed at the time of the study ( $n = 14$ ). Interviews conducted were conducted with a next of kin in ~50% of the cases. Little difference by interview status were observed within the case group, but analyses are not shown. |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Never                                                                  | 293                     | 1                      |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Ever                                                                   | 20                      | 1.2 (0.6–2.3)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | 1–2 yr                                                                 | 8                       | 0.9 (0.3–2.4)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | 3–5 yr                                                                 | 6                       | 1.3 (0.3–4.5)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | > 5 yr                                                                 | 6                       | 1.7 (0.4–6.5)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Trend-test $P$ value, 0.22                                             |                         |                        |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Kidney (RCC)                                         | Duration of employment in petroleum-related occupations, men (OR):     |                         |                        |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Never                                                                  | 197                     | 1                      |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | Ever                                                                   | 116                     | 1.0 (0.7–1.4)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | 1–2 yr                                                                 | 32                      | 0.9 (0.5–1.6)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | 3–10 yr                                                                | 39                      | 1.0 (0.6–1.6)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                      | > 10 yr                                                                | 45                      | 1.1 (0.7–1.7)          |                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                       | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                           | Exposed cases or deaths  | Risk estimate (95% CI)                           | Covariates controlled                                                        | Comments                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Siemiatycki et al. (1987a)</a><br>Montreal, Canada<br>1979–1985<br>(incidence)<br>Case–control                             | <i>Cases:</i> 181 kidney, 486 bladder; patients (aged 35–70 yr) with a cancer diagnosed at one of the 19 participating hospitals. Response rate 82%.<br><i>Controls:</i> 2196 other cancers (excluding lung, kidney, bladder). Controls were selected among the other cancer sites (excluding lung, kidney, and bladder). No matching.<br><br><i>Exposure assessment method:</i> See <a href="#">Table 2.1</a> . | Kidney<br><br>Urinary bladder                        | Exposure to automotive gasoline (OR):<br>Never<br>Ever<br><br>Exposure to automotive gasoline (OR):<br>Never<br>Ever | NR<br>24<br><br>NR<br>64 | 1<br>[1.2 (0.8–1.8)]<br><br>1<br>[1.2 (0.9–1.6)] | Age, SES, ethnic group, cigarette smoking, and blue-white-collar job history | <i>Exposure assessment critique:</i> See <a href="#">Table 2.1</a> .<br><br><i>Design and analysis:</i><br><br><i>Strengths:</i> Assessment of exposures to occupational hazards made by experts in industrial hygiene, including automotive gasoline. Adjustment for potential confounders.<br><br><i>Limitations:</i> Cases of cancer at other sites used as controls. |
| <a href="#">Sharpe et al. (1989)</a><br>Montreal, Canada<br>Diagnoses<br>1 January 1982 to 30 June 1987<br>(incidence)<br>Case–control | <i>Cases:</i> 164; RCC diagnosed and histologically confirmed at one of four Montreal-area hospitals between 1 January 1982 and 31 December 1986 or at another five Montreal-area hospitals between 1 July 1982 and 30 June 1987 and known to have been alive at the time of the chart review in 1987.                                                                                                           | Kidney (RCC)                                         | Self-reported occupational gasoline exposure (OR):<br>Never<br>Ever                                                  | NR<br>23                 | 1<br>1.09 (0.55–2.15)                            | Age, sex, urologist                                                          | <i>Limitations:</i> Small number of study participants; low response rate among the cases; the choice of patients with renal disease as the controls; and self-declared exposure to gasoline and other hydrocarbons.                                                                                                                                                     |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                    | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                                                                                                     | Exposed cases or deaths                           | Risk estimate (95% CI)                                                                  | Covariates controlled                                                                                                                                        | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|-----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Sharpe et al. (1989)</a><br>(cont.)                                               | <p><i>Controls:</i> 161; one control per case, matched on sex, date of birth, and urologist from patients who were suspected of having RCC because they presented with haematuria, but for whom a non-neoplastic diagnosis was given or a urinary tract tumour was ruled out.</p> <p><i>Exposure assessment method:</i> Self-reported exposure to gasoline.</p>                                                               |                                                      |                                                                                                                                                                                                                                                |                                                   |                                                                                         |                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <a href="#">Partanen et al. (1991)</a><br>Finland<br>1977–1978<br>(incidence)<br>Case-control | <p><i>Cases:</i> 408; individuals (aged &gt; 20 yr) with primary renal adenocarcinoma (1977–1978) identified through the Finnish Cancer Registry. Response rate, 68.6%, [77%] next-of-kin interviews.</p> <p><i>Controls:</i> 819; matched on year of birth, sex, age and survival status at time of data collection identified through Population Register Centre. Response rate, 68.1%, [75.9%] next-of-kin interviews.</p> | <p>Kidney (RCC)</p> <p>Kidney (RCC)</p>              | <p>Summary indicator of exposure to gasoline (OR):</p> <p>Unexposed</p> <p>Exposed</p> <p>Summary indicator of exposure to gasoline, men (OR):</p> <p>Unexposed</p> <p>Exposed</p> <p>Exposed (excluding white-collar workers and farmers)</p> | <p>NR</p> <p>39</p> <p>NR</p> <p>39</p> <p>NR</p> | <p>1</p> <p>1.72 (1.03–2.87)</p> <p>1</p> <p>1.63 (0.97–2.75)</p> <p>3.9 (1.4–10.6)</p> | <p>Sex, year of birth, survival status at data collection</p> <p>Year of birth, survival status at data collection, obesity, smoking, coffee consumption</p> | <p><i>Exposure assessment critique:</i> The estimates of exposure by the industrial hygienist were semiquantitative, but probably imprecise. A strength was that co-exposures were assessed and limited the quality of the exposure assessment. The misclassification error in exposure was probably nondifferential.</p> <p><i>Design and analysis:</i></p> <p><i>Strengths:</i> Lifelong job history. Adjustment for non-occupational risk factors.</p> |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design | Population size, description<br>Exposure assessment method                                                                                                                                                             | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                 | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                                   | Comments                                                                                                                                                                                                                         |
|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Partanen et al. (1991)</a><br>(cont.)              | <i>Exposure assessment method:</i> Semiquantitative assessment on the basis of self-reported occupational history. The level of exposure was scored by an industrial hygienist on the basis of job title and duration. | Kidney (RCC)                                         | Level of exposure to gasoline (ppm equivalent of benzene), men (OR):       |                         |                        | Year of birth, survival status at data collection, obesity, smoking, coffee consumption | <i>Limitations:</i> Response rate was 68.6% among eligible cases and 68.1% among eligible controls. Next-of-kin interviews for deceased cases (66.7%) and deceased controls (65.3%). Crude adjustment for smoking (ever, never). |
|                                                                |                                                                                                                                                                                                                        |                                                      | 0                                                                          | NR                      | 1                      |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 0.1–0.19 ppm                                                               | 4                       | 0.63 (0.19–2.12)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 0.2–0.9 ppm                                                                | 25                      | 1.55 (0.83–2.91)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 1.0–2.0 ppm                                                                | 10                      | 7.39 (1.58–34.6)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        | Kidney (RCC)                                         | Duration of exposure to gasoline, men (OR):                                |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 0                                                                          | NR                      | 1                      |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 5–11 yr                                                                    | 13                      | 1.39 (0.61–3.13)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 12–20 yr                                                                   | 13                      | 2.02 (0.83–4.90)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 21–51 yr                                                                   | 13                      | 1.58 (0.69–3.63)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        | Kidney (RCC)                                         | Cumulative exposure to gasoline (ppm-years; benzene equivalent), men (OR): |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 0                                                                          | NR                      | 1                      |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 0.5–1.9 ppm-years                                                          | 7                       | 1.28 (0.45–3.65)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 2.0–13 ppm-years                                                           | 23                      | 1.39 (0.75–2.58)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        |                                                      | 14–102 ppm-years                                                           | 9                       | 4.34 (1.15–16.4)       |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                |                                                                                                                                                                                                                        | Kidney (RCC)                                         | Latency of exposure to gasoline, men (yr):                                 |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                | 0                                                                                                                                                                                                                      | NR                                                   | 1                                                                          |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                | 17–26 years                                                                                                                                                                                                            | 10                                                   | 1.23 (0.51–2.96)                                                           |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                | 27–33 yr                                                                                                                                                                                                               | 17                                                   | 2.82 (1.17–6.80)                                                           |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |
|                                                                | 34–58 yr                                                                                                                                                                                                               | 12                                                   | 1.28 (0.56–2.98)                                                           |                         |                        |                                                                                         |                                                                                                                                                                                                                                  |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                      | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                       | Exposed cases or deaths | Risk estimate (95% CI)                                        | Covariates controlled                                                         | Comments                                                                                                                                                                                                                                                                                                                                                                                                               |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Partanen et al. (1991)</a><br>(cont.)                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Kidney (RCC)                                         | Summary indicators of exposure, men (OR):<br>Neither gasoline, diesel, distilled oils<br>Gasoline only<br>Diesel or other distilled fuel only<br>Exposed to both | NR<br>23<br>6<br>13     | 1<br>2.05 (1.05–3.98)<br>0.68 (0.23–2.01)<br>1.29 (0.55–3.02) | Year of birth, survival status at data collection                             |                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <a href="#">McCredie and Stewart (1993)</a><br>(some RCC cases also included in <a href="#">Mandel et al., 1995</a> ) Australia, New South Wales 1989–1990 (incidence)<br>Case–control | <i>Cases:</i> 489 RCC, 147 cancers of the renal pelvis; aged 20–79 yr at diagnosis (population-based) of RCC or renal pelvic cancer. Identified through cancer registry.<br><i>Controls:</i> 523; electoral rolls, randomly selected (population-based).<br><i>Exposure assessment method:</i> Face-to-face interviews. Employment in certain industries and occupations, exposure to chemicals with suspected associations with kidney cancer. Question on employment as welder (among other). | Kidney (RCC)<br><br>Kidney (urinary pelvis/UUT)      | Employment as petrol station attendant (OR):<br>Never<br>Ever<br><br>Employment as petrol station attendant (OR):<br>Never<br>Ever                               | NR<br>25<br><br>NR<br>9 | 1<br>0.69 (0.40–1.21)<br><br>1<br>1.03 (0.46–2.30)            | Age, sex, method of interview<br><br>Age, sex, method of interview, education | <i>Strengths:</i> Relatively large study size for renal cell carcinomas. One of the rare studies to examine cancer of the renal pelvis.<br><i>Limitations:</i> Participation rate for RCC cases, 67.6%. Participation rate for cases of cancer of the renal pelvis, 74.5%. The questionnaire elicited information on selected occupations and occupational exposures of primary interest only, and were self-declared. |



Table 2.4 (continued)

| Reference, location, enrolment/ follow-up period, study design                                                                                                                         | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Cancer type (histopathology), incidence or mortality | Exposure category or level                               | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                       | Comments                                                                                                                                                                                                                               |  |  |  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------|-------------------------|------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|
| <a href="#">Mandel et al. (1995)</a><br>(supersedes <a href="#">Mellemgaard et al., 1994</a> )<br>Australia, Denmark, Germany, Sweden, USA<br>1989–1991<br>(incidence)<br>Case–control | <i>Cases:</i> 1732; men and women aged 20–79 yr, incident RCC, confirmed by histopathology or cytology, identified through cancer registries (except in Berlin and Heidelberg where cases were ascertained from clinical department).<br><br><i>Controls:</i> 2309; frequency-matched on sex, age, drawn from registers covering the entire population (Denmark, Uppsala), electoral rolls (Sydney). Residential lists (Berlin, Heidelberg) or Health Care Financing Administration lists (Minnesota; aged 65–79 yr), or random-digit dialling (Minnesota; aged 20–64 yr).<br><br><i>Exposure assessment method:</i> Germany collected full occupational histories, other centres asked about specific occupations. | Kidney (RCC)                                         | Employment as gas station attendant, men (OR):           |                         |                        | Age, smoking status, BMI, education, study centre                           | <i>Strengths:</i> Large number of study participants. Specific assessment of exposure to gasoline.<br><br><i>Limitations:</i> Self-reported exposure to gasoline. Some differences in data collection procedures across study centres. |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Kidney (RCC)                                         | Never                                                    | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Ever                                                     | 56                      | 1.3 (0.9–1.9)          |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Duration of occupational exposure to gasoline, men (OR): |                         |                        |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Never                                                    | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Ever                                                     | 164                     | 1.6 (1.2–2.0)          |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Kidney (RCC)                                         | 1–5 yr                                                   | 56                      | 1.6 (1.1–2.4)          | Age, smoking status, BMI, education, study centre, other petroleum products |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | 6–27 yr                                                  | 56                      | 1.4 (0.9–2.1)          |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | 28–62 yr                                                 | 52                      | 1.6 (1.1–2.5)          |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Duration of occupational exposure to gasoline, men (OR): |                         |                        |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | Never                                                    | NR                      | 1                      |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | 1–5 yr                                                   | 56                      | 1.5                    |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | 6–27 yr                                                  | 56                      | 1.1                    |                                                                             |                                                                                                                                                                                                                                        |  |  |  |
|                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                      | 28–62 yr                                                 | 52                      | 1.2                    |                                                                             |                                                                                                                                                                                                                                        |  |  |  |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                     | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Cancer type (histopathology), incidence or mortality                                                                                    | Exposure category or level                                                                                                                                                                                                 | Exposed cases or deaths                  | Risk estimate (95% CI)                                        | Covariates controlled                | Comments                                                                                                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|---------------------------------------------------------------|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Silverman et al. (1983)</a><br>Detroit, Michigan, USA<br>December 1977 to November 1978<br>(incidence)<br>Case-control | <i>Cases:</i> 303; White men with histologically confirmed newly diagnosed carcinoma (or papilloma not specified as benign) of the lower urinary tract (bladder, [95%]; renal pelvis, ureter, urethra), aged 21–84 yr, identified through hospitals.<br><br><i>Controls:</i> 296; general population controls (white males) from same area frequency-matched on age. Controls aged 21–64 yr selected through random-digit dialling from Detroit residents, those aged 65–84 yr by random sampling from Health Care Financing Administration list of Detroit.<br><br><i>Exposure assessment method:</i> Self-reported full job history, followed by exposure details for each job. | Urinary bladder (lower urinary tract cancers)<br><br>Urinary bladder (lower urinary tract)<br><br>Urinary bladder (lower urinary tract) | Employment in gasoline service industry (RR):<br>Never<br>Ever<br><br>Employment in gasoline service industry (RR):<br>Never<br>Ever<br><br>Employment as a garage worker and gas station attendant (OR):<br>Never<br>Ever | NR<br>18<br><br>NR<br>18<br><br>NR<br>18 | 1<br>1.6 (0.80–3.5)<br><br>1<br>1.3<br><br>1<br>1.2 (0.6–2.4) | None<br><br>Age, smoking<br><br>None | <i>Strengths:</i> Cases of cancer of the lower urinary tract histologically confirmed. Incident cases. Population controls frequency-matched on age. High response rate for cases and for controls (> 80%). Complete work history information through in-person interviews. Adjustment for smoking.<br><br><i>Limitations:</i> Analysis on the basis of job and industry titles only. No assessment of exposure to gasoline. |

| Reference, location, enrolment/follow-up period, study design                                                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                         | Exposed cases or deaths  | Risk estimate (95% CI)                 | Covariates controlled                                                                                                                   | Comments                                                                                                                                                                                                                                                              |
|------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|--------------------------|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Mommensen and Aagard (1984)</a><br>Aarhus Hospital, Denmark<br>1977–1980 (incidence)<br>Case-control | <i>Cases:</i> 212 (165 men, 47 women); patients with newly diagnosed bladder cancer admitted to the department of oncology or radiotherapy (1977–1979). Response rate, NR.<br><br><i>Controls:</i> 259 (165 men, 94 women); matched on sex, age, regional area. Drawn from national registries (unspecified). 61% responded to the questionnaire.<br><br><i>Exposure assessment method:</i> Self-reported occupational history and occupational exposure. | Urinary bladder<br><br><br><br>Urinary bladder       | Work with oil or gasoline, men (OR):<br>Never<br>Ever<br><br>Work with oil or gasoline, men (OR):<br>Never<br>Ever | NR<br>20<br><br>NR<br>20 | 1<br>2.71 (1.18–6.17)<br><br>1<br>1.72 | Age, region<br><br><br>Age, region, industrial work, work with kerosene or asphalt, blacksmiths/mechanics, work with chemical materials | <i>Strengths:</i> Lifetime occupational history elicited from the questionnaire.<br><br><i>Limitations:</i> Self-declared exposure to chemicals. Broad category of exposure to “oil or gasoline”. No adjustment for smoking. Exposure characterized as ever vs never. |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                           | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Cancer type (histopathology), incidence or mortality | Exposure category or level                                            | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled              | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------|-------------------------|------------------------|------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Schoenberg et al. (1984)</a><br>New Jersey, USA<br>February 1978 to January 1979 (incidence)<br>Case-control | <i>Cases:</i> 658; White male patients (aged 21–84 yr) with newly diagnosed bladder cancer. Identified through hospital pathology records; participation rate, 89.7%.<br><i>Controls:</i> 1258 age-stratified random samples of White men (aged 21–84 yr) identified through random-digit dialling (aged 21–64 yr) or Health Care Financing Administration for Medicare (aged 65–84 yr); participation rate, 86.6%.<br><i>Exposure assessment method:</i> On the basis of the full job history, employment categories were designed by an industrial hygienist to exclude job titles (e.g. clerical, administrative) that were unlikely to have substantial exposures. | Urinary bladder                                      | Employment as garage and/or gas station worker (OR):                  |                         |                        | Age, duration of cigarette smoking | <i>Strengths:</i> Incident cases. Population controls. High participation (> 85%). Job categories defined by industrial hygienist. Participants reported exposures to known or suspected carcinogens classified in 13 groups.<br><br><i>Limitations:</i> Exposure to gasoline can only be inferred from the employment categories, mainly for those ever employed as garage and/or gas station workers. Gasoline was not included as one of the 13 groups of carcinogens assessed in the analysis. |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                 | NR                      | 1                      |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Ever                                                                  | 43                      | 2.35 (1.47–3.78)       |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Urinary bladder                                      | Age at first employment as garage and/or gas station worker (OR):     |                         |                        |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                 | NR                      | 1                      |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | ≤ 25 yr                                                               | NR                      | 2.15                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 26–40 yr                                                              | NR                      | 5.44                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | ≥ 41 yr                                                               | NR                      | 4.75                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Trend-test <i>P</i> value, 0.25                                       |                         |                        |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Urinary bladder                                      | Time since first employment as garage and/or gas station worker (OR): |                         |                        |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                 | NR                      | 1                      |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | < 20 yr                                                               | NR                      | 3.06                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 20–39 yr                                                              | NR                      | 3.16                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | ≥ 40 yr                                                               | NR                      | 1.66                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Trend-test <i>P</i> value, 0.29                                       |                         |                        |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Urinary bladder                                      | Duration of employment as garage and/or gas station worker (OR):      |                         |                        |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Never                                                                 | NR                      | 1                      |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | < 2 yr                                                                | NR                      | 1.58                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 2–4.9 yr                                                              | NR                      | 5.02                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | 5–9.9 yr                                                              | NR                      | 4.08                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | ≥ 10 yr                                                               | NR                      | 0.63                   |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                      | Trend-test <i>P</i> value, 0.45                                       |                         |                        |                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

Table 2.4 (continued)

| Reference, location, enrolment/ follow-up period, study design                                                                                    | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                           | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                                                                                                            | Exposed cases or deaths | Risk estimate (95% CI)                        | Covariates controlled                                                                                                                                                 | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">Teschke et al. (1997)</a><br>British Columbia, Canada<br>September 1990 to May 1991 (incidence)<br>Case-control                       | <i>Cases:</i> 105; incident cases, aged $\geq 19$ yr, with histologically confirmed primary malignant tumours of the urinary bladder, identified through the British Columbia cancer registry.<br><i>Controls:</i> 139 population controls randomly selected from 5-yr age and sex strata of the provincial voters list. Frequency-matched on age and sex.<br><i>Exposure assessment method:</i> Self-reported occupational history. | Urinary bladder<br><br>Urinary bladder               | Service station attendants and managers occupation (OR):<br>Never<br>Ever<br><br>Service station attendants and managers occupation, 20-yr lag (OR):<br>Never<br>Ever | NR<br>6<br><br>NR<br>5  | 1<br>0.3 (0.1–0.99)<br><br>1<br>0.4 (0.1–1.1) | Sex, age, smoking                                                                                                                                                     | <i>Strengths:</i> High response rates for cases (88.2%) and controls (80.3%). Questionnaire on work history.<br><i>Limitations:</i> Small study size.                                                                                                                                                                                                                                                                                                                             |
| <a href="#">Kogevinas et al. (2003)</a><br>Germany, France, Greece, Spain, Denmark (pooled case-control)<br>1976–1996 (incidence)<br>Case-control | <i>Cases:</i> 3346; incident (< 2 yr between diagnosis and interview) bladder cancer cases in men aged 30–79 yr.<br><i>Controls:</i> 6840 men, matched to cases on age (within 5 yr) and geographical area. Three studies had population controls, one both hospital and population controls, and the rest had hospital controls. Case-control ratio ranged from 1:1 to 1:4.5 across studies.                                        | Urinary bladder                                      | Gasoline exposure categorized in low and high (FINJEM) (OR):<br>Never<br>Lower exposure<br>Higher exposure                                                            | 2733<br>315<br>298      | 1<br>1.07 (0.915–1.24)<br>1.21 (1.03–1.42)    | Age (5-yr age groups), smoking status (categorical never/ex/ current in tertiles of pack-years), study centre, and interaction product term for age and study centre. | <i>Exposure assessment critique:</i> The systematic use of a consistent approach for identifying jobs with exposure to gasoline across the different studies was a particular strength. JEM might not accurately capture individual exposure, resulting in nondifferential misclassification.<br><i>Design and analysis:</i><br><i>Strengths:</i> Large sample size, adjustment for smoking. Covered multiple countries.<br><i>Limitations:</i> Participation rates were unclear. |

**Table 2.4 (continued)**

[illegible]



Table 2.4 (continued)

| Reference, location, enrolment/ follow-up period, study design                          | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Cancer type (histopathology), incidence or mortality | Exposure category or level                  | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                           |
|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|---------------------------------------------|-------------------------|------------------------|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Colt et al. (2004)<br>New Hampshire, USA<br>1 July 1994 to 30 June 1998<br>Case-control | Cases: 424; New Hampshire residents, aged 25–74 yr, diagnosed with primary bladder cancer were identified from the New Hampshire State Cancer Registry.<br><br>Controls: 645; selected from non-melanoma skin cancer study. Additional controls were frequency-matched to these cases on age (25–34, 45–54, 55–64, 65–69, 70–74 yr) and gender using population lists of driving licence holders obtained from the New Hampshire Department of Transportation. Controls aged ≥ 65 yr were chosen from data files provided by the Centers for Medicare and Medicaid Services of New Hampshire.<br><br><i>Exposure assessments method:</i> Lifetime occupational histories were obtained from interviews, detailed job descriptions provided by participants were reviewed to assess potential exposures. | Urinary bladder                                      | Garage and/or gas station worker, men (OR): |                         |                        | Age and smoking       | <i>Exposure assessment critique:</i> The retrospective nature of the study could lead to recall bias with higher reports in cases.<br><br><i>Design and analysis:</i><br><i>Strengths:</i> Adjustment for smoking.<br><br><i>Limitations:</i> Broad exposure definition including garage workers who may have had other exposures. |
|                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Urinary bladder                                      | Ever                                        | 19                      | 1.7 (0.8–3.4)          |                       |                                                                                                                                                                                                                                                                                                                                    |
|                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | Garage and/or gas station worker, men (OR): |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                    |
|                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | < 5 yr                                      | 14                      | 1.4 (0.6–2.9)          |                       |                                                                                                                                                                                                                                                                                                                                    |
|                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | > 5 yr                                      | 5                       | 6.3 (0.7–54.8)         |                       |                                                                                                                                                                                                                                                                                                                                    |
|                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                      | Trend-test <i>P</i> value, 0.066            |                         |                        |                       |                                                                                                                                                                                                                                                                                                                                    |

**Table 2.4 (continued)**

| Reference, location, enrolment/ follow-up period, study design                                                                                                                           | Population size, description<br>Exposure assessment method                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Cancer type (histopathology), incidence or mortality | Exposure category or level                                                 | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled                                                           | Comments                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------|-------------------------|------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <a href="#">Colt et al. (2011)</a><br>USA (Maine, New Hampshire, Vermont)<br>Maine, Vermont: September 2001 to October 2004;<br>New Hampshire: January 2002 to July 2004<br>Case-control | <i>Cases:</i> 1158; all residents in Maine, Vermont, and New Hampshire newly diagnosed with histologically confirmed carcinoma of the urinary bladder, aged 30–79 yr, with a completed interview. Response rate, 65%.<br><br><i>Controls:</i> 1402; population controls randomly selected from the Department of Motor Vehicle records (aged 30–64 yr) or Centers for Medicare and Medicaid Services (aged 65–79 yr), frequency-matched by state, gender, and age. Response rate, 65%.<br><br><i>Exposure assessment method:</i> Self-reported occupational history obtained by personal interviews. | Urinary bladder                                      | Garage and service station-related occupations, men (OR):<br>Never<br>Ever | NR<br>42                | 1<br>1.0 (0.7–1.6)     | Age, race/ ethnicity, state, smoking status, employment in high-risk occupation | <i>Strengths:</i> Large sample size.<br><i>Limitations:</i> Broad category of occupation. |

AML, acute myeloid leukaemia; BMI, body mass index; CI, confidence interval; FINJEM, Finnish Job-Exposure Matrix; HWSB, healthy-worker survivor bias; ICD, International Classification of Diseases; JEM, job-exposure matrix; mo, month(s); NC, not calculated; NR, not reported; OR, odds ratio; PA, Pennsylvania; PAH, polycyclic aromatic hydrocarbon; ppm, parts per million; RCC, renal cell carcinoma; RR, relative risk; SES, socioeconomic status; SIR, standardized incidence ratio; SMR, standardized mortality ratio; SRR, standardized registration ratio; UK, United Kingdom; USA, United States of America; US EPA, United States Environmental Protection Agency; UUT, upper urinary tract; vs, versus; yr, year(s).

A study of mortality in service station attendants in Denmark ([Grandjean and Andersen, 1991a](#)), a linkage study on work-related cancer in the Nordic countries ([Andersen et al., 1999](#)), and a linkage study on cancers of the kidney, bladder, and renal pelvis in Sweden ([Wilson et al., 2008](#)) overlapped with the cohort study on cancer incidence in filling station attendants in four Nordic countries ([Lynge et al., 1997](#)) and were excluded from the present evaluation. For the study on urinary bladder cancer in British Columbia conducted in 1983–1990 ([Band et al., 2005](#); [Richardson et al., 2007](#)), only the results from [Band et al. \(2005\)](#) are reported below, because [Richardson et al. \(2007\)](#) included only gasoline as a combustion product.

#### 2.4.1 Cohort studies

In the follow-up study of the Canadian Labour Force Ten Percent Sample Study that included 415 201 male employees in 1965–1969, 124 deaths from bladder cancer and 70 deaths from cancer of other organs of the urinary tract were identified up to 1973 from the mortality database of Statistics Canada ([Howe and Lindsay, 1983](#)) (see Section 2.1.1). In service station attendants, the SMR for cancer of the bladder and other urinary organs was 5.29 ( $P \leq 0.001$ ), based on 7 observed deaths. [The Working Group noted that the SMRs by occupation were given for cancer of the bladder and other organs of the urinary tract without distinction between cancer subtypes. No information on employment duration was available.]

In Sweden, [McLaughlin et al. \(1987\)](#) used the Cancer-Environment Registry for the entire population employed in 1960, based on employment data from the 1960 census, to calculate an SIR for kidney cancer among people with the occupation “gas station workers” was [0.59 (95% CI, 0.27–1.12)], based on 9 exposed cases. Using the same study design within the same data set, [Malker et al. \(1987\)](#) calculated an SIR for bladder

cancer among people with the occupation “gas station workers” of [1.34 (95% CI, 0.90–1.92)] based on 29 exposed cases. [The Working Group noted as strengths the use of high-quality population-based exposure assessment and outcome registries with near-complete national follow-up; however, a limitation was the cross-sectional exposure assessment at single time point in 1960.]

In a cohort study in 16 524 male and 2445 female service station attendants in four Nordic countries who were identified from the 1970 censuses and followed for about 20 years (see Section 2.1.1), [Lynge et al. \(1997\)](#) reported an SIR for kidney cancer in men of 1.3 (95% CI, 1.0–1.7; all four countries) based on 53 observed cases, which was consistent across the four countries (Denmark, Sweden, Norway, and Finland). In women, the SIR for kidney cancer was 1.2 (95% CI, 0.3–3.0) based on 4 observed cases. For cancer of the renal pelvis, the SIR was increased in men (2.0; 95% CI, 1.0–3.7) but no cases were observed in women. The SIRs for bladder cancer were 1.1 (95% CI, 0.9–1.3) and 0.5 (95% CI, 0.0–1.9) in men and women, respectively. [The Working Group noted as a strength that this was a large cohort of service station attendants exposed to gasoline during the filling of the car tanks before self-service by the customers became normal practice, i.e. at a time when exposure to gasoline occurred during refuelling operations. The main limitations included the absence of adjustment for smoking and other risk factors for kidney cancer, such as BMI and hypertension, although it was not expected that smoking habits in this profession differed from those in the general population; and the lack of data on employment duration and latency since first exposure, because job information was available at one point in time at the census in 1970. Exposure misclassification and failure to identify the most highly exposed participants may have resulted in SIR estimates that were biased towards the null.]

In a cohort of economically active people in the 1970 census in Finland who were followed for cancer incidence from 1971 to 1995 through the Finnish Cancer Registry, 10 277 cases of bladder cancer and 9954 cases of renal cell cancer were identified ([Lohi et al., 2008](#)) (see Section 2.1.1). Risk ratios were calculated for individuals who were exposed to gasoline and hydrocarbon solvents compared with individuals who were not exposed, as determined using FINJEM. For kidney cancer, the risk ratio of 1.13 (95% CI, 0.99–1.28) associated with low-level exposure to gasoline in men, adjusted for smoking, BMI, and social class at a group level (by occupation), decreased to 1.05 (95% CI, 0.81–1.35) and 0.83 (95% CI, 0.49–1.40) for medium and high exposure levels, respectively. The risk ratios associated with bladder cancer in men were close to 1 in each exposure category. The numbers of gasoline-exposed cases among women were too small for meaningful analysis. [The Working Group considered the very large sample size and the assessment of exposure to gasoline using a JEM as strengths. Adjustment for non-occupational risk factors was also a strength, although it was made at an occupational group level, possibly resulting in overadjustment. Nevertheless, the absence of a risk ratio that increases with increasing intensity of exposure to gasoline does not provide strong support for a causal link. Because only the occupation held at entry in the cohort in 1970 was known, no analysis by duration of exposure, cumulative exposure, or by latency since first exposure was possible, possibly resulting in risk ratio estimates that were biased towards the null.]

In Canada, [Schnatter et al. \(1993\)](#) conducted an industry-funded study of mortality in 6672 male workers in petroleum marketing and distribution, of whom 4889 were classified as being exposed to hydrocarbons ([Schnatter et al., 1993](#)) (see Section 2.1.3). For kidney cancer, the SMR of 1.58 (95% CI, 0.63–3.25; 7 deaths) in exposed workers increased to 2.08 (95% CI, 0.67–4.85; 5 deaths) in the subset of workers who were exposed

daily. The SMRs by duration of employment and by latency time after first exposure did not show any clear trend. Two deaths from kidney cancer were observed among tanker drivers with probably high exposure, leading to an SMR of [2.10]. In internal analyses in the cohort using Poisson regression modelling to investigate the effect of exposure frequency on risk, controlling for age, socioeconomic status, year of hire and period of death, the relative risk (RR) for kidney cancer was 3.80 (95% CI, 0.45–32.29) for workers exposed daily compared with non-exposed workers, replicating the observation that the risk estimate increased with exposure frequency. For bladder cancer, the SMR among exposed workers was 0.49 (95% CI, 0.09–1.43) on the basis of only 3 deaths. The SMRs by duration of employment or latency time were close or below 1, without any dose–response trend. [The Working Group noted that this was a well-conducted study among petroleum marketing and distribution workers, with information available on work history, and a basic assessment of hydrocarbon exposure based on exposure frequency. The use of internal Poisson regression analyses was noted as a strength that reduced the HWE. However, the results were constrained by the small number of cancer deaths, leading to wide confidence intervals. The lack of information on exposure intensity and on cumulative exposure to hydrocarbons did not allow exposure–response trends to be examined in more detail. The Working Group also noted that no data were available on non-occupational risk factors for kidney or bladder cancer, such smoking, BMI, or hypertension.]

[Sorahan \(2007\)](#) investigated mortality and morbidity from cancer in an industry-funded cohort of 16 477 male petroleum distribution workers in the UK (see Section 2.1.3). For kidney cancer, the SMR was slightly above 1 (SMR, [1.14]; 95% CI, [0.86–1.49]), and the SRR (based on cancer morbidity) was slightly below unity (SRR, [0.95]; 95% CI, [0.75–1.19]). The number

of deaths from bladder cancer was close to that expected (SMR, [1.01]; 95% CI, [0.81–1.25]), and the SRR for bladder cancer was slightly increased (SRR, [1.19]; 95% CI, [1.05–1.35]). [The Working Group noted as strengths of this study the large size of the cohort of petroleum distribution workers and the long follow-up period, allowing the calculation of precise risk estimates for the most frequent cancers. The availability of data on morbidity, in addition to mortality, was also a valuable feature. Risk estimates by occupation, duration of employment, or latency period were not provided for cancers of the kidney, bladder, and other parts of the urinary tract. The Working Group considered that the estimates may be subject to downward bias because of the HWE and HWSB because of the restriction of the cohort to workers with 1 year of employment. A limitation was the lack of adjustment for confounding by lifestyle factors or co-exposures among distribution workers.]

[Wong et al. \(1993\)](#) conducted an industry-funded cohort study on mortality from cancer and other diseases in relation to gasoline exposure among petroleum distribution workers in the USA (see Section 2.1.3). The risk of kidney cancer in relation to gasoline exposure was re-examined later using a nested case-control study design ([Wong et al., 1999](#)), including the 12 deaths from kidney cancer identified among land-based terminal employees in the cohort and up to 5 controls who were individually matched to the cases on company, date of birth, and sex, and randomly selected from employees who were alive at the time of death of the case. [Wong et al. \(1999\)](#) also carried out an SMR analysis for several cancer sites (including kidney and bladder) in a cohort of marine or land-based distribution workers exposed to gasoline. Exposure to gasoline was assessed using employment records. The RRs for kidney cancer associated with duration of employment, duration of exposure, cumulative exposure, and frequency of peak exposure were close to unity

in all analyses. For bladder cancer, the SMR was 0.72 (95% CI, 0.49–1.01; 33 cases). [The Working Group noted that a main strength of this study was the quantitative assessment of exposure to proxies for gasoline, allowing dose-response trends by duration of exposure and cumulative exposure to be examined for kidney cancer. Limitations included the use of mortality rather than incidence data, the very small number of cases of kidney cancer in the cohort, downward bias because of the HWE, and the lack of information on smoking and other risk factors for cancers of the kidney or bladder, which limited the informativeness of this study for the present evaluation of these cancer outcomes.]

In the industry-funded study in employees in the petroleum industry in Australia ([Gun et al., 2006](#)) (see Section 2.1.3), the SIR for kidney cancer in tanker drivers probably exposed to gasoline was 2.05 (95% CI, 1.06–3.59). [The Working Group noted that, with the exception of the tanker drivers, exposure to gasoline could not be inferred from the data provided in this paper. A limitation was that smoking was not taken into account in the analysis, and there was the potential for downward bias from the HWE.]

In the cohort study on causes of mortality from non-malignant and malignant diseases in service station attendants in the Latium region, Italy ([Lagorio et al., 1994a](#)) (see Section 2.1.4), no deaths from kidney cancer were observed (versus 2.3 expected deaths in men and 0.1 in women). There were 6 deaths from bladder cancer in men, resulting in an SMR of 1.22 (95% CI, [0.45–2.67]); no deaths from bladder cancer were observed in women (versus 0.1 expected). Although exposure to gasoline was expected to be higher in service station attendants working in small stations than in those working in large stations, the SMR for bladder cancer did not differ by station size. [The Working Group noted that exposure to gasoline was probably high among service station attendants in Italy because refuelling activities were common practice at the time of the study.



However, the very small sample size and the short follow-up period reduced the informativeness of the study for the present evaluation.]

In the community-based cohort of residents in Hazle Township and Hazleton, Pennsylvania, USA, who were exposed to gasoline leaked from underground storage tanks ([Patel et al., 2004](#)) (see Section 2.1.6), the SIR for urinary bladder was 1.28 (95% CI, 0.26–3.73) based on only 3 cases. No cases of kidney cancer were reported. [The Working Group noted that this was the only study on exposure from an environmental source, and therefore it provides a novel exposure route for consideration in the present evaluation. Although information was collected on potential confounders such as smoking and chronic diseases, this information was not used in the analysis. However, the informativeness of this study for the present evaluation was reduced because of the small number of cases, low participation rate, and indirect assessment of exposure to gasoline.]

#### 2.4.2 Case-control studies

This section describes the case-control studies on cancers of the kidney and bladder, starting from those focused on kidney cancer.

Among the six studies on kidney cancer, exposure to gasoline was assessed by industrial hygiene experts in two studies ([Siemiatycki et al., 1987a](#); [Partanen et al., 1991](#)), was self-reported by study participants in two studies ([Sharpe et al., 1989](#); [Mandel et al., 1995](#)), and relied on the job title alone in two studies ([McLaughlin et al., 1985](#); [McCredie and Stewart, 1993](#)).

In nine case-control studies, information was provided on bladder cancer in relation to gasoline exposure. Exposure to gasoline was assessed by industrial hygienists in a study by [Siemiatycki et al. \(1987a\)](#), by a JEM in a study by [Kogevinas et al. \(2003\)](#), and by self-report in a study by [Mommensen and Aagard \(1984\)](#). In six studies, relevant exposures were assigned using

job title alone ([Silverman et al., 1983](#); [Schoenberg et al., 1984](#); [Teschke et al., 1997](#); [Colt et al., 2004](#), 2011; [Band et al., 2005](#)).

##### (a) *Kidney cancer*

In a population-based case-control study conducted in the Minneapolis-Saint Paul area, Minnesota, USA ([McLaughlin et al., 1985](#)), which superseded a previous analysis by [McLaughlin et al. \(1984\)](#), 506 cases of renal cell carcinoma were identified from hospital records in patients diagnosed between January 1974 and June 1979, and 714 controls in the same area, frequency-matched on age and sex, were selected from lists of telephone subscribers and from the Health Care Financing Administration. Information on smoking, diet, and drug use, as well as medical, occupational, and residential history was obtained for 495 cases and 695 controls from interviews with the study participants themselves or with the next of kin for the case participants who were deceased ( $n = 237$ ) or too ill to be interviewed directly ( $n = 14$ ) at the time of the study. No clear association with duration of employment in petroleum-related occupations among men was seen, but it was noted that the ORs for kidney cancer increased with years of employment as a gasoline station attendant (OR for > 5 years of employment compared with men never employed as a gasoline station attendant, 1.7; 95% CI, 0.4–6.5). [The Working Group noted that the study included a very high proportion of cancer patients who were deceased or too ill to be interviewed, requiring next-of-kin responses; however, matching on vital status was done to ensure a similar next-of-kin interview percentage in the controls. The poorer quality of information provided by the next of kin may have led to underreporting of occupations, particularly those of short duration, and may potentially bias the risk estimates towards the null. A strength included the adjustment for cigarette smoking.]



In the multicancer case-control study conducted in Montreal, Canada ([Siemiatycki et al., 1987a, b](#)) (see Section 2.1.10), 181 patients with kidney cancer were compared with 2196 patients with cancer of other types (excluding bladder and lung) who served as a control group. Exposure to automotive gasoline, and to other petroleum-derived liquids, was assessed by experts using work history data obtained from a detailed occupational questionnaire. The OR for kidney cancer associated with exposure to automotive gasoline, adjusted for age, socioeconomic status, ethnic group, cigarette smoking, and blue- or white-collar job history was 1.2 (95% CI, [0.8–1.8]). [The Working Group noted the assessment of exposure to automotive gasoline by experts using detailed questionnaires on each job held by the study participants during the lifetime as a notable strength of this study. A limitation was the lack of analysis by duration of exposure or latency time since first exposure for automotive gasoline.]

[Sharpe et al. \(1989\)](#) conducted a case-control study on kidney cancer in Montreal, Canada. The group of cases was composed of patients with renal cell carcinoma diagnosed between 1 January 1982 and 30 June 1987 at five Montreal-based hospitals. The control group included patients presenting with haematuria for whom a non-neoplastic diagnosis was given, and who were matched to the cases on sex, date of birth, and urologist. Case and control participants were sent a self-administered questionnaire on non-occupational exposure that was subsequently reviewed with the participant by telephone. Exposure to hydrocarbon compounds was self-declared. Among the 403 patients with renal cell carcinoma who were identified, 164 (41%) responded to the questionnaire and were matched to 161 controls. The OR for kidney cancer associated with exposure to gasoline, as declared in the questionnaire, was 1.09 (95% CI, 0.55–2.15). [The Working Group noted that this study was less informative for the present

evaluation because of the small number of study participants; the low response rate among the cases; the choice of patients with renal disease as the controls, which may induce bias towards the null; and the self-declared exposure to gasoline and other hydrocarbons, which could induce differential exposure misclassification bias away from the null.]

In a population-based study in residents of Finland ([Partanen et al., 1991](#)), cases of renal cell cancer diagnosed in 1977 and 1978 were identified through the Finnish Cancer Registry and were individually matched to population controls, identified in the Population Register Centre, on year of birth, sex, and survival status at the time of data collection. A questionnaire requesting information on job history and non-occupational risk factors was sent to the study participants or to the next of kin of deceased case and control participants. After excluding participants who completed the questionnaire but did not report any history of employment, the study included 408 cases and 819 controls (in both groups, 76% of interviews were with the next of kin). The individual occupational histories for the years 1920 through 1968 (to allow for a latency period of  $\geq 10$  years) were scored blindly as to case-control status by an industrial hygienist for nine occupational exposures, including gasoline and diesel fuel and other distilled fuel oils. Participants were classified as exposed to gasoline if they had “at least five years of high- or low-level exposure before 1968 or less than five years of exposure but at least one year of high-level exposure during 1920–1968”. The exposed participants included people with a variety of occupations, such as vehicle mechanics, printers, oil refinery workers, service station attendants, or taxi drivers. Among the cases, 39 men and no women were exposed to gasoline, resulting in an OR of 1.63 (95% CI, 0.97–2.75) for renal cell cancer in men, after adjustment for obesity, smoking, and coffee consumption. This OR increased to 3.9 (95% CI, 1.4–10.6) when the analysis was restricted by

excluding white-collar workers and farmers (i.e. restricting to blue-collar workers). There was a clear trend for the OR to increase with the level of exposure to gasoline (based on parts per million of benzene equivalent) and with cumulative exposure to gasoline (based on ppm-years of benzene equivalent), but no trend with exposure duration was seen. In a further analysis that controlled for concomitant exposure to diesel or other distilled fuel in men, the OR for kidney cancer and exposure to gasoline only was 2.05 (95% CI, 1.05–3.98). [The principal strengths of this study included individual assessment of exposure to gasoline by an industrial hygienist, the dose–response trend analysis, and the exclusion of participants with co-exposure to diesel fuel in a sub-analysis. The study also had several limitations. The low participation rate (68.3%) was a potential source of bias if participation depended both on exposure to gasoline and on the case–control status. Another source of potential bias was the high proportion of next-of-kin interviews (76%), and consequently poorer-quality information on job history, but this proportion was highly similar in cases and in controls. This could have resulted in nondifferential exposure misclassification, with bias towards the null. Moreover, the adjustment was based only on smoking categorized as ever or never smoking, leaving the possibility of residual confounding by tobacco smoking, and other risk factors for kidney cancer, such as obesity and hypertension, were not taken into account. Finally, exposure misclassification could not be ruled out, but it was expected to be nondifferential, because exposure scoring was performed blind to case–control status, and would be more likely to bias the risk estimates towards unity. The increase in the OR associated with gasoline exposure in the analysis restricted to blue-collar workers was noteworthy. This could be explained by lower exposure misclassification in this subgroup, and higher exposure levels in exposed blue-collar workers than in exposed workers in the rest of the population.]

[McCredie and Stewart \(1993\)](#) conducted a population-based case–control study on renal cell cancer and cancer of the renal pelvis in New South Wales, Australia ([McCredie and Stewart, 1993](#)); a subset of the cases of renal cell carcinoma were included in a larger pooled study by [Mandel et al. \(1995\)](#). The groups of cases comprised 489 cases of renal cell cancer and 147 cases of cancer of the renal pelvis with confirmed diagnosis in 1989–1990, identified through the New South Wales cancer registry ([McCredie and Stewart, 1993](#)). A common group of controls was composed of 523 men and women identified from electoral rolls. A face-to-face or telephone interview was conducted using a structured questionnaire that elicited information on selected occupations and occupational exposures of primary interest, as well as on non-occupational risk factors. The OR for renal cell cancer associated with employment as petrol station attendant was 0.69 (95% CI, 0.40–1.21). The corresponding OR for cancer of the renal pelvis was 1.03 (95% CI, 0.46–2.30). [The Working Group noted that this was a relatively large study on renal cell cancer and was one of the few studies to report on occupational factors in cancer of the renal pelvis, which, however, was limited by small numbers. Limitations included the lack of detailed occupational history, the potential for recall bias, and the lack of analysis by duration of employment.]

In an international investigation of population-based case–control studies, [Mandel et al. \(1995\)](#) reported on the risk of renal cell cancer in relation to occupation and occupational exposures using pooled data from Australia, Denmark, Germany, Sweden, and the USA. All data were collected according to a common protocol, and the questionnaires for data collection were similar (the response rate was not reported). Details concerning data collection in Denmark were published separately ([Mellemgaard et al., 1994](#)). In Australia, a subset of cases and controls included in the pooled analysis were part of a study published by [McCredie and Stewart \(1993\)](#),

reported above. The pooled analysis included 1732 cases of incident renal cell cancer, ascertained from local cancer registries or from an active surveillance system, and 2309 population controls, frequency-matched to the cases on sex and 5-year age group, who were selected from population registers or electoral rolls. Information on occupation, industry, and exposures of primary interest was elicited during face-to-face interviews with case and control participants. The response rate was 72.3% in cases and 74.2% in controls ([McLaughlin et al., 1995b](#)). In the pooled analysis, the OR for men who had worked as gas station attendants was 1.3 (95% CI, 0.9–1.9). Ever exposure to gasoline, as reported by the study participant, was associated with an OR of 1.6 (95% CI, 1.2–2.0), but no clear trend was seen with increasing duration of gasoline exposure. Adjustment for “other petroleum products” decreased the ORs for gasoline in all categories of exposure duration. [The Working Group noted that this was the largest case–control study on kidney cancer and occupational risk factors to examine exposure–response trends with duration of exposure. Despite the elevated ORs, the absence of a trend with duration of exposure did not support a causal association. Furthermore, as occupational exposures were self-reported by study participants during the interview, it was possible that case participants were more prone to report exposure to gasoline and other agents than were the population controls. This recall bias may have led to overestimation of risk estimates.]

(b) *Bladder cancer*

A population-based case–control study on occupation and cancer of the lower urinary tract was carried out among men in Detroit, Michigan, USA, as part of the National Bladder Cancer Study ([Silverman et al., 1983](#)). The cases diagnosed in December 1977 through November 1978 were identified from hospital records; for 95%, urinary bladder was specified

as the primary cancer site. General population controls, frequency-matched on age to the cases, were selected by random-digit dialling (controls aged 21–64 years) or from Health Care Financing Administration lists in Detroit (controls aged 65–84 years). Data on job history and non-occupational risk factors were collected during in-person interviews. After exclusion of non-White people, participants who had never held jobs for  $\geq 6$  months, or refusals, the study included 303 cases and 296 controls. For workers in the gasoline service industry, the crude OR was 1.6 (95% CI, 0.8–3.5), which decreased to 1.3 (95% CI not reported) after adjustment for age and smoking. The OR for those who had worked as garage workers and gas station attendants was 1.2 (95% CI, 0.6–2.4) (adjustment for smoking not shown). [The Working Group considered the exposure classification to be a suitable proxy for exposure to automotive gasoline, with some additional exposure misclassification and confounding from co-exposures compared with job groupings for service station attendants only.]

[Mommensen and Aagard \(1984\)](#), superseding [Mommensen et al. \(1982, 1983\)](#), conducted a case–control study in Aarhus, Denmark that included 165 male patients with urinary bladder cancer who were admitted to the Department of Oncology and Radiotherapy of Aarhus hospital and 165 male controls selected from the regional division of the population register, matched on age and current residence ([Mommensen and Aagard, 1984](#)). Information on sociodemographic factors, alcohol consumption, cigarette smoking, and occupational history was obtained during a telephone interview. The response rate was not reported for cases, but it was stated that 61% of controls responded to the questionnaire. Possible occupational exposures to chemicals were self-declared by the study participants. Logistic regression analysis showed that the OR for urinary bladder cancer associated with “work with oil or gasoline” was 2.71 (95% CI, 1.18–6.17). This OR decreased only slightly (OR, 1.72) after

adjustment for other occupational determinants. [Exposure to gasoline was self-declared and could not be distinguished from exposure to oil. The elevated OR for exposure to “work with oil or gasoline” may have been confounded by tobacco smoking.]

Another part of the National Bladder Cancer Study was based in New Jersey, USA, in 1978 ([Schoenberg et al., 1984](#)). The design was similar to that described by [Silverman et al. \(1983\)](#) and included 658 cases of incident bladder cancer and 1258 population controls among White men. Home interviews with the study participants provided information on personal risk factors, as well as on work history and occupational exposures. Industry-specific employment categories were designed by industrial hygiene experts to exclude job titles (e.g. clerical, administrative) that were unlikely to have substantial exposure. Being ever employed in the category “garage and/or gas station workers” was associated with an OR of 2.35 (95% CI, 1.47–3.78), adjusted for age and duration of cigarette smoking. There was no clear trend in risk in relation to latency time since first exposure, age at first exposure, or duration of employment in this category. [The Working Group considered the exposure classification to be a suitable proxy for exposure to automotive gasoline, although noting that this may include some additional exposure misclassification, and upwards bias from co-exposures to engine oil.]

In the multicancer case-control study conducted in Montreal, Canada ([Siemiatycki et al., 1987a, b](#); described in Section 2.1.1), 486 patients with bladder cancer were compared with 2196 patients with cancers at other sites (excluding kidney and lung) who served as a control group. The OR for bladder cancer associated with exposure to automotive gasoline as assessed by experts from detailed work history data was 1.2 (95% CI, [0.9–1.6]). [The assessment of exposure to automotive gasoline by experts in this study was a notable strength of this study.]

[Teschke et al. \(1997\)](#) conducted a case-control study in British Columbia, Canada, that included 105 cases of histologically confirmed bladder cancer registered by the British Columbia Cancer Agency between 1 September 1990 and 31 May 1991, and 139 population controls selected from provincial voter lists. Because parallel case-control studies were conducted for cancers of the nasal cavity or sinuses using a common control group, the controls were frequency-matched on the age and sex distribution of all three cancer sites. Face-to-face or telephone interviews were conducted using a standard questionnaire in which information was requested on occupational, residential, smoking, and medical histories, as well as on exposure to known or suspected carcinogens. The OR associated with employment as service station attendant or manager was 0.3 (95% CI, 0.1–0.99). [The Working Group noted that this was a small study and included a small number of service station attendants. The authors used an in-house classification of occupations that may not have adequately reflected exposure to gasoline.]

In a multicentre case-control study in France, Germany, Greece, Denmark, and Spain, [Kogevinas et al. \(2003\)](#) reported on risk of bladder cancer; the study included 3346 cases and 6840 population controls or hospital controls with various diseases who were matched to the cases on age and geographical area. Data on occupational history collected for the cases and controls were coded using ISCO codes, and the FINJEM was applied to assess occupational exposures to a list of predefined agents and to gasoline. The prevalence of exposure and average levels of exposure were evaluated for each occupation, and the overall exposure was defined as the product of these two continuous variables. Gasoline-exposed participants were then classified according to the maximum achieved exposure during work history in high and low exposure groups. The OR increased from 1.07 (95% CI, 0.915–1.24) to 1.21 (95% CI, 1.03–1.42)



in participants in the groups with low (assumed to be first quartile) and high (assumed to be quartiles 2 and 3 combined) exposure, respectively, compared with the non-exposed participants. [The Working Group noted that this study had several strengths, including the large sample size, adjustment for smoking, and the use of a JEM to assess occupational exposure. The use of the FINJEM for assessing exposure to gasoline may have led to exposure misclassification, most probably nondifferential.]

[Band et al. \(2005\)](#) conducted a case-control study on bladder cancer in men diagnosed between 1983 and 1990 and ascertained by the British Columbia cancer registry. Of the 1744 eligible cases, the authors included 1129 men (64.7%) who responded to a self-administered questionnaire on lifetime occupations and industries, ethnic origin, education, lifetime smoking habits, and alcohol drinking. In the event of a patient death, the spouse or the closest living relative responded to the questionnaire. The controls comprised 8492 patients with other cancers ascertained by the cancer registry (excluding lung cancer and cancers of unknown primary site) who responded to the questionnaire (participation, 60%) and were matched to 1125 of the cases on age and year of diagnosis. The ORs adjusted for important confounders (smoking, alcohol consumption, proxy response, ethnicity, and education) were 2.66 (95% CI, [1.69–4.19]) for ever employment as a service station attendant and 1.75 (95% CI, [1.21–2.53]) for ever employment in the gasoline service station industry. The ORs for usual employment in this occupation or usual employment in this industry were 9.35 (95% CI, [1.77–49.51]) and 1.69 (95% CI, [0.68–4.18]), respectively. [The Working Group noted that, despite the large study size, the small number of cases with usual employment as service station attendant or in the gasoline service station industry was a limitation. Other limitations included the low participation rate, the use as controls of patients with other cancer

types that may share the same risk factors as the case group, and the use of a self-administered questionnaire.]

[Colt et al. \(2004\)](#) conducted a case-control study on urinary bladder cancer in New Hampshire, USA, that included 424 cases diagnosed between 1 July 1994 and 30 June 1998 that were identified from the New Hampshire State cancer registry (participation rate, 68%) and 645 population controls that were identified from files provided by the Centers for Medicare and Medicaid Services or lists provided by the New Hampshire Department of Transportation (overall participation rate, 65%). A calendar of work history information was obtained by questionnaire. The exposure of “garage and/or gas station workers” was coded using Standard Occupational Classification (SOC) 1980 code 873, which includes filling gas tanks; checking and adding oil to engines, transmissions, and other hydraulic equipment; checking air pressures in tyres and tanks; and cleaning windshields and headlights. The OR adjusted for age and smoking in men who had worked as garage and/or gas station workers (SOC 1980 code 873) was 1.7 (95% CI, 0.8–3.4). The OR increased by duration of employment and was 6.3 (95% 0.7–54.8) in men who had been employed in this occupation for > 5 years. [The Working Group considered the exposure classification to be a suitable proxy for exposure to automotive gasoline, with some exposure misclassification, and considered the co-exposures to be more modest than for mechanics, although some co-exposure to engine oil, which could lead to confounding, was noted.]

[Colt et al. \(2011\)](#) conducted a case-control study on urinary bladder cancer in Maine, New Hampshire, and Vermont, USA, which included 1158 cases (response rate, 65%) and 1402 population controls (response rate, 65%) that did not overlap with the previous case-control study conducted by the same author ([Colt et al., 2004](#)). The OR adjusted for age, state, smoking

status, and employment in any high-risk occupation associated with work as garage or service station worker was 1.0 (95% CI, 0.7–1.6). [As in the previous study, the Working Group noted the broader exposure classification of garage or service station worker, although it considered confounding from co-exposures to mechanical tasks to be modest.]

## 2.5 Cancer of the digestive and respiratory systems

See Table S2.5 (Annex 2, Supplementary tables for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>).

Thirteen cohort or nested case-control studies on the association between occupational exposure to automotive gasoline and cancers of the digestive system were found to be informative for the present evaluation ([Norell et al., 1986](#); [Schnatter et al., 1993](#); [Wong et al., 1993, 1999](#); [Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Aragonés et al., 2002](#); [Weiderpass et al., 2003](#); [Patel et al., 2004](#); [Sorahan, 2007](#); [Tarvainen et al., 2008](#); [Lindbohm et al., 2009](#); [Talibov et al., 2019](#)), together with five case-control studies ([Stemhagen et al., 1983](#); [Siemiatycki et al., 1987a](#); [Gerhardsson de Verdier et al., 1992](#); [Arbman et al., 1993](#); [Parent et al., 1998](#)).

For cancer of the respiratory system, seven cohort studies ([Schnatter et al., 1993](#); [Wong et al., 1993, 1999](#); [Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Patel et al., 2004](#); [Sorahan, 2007](#)), and 13 case-control studies ([Siemiatycki et al., 1987a](#); [Brown et al., 1988](#); [Ahrens et al., 1991](#); [Wortley et al., 1992](#); [Teschke et al., 1997](#); [De Stefani et al., 1998, 2005](#); [Shangina et al., 2006](#); [Consonni et al., 2010](#); [Wang et al., 2014](#); [Mattei et al., 2016](#); [Carton et al., 2017](#); [Barul et al., 2018, 2019](#)) were included in the present evaluation.

The Working Group also considered studies on cancer risk in gasoline attendants on an occupational level (three-digit) who had probable predominant occupational exposure to automotive gasoline but for whom individual-level exposure to gasoline was not specifically assessed ([Stemhagen et al., 1983](#); [Norell et al., 1986](#); [Gerhardsson de Verdier et al., 1992](#); [Wortley et al., 1992](#); [Arbman et al., 1993](#); [Teschke et al., 1997](#); [Aragonés et al., 2002](#); [Consonni et al., 2010](#)).

The cohort study by [Grandjean and Andersen \(1991a\)](#), who reported specifically on gas station workers, was excluded because the study was superseded by completely overlapping later analyses by [Lynge et al. \(1997\)](#). The Working Group also excluded studies in which risk estimates were reported according to exposure to gasoline combined with another agent, such as lubricating oil ([Bradshaw and Schonland, 1969](#)) and dry-cleaning agents ([Lin and Kessler, 1981](#)). A non-systematic review with a meta-analysis on occupational exposures and pancreatic cancer, which included a risk estimate for gasoline exposure using FINJEM, was considered by the Working Group to be uninformative for the present evaluation because of the unclear description of the four populations exposed to gasoline ([Ojajärvi et al., 2000](#)).

### 2.5.1 Cancer of the digestive system

#### (a) Cohort analyses and nested case-control studies

[Norell et al. \(1986\)](#) conducted a retrospective cohort study based on linkage of the Swedish 1960 census that comprised approximately 2 million men for whom information was available on age, sex, county, and branch of industry. Cases of incident pancreatic cancer were retained from the Swedish Cancer Register for follow-up in the period 1961–1979. Excess risk of pancreatic cancer was reported for men employed in petrol stations at the time of census (standardized



morbidity ratio, 1.6; 95% CI, [1.0–2.5]; 20 cases), using employed men in the same region as the reference population. [Aragonés et al. \(2002\)](#) also used data from the Swedish census. This study was carried out in a cohort of men and women who were employed at the time of the Swedish census in 1970 (1 779 646 men and 1 066 346 women). A subcohort included workers reporting the same occupation in both 1960 and 1970 censuses (693 501 men and 231 858 women). An excess of stomach cancer was reported in those registered as petrol station workers in the 1970 census (RR, 1.52; 95% CI, 1.07–2.16; 33 cases) and in those registered as petrol station workers in both the 1960 and 1970 censuses (RR, 1.67; 95% CI, 0.97–2.87; 14 cases), after adjustment for age, period, and geographical risk area. [The Working Group noted that both cohorts were based on high-quality nationwide registries, but categorization as a gas station filling attendant was based on occupation and industry from the 1960 and 1970 censuses, hence inferring exposure from single point in time and not permitting an assessment of cumulative exposure. Exposure misclassification, including failure to identify the most highly exposed participants, may have resulted in biased risk estimates towards the null. For both studies, the Working Group noted that at the time of the censuses it was likely that the gasoline station attendants were exposed to gasoline during car refuelling, since it was before self-service by the customer became normal practice in Sweden. As the study included occupational data from the Swedish 1960 census, the Working Group considered that it complemented the study by [Lyngé et al. \(1997\)](#) in that it included 20 cases (follow-up, 1961–1979) partly registered during the decade before the establishment of the cohort of service station attendants from the 1970 census (15 cases; follow-up, 1970–1989).]

In a cohort study of 16 524 male and 2445 female service station attendants in four Nordic countries who were identified from the 1970 censuses and followed for about 20 years, [Lyngé](#)

[et al. \(1997\)](#) reported an excess risk of cancer of mouth in men (SIR, 1.7; 95% CI, 0.8–3.0; 11 cases) and pharynx (SIR, 1.7; 95% CI, 0.9–2.8; 15 cases). Other than a slight excess of colon cancer (SIR, 1.3; 95% CI, 0.8–2.2; 16 cases), the number of cases of digestive cancer among the women was too low to allow for interpretation of the estimates. The excess risk of cancer of the pancreas reported for petrol stations in the study by [Norell et al. \(1986\)](#) was below unity in the study by [Lyngé et al. \(1997\)](#). [The Working Group noted that this was a large cohort of service station attendants who were most probably exposed to gasoline during car refuelling before self-service by the customers became normal practice in the 1970s and 1980s in the Nordic countries. Limitations included that there were few cases of most of the digestive cancers, and the lack of adjustment for potential confounders such as smoking and alcohol consumption. Exposure misclassification because of the consideration of occupation at a single time point (1970 census) and failure to identify the most highly exposed participants may have resulted in SIR estimates that were biased towards the null.]

The association between occupational exposure to gasoline and several cancers of the gastrointestinal tract has been investigated in a cohort of all economically active Finnish people born between 1906 and 1945 and followed for 46.8 million person-years between 1971 and 1995 (see Section 2.1.1) ([Weiderpass et al., 2003](#); [Tarvainen et al., 2008](#); [Lindbohm et al., 2009](#)). Categorization using combined information on industry and occupation, applying FINJEM, provided exposure metrics for annual average exposure to gasoline (high, middle, and low) for the periods 1945–1959 and 1960–1984. Cases of incident cancer were identified through the Finnish Cancer Registry (follow-up, 1971–1995). [Weiderpass et al. \(2003\)](#) investigated the association between gastrointestinal cancers and exposures to 31 occupational agents, using FINJEM, in 413 877 women employed in the industry and

service sectors (181 job titles). The prevalence of participants exposed to gasoline was 0.7%. Overall, a total of 7944 cases of gastrointestinal cancer were identified, including cancers of the oesophagus (389 cases), stomach (1881 cases), colon (2009 cases), rectum (1323 cases), liver (389 cases), gallbladder (651 cases), and pancreas (1302 cases). An excess risk of rectal cancer in gasoline-exposed workers was reported (RR for low exposure, 2.46; 95% CI, 1.02–5.94; RR for medium/high exposure, 2.76; 95% CI, 0.39–19.6). Risks for cancer of the stomach, colon, and gall bladder were close to unity or imprecise, and risks were not estimated for cancers of the oesophagus, liver, and pancreas because of the few data available. [Tarvainen et al. \(2008\)](#) used the whole cohort including both men and women (725 868 men, 825 528 women) and estimated risk of cancer of the mouth and pharynx (excluding nasopharynx) according to exposure to gasoline. A total of 2708 cases of cancer of the mouth and pharynx were identified, and the SIR estimates for the lowest, intermediate, and highest levels of gasoline exposure, lagged by 10 years, and adjusted for social class (small variation), were 1.22 (95% CI, 0.96–1.53; 74 cases), 1.13 (95% CI, 0.67–1.78, 18 cases), and 1.51 (95% CI, 0.55–3.28; 6 cases), respectively. [Lindbohm et al. \(2009\)](#) also used the whole cohort of economically active Finnish people (about 1.2 million) to investigate the association between occupational exposure to gasoline and cancer of the liver. Based on 2474 cases, the RR estimate for liver cancer (all types), adjusted for lifestyle factors on an occupational group level, was 1.24 (95% CI, 0.70–2.20) in the highest exposure category. Risk estimates increased with increasing cumulative exposure.

[For these studies, the Working Group noted that, in addition to the use of high-quality registers, cancer incidence as an outcome, and the high number of person-years at follow-up, a notable strength was the comprehensive coverage for both genders, allowing separate analyses for female workers. Also, use of FINJEM (not

gender-specific), allowing exposure to gasoline to be distinguished from exposures to other carcinogenic agents as well as providing some contrast in cumulative exposure across occupational groups, was a strength. However, the Working Group also noted that the occupational categories used were still crude and were assigned average exposures, resulting in potential misclassification of exposure because of the inability of FINJEM to account for exposure variability within occupations. Also, since only the occupation held at the 1970 census was known, no analysis by duration of exposure, cumulative exposure, or by latency since first exposure was possible. This nondifferential misclassification of exposure would generally bias the estimates towards the null. Although adjustment for potential confounders was made by using social class, smoking habits, and alcohol consumption on a group level (by occupation) in FINJEM, the lack of data on lifestyle factors and co-exposure to other carcinogenic agents on an individual level hampered control for confounders and may have induced overadjustment. Finally, another limitation was also the low proportion of workers exposed to gasoline, which for some of the outcomes probably contributed to the uncertain risk estimates with wide confidence intervals. The Working Group noted the overlap between the results for the colon and rectum presented by Weiderpass et al. and those in a later study by [Talibov et al. \(2019\)](#), described below, but the analysis limited to women only was considered to be informative as a sensitivity analysis.]

[Talibov et al. \(2019\)](#) conducted a case-control study nested in the census-based NOCCA cohort study ([Pukkala et al., 2009](#)). The NOCCA-JEM provides country-specific exposure estimates and was used to assess the association between occupational exposure to gasoline and risk of colorectal cancer. A total of 181 709 cases of colon cancer and 109 227 cases of rectal cancer, diagnosed between 1961 and 2005 (in Finland, Iceland, Sweden, and Norway) were identified

and were matched on country, birth year, and sex. The estimates were adjusted for benzene, perceived physical workload, formaldehyde, ionizing radiation, chlorinated hydrocarbons, chromium, and wood dust. For cancers of the colon, rectum, and colorectum, ORs of 1.01 (95% CI, 0.94–1.08), 0.93 (95% CI, 0.86–1.02), and 0.98 (95% CI, 0.93–1.03), respectively, for ever-versus-never exposed were reported. For high exposure to gasoline compared with never exposed, the ORs were 1.02 (95% CI, 0.94–1.11) for colorectal cancer, 0.92 (95% CI, 0.80–1.06) for rectal cancer, and 1.07 (95% CI, 0.97–1.19) for colon cancer. A trend for decreasing risk with increasing exposure was reported for cancers of the descending colon (*P* for trend, 0.09). After adjustment for life-style factors on an aggregated level in the Finnish part of the study, the ORs for high exposure to gasoline compared with never exposed were 0.77 (95% CI, 0.56–1.06) for colorectal cancer, 0.65 (95% CI, 0.38–1.10) for rectal cancer, and 0.87 (95% CI, 0.58–1.29) for colon cancer. [The Working Group noted the large number of cases, accuracy, and completeness of the incidence data, occupational data retrieved from nationwide census record, and use of a country-specific JEM. The NOCCA-JEM also allowed for adjustment for co-exposures to other carcinogenic agents. However, exposure misclassification was anticipated because the earliest registered job code defined the occupational category of the person. In addition, the NOCCA-JEM cannot account for exposure heterogeneity within one occupation because it assigns average exposure to all members of the occupational group. This nondifferential misclassification of exposure would introduce bias of the effect estimates towards the null. Also, gasoline was included in the JEM using the proxy benzene (ppm). In the model, benzene was included in the final model as an adjustment factor since it was not strongly correlated with gasoline or diesel exhaust. Adjusting for benzene in the model might have caused overadjustment, potentially masking a true association between

gasoline exposure and colorectal cancer. Finally, lack of confounder controls for physical activity, diet, smoking, alcohol intake, and BMI on an individual level might cause potential bias that could create a false association or mask a true association.]

[Schnatter et al. \(1993\)](#) (in an update of the study by [Schnatter et al., 1992](#)) conducted an industry-funded cohort study in 4889 active employees and living retired male workers between 1964 and 1983 who had worked for  $\geq 1$  year in the marketing or distribution operating sector of a Canadian petroleum company and who were also classified as ever exposed to hydrocarbons (approximately 75% of the full cohort of 6672 male workers) (see Section 2.1.3). The SMRs for marketing/distribution workers exposed to hydrocarbons were above unity for cancers of the oesophagus (SMR, 1.41; 95% CI, 0.51–3.07) and large intestine (SMR, 1.50; 95% CI, 0.97–2.21) and, to a lesser extent, pancreas (1.13; 95% CI, 0.58–1.97), although not for cancers of the stomach or rectum. [The Working Group noted the imprecise estimates as a limitation. Even with restriction of the analyses to workers ever exposed to hydrocarbons, which was a strength of the study, co-exposure to other potential occupational carcinogens was likely for petroleum distribution workers and was a notable limitation that was likely to induce confounding. The Working Group noted the likelihood of HWEs and HWSB because of the inclusion of living retired workers only, excluding those who may have died from occupational cancers before retirement, potentially causing an underestimation of risk.]

[Sorahan \(2007\)](#) conducted an industry-sponsored cohort study in male petroleum distribution workers in the UK including 16 477 men first employed in the period 1946–1974 for  $\geq 12$  months and with some employment after 1950 (see Section 2.1.3). Follow-up was conducted for cancer mortality between 1951 and 2003 and for cancer incidence between 1971 and

2003. Except for an excess incidence of cancer of the stomach, with an SRR of [1.28] (95% CI, [1.12–1.45]), SRRs were not far from unity or were imprecise for the other cancers of the digestive system that were investigated. [The Working Group noted as a strength that the cohort study was relatively large, but a notable limitation was the lack of adjustment for co-exposure to other carcinogenic refinery products and other agents, which could induce upwards bias in the estimates for gasoline. Limiting the cohort to those with  $\geq 12$  months might have introduced some health-worker bias effects.]

[Wong et al. \(1993\)](#) performed an industry-funded retrospective cohort study in land-based terminal petroleum workers (1946–1985, employed for  $\geq 1$  year) who were primarily exposed to gasoline vapour ( $n=9026$ ). They assessed mortality until June 1989 from a range of cancers (see Section 2.1.3), including cancers of the digestive system and peritoneum (151 cases). [Crude SMRs for overall mortality (0.513) and all cancers combined (0.664) were below unity, indicating a substantial HWE bias in this cohort.] In a later analysis in an overlapping cohort with extended follow-up ([Wong et al., 1999](#)), the SMRs for petroleum distribution workers (land-based terminal and marine workers combined) exposed to gasoline compared with a national referent were below unity for most cancer types, including cancers of the oesophagus, stomach, large intestine, rectum, liver and biliary tract, and pancreas. [The Working Group noted that the study was based only on mortality data, that co-exposure to other potential occupational carcinogens was likely for petroleum distribution workers, and that no occupational or lifestyle-related confounders were considered in the analysis, collectively limiting the informativeness of the study for the present evaluation. The confounding by co-exposures might have biased the estimates upwards. A substantial HWE bias was noted, which was probably partly explained by the limiting of the cohort to those with

$\geq 12$  months of employment, suggesting cohort selection bias on the basis of health status at the time of hire. The need to take healthy-worker bias into account in cohorts of petroleum workers in general, and in this study in particular, has been emphasized previously ([Dement et al., 1997](#)).]

In the cohort study of mortality from non-malignant and malignant diseases among service station attendants in the Latium region, Italy, where workers were followed for 11 years (1981 through 1992) ([Lagorio et al., 1994a](#)), an excess risk of oesophageal cancer (SMR [3.51]; 95% CI [0.96–8.99]) was found among male gasoline attendants working in small stations (only 4 observed cases), where exposure to gasoline was expected to be higher than in large stations (no observed cases). No excess was found for other cancers of the digestive system, including the oral cavity, pharynx, stomach, colon, liver, and pancreas among men, women, or workers employed at small or large stations. [The Working Group noted that exposure to gasoline was probably high among service station attendants in Italy because refuelling activities were common practice at the time of the study. However, notable limitations included the small sample size and short follow-up period, leading to imprecise estimates, and the use of mortality rather than incidence data. Lack of information on lifestyle factors known to be risk factors for oesophageal cancer, such as smoking and alcohol consumption habits, did not allow for any confounder control.]

[Patel et al. \(2004\)](#) conducted a historical cohort study of individuals resident in the area affected by gasoline spill between 1990 and 2000 in Pennsylvania, USA (see Section 2.1.6). Among the 663 cohort members, 2 cases of stomach cancer and 3 cases of colon cancer were observed, with SIRs of 1.79 (95% CI, 0.21–6.45) and 0.65 (95% CI, 0.13–1.91), respectively, compared with referents for the state of Pennsylvania. [The Working Group noted that the source of exposure investigated in this study was different



from that in the other studies, meaning that the results could complement the other evidence. However, the small number of outcomes and imprecise confidence intervals hindered interpretation. It was also possible that the retrospective study design, based on self-reported incident cancers, resulted in incomplete ascertainment of cancer cases, causing a downward bias in the SIR. Finally, latency was not considered in the analysis, possibly diluting the estimates of risk from the etiologically relevant exposure period. The Working Group noted that in an unpublished report on the gasoline spill, an excess of stomach cancer was reported during the period from 1985 to 2000 (SIR, 3.29; 5 cases), and from 1990 to 2000 (SIR, 4.00; 4 cases) when comparing rates for the local residents with those in Pennsylvania ([Pennsylvania Department of Health, 2001](#)). It was noted in the report that this area has had some of the highest rates of stomach cancer in both Pennsylvania and the USA, and that this could be partly explained by the presence of coal mining in this geographical area.]

#### (b) Case-control studies

[Stemhagen et al. \(1983\)](#) conducted a case-control study in New Jersey, USA, to investigate the association between occupational exposures and primary liver cancer. Using hospital records, the tumour registry, and death certificates, the authors identified 959 cases diagnosed between 1 January 1975 and 1 March 1980; of these, 335 cases were confirmed. Interviews covering information on occupational history since age 12 years, lifetime residence, medical history, smoking habits, and consumption of alcohol were completed for 265 people with liver cancer; of these interviews, 95% were conducted with family members of the deceased case participant or of case participants who were not able to respond. The study included 530 controls matched on age, race, sex, county of residence, and vital status. The OR for liver cancer among people who had ever been employed at a gasoline

service station for  $\geq 6$  months was 2.88 (95% CI, 1.20–6.88; 12 cases) for all types combined and was higher when only cases of hepatocellular carcinoma were included (OR, 4.20; 95% CI, 1.55–11.35; 11 cases). [The Working Group noted as a limitation that the study included a small sample size for service station attendants, leading to imprecision in estimates. Also, the number of cases ( $n = 12$ ) within the group of employees at gasoline service stations was too low for the estimates to be adjusted for reported level of alcohol consumption, and information on hepatitis B or cirrhosis was lacking. The focus of the study was on pesticide exposure and, hence, questions on agricultural occupations were somewhat more detailed than those on other occupations. Limitations also included potential information bias, because only a minor proportion of living cases were interviewed, and differential exposure misclassification bias (recall bias), because information on occupational exposure and potential confounders was reported retrospectively. These limitations reduced the informativeness of the study for the present evaluation.]

[Siemiatycki et al. \(1987a\)](#) conducted a population-based case-control study on the association between occupational exposure to 12 petroleum-derived liquids and risk of 20 cancer types (Section 2.1.10). No associations between exposure to automotive gasoline and risk of colon, rectal, or rectosigmoid cancers were observed, but automotive gasoline was associated with risk of stomach cancer (OR, 1.5; [95% CI, 1.1–2.0]). From the same case-control set, [Parent et al. \(1998\)](#) undertook a more detailed study of risk of stomach cancer in men according to level of exposure to leaded gasoline (unexposed, non-substantial, substantial) and exposure pattern (frequency, concentration, duration). The controls included a group of patients with cancer at other sites (excluding cancer of the lung and oesophagus) and a group of population-based controls drawn from electoral lists and random-digit dialling. Three separate analyses were conducted: one

using the controls with cancer, one using the population controls, and one pooling the two groups of controls. The results were considered similar across the three analyses and, hence, only the analysis with the pooled group of controls was reported. Of the interviews collecting information on socioeconomic characteristics, lifestyle factors, and occupational history, 81% were with the cases, and the rest were with proxy respondents. Elevated ORs for stomach cancer were found for the highest levels of exposure to leaded gasoline (substantial) for concentration (OR for high concentration, 2.0; 95% CI, 1.1–4.0) and duration (OR for  $\geq 20$  years exposure, 1.6; 95% CI, 1.0–2.7), compared with never exposure. An exposure–response relation with risk of stomach cancer was noted when using combinations of the exposure metrics concentration and duration. Three reference populations were used – other cancer patients, population controls, and a pooled group of cancer patients and population controls; in the estimates presented, the pooled group was used as the reference population. [The Working Group noted as strengths the use of cases of incident cancer, and the detailed exposure assessment comprising different exposure metrics for gasoline, as well as adjustment for education and smoking. In contrast to the study by [Siemiatycki et al. \(1987a\)](#), the study by [Parent et al. \(1998\)](#) also included a control group of non-cancer participants, which was a strength. Limitations included the small numbers of exposed cases.]

[Gerhardsson de Verdier et al. \(1992\)](#) conducted a population-based case–control study in Stockholm, Sweden, in 1986–1988, to assess the association between occupational exposures and colorectal cancer. The study was limited to participants born in Sweden between 1907 and 1946, and included 568 cases diagnosed with adenocarcinoma of the colon (352 cases) or rectum (217 cases), and 512 controls randomly selected from a population register and frequency-matched on year of birth and sex. Information

on occupational history and lifestyle factors (diet, physical activity, and BMI) were mainly collected through self-administered questionnaires. The risk (OR) of colon cancer among men for the combined occupational group of petrol station/automobile-repair workers, after adjusting for age, diet, physical activity, BMI, and family history of colorectal cancer, was 1.8 (95% CI, 0.6–5.2). The corresponding risk estimate (OR) for rectal cancer among men was 1.5 (95% CI, 0.4–5.6; 7 exposed cases). [The Working Group noted the adjustment for potential confounders (diet, physical activity, and BMI) as strengths. However, because of the small number of participants with the combined occupations, it was not possible to distinguish gasoline from other risk factors for colon cancer to which an automobile-repair worker would be exposed to (e.g. 7 of the 12 case participants who reported having worked at a petrol station or in automobile repair also reported occupational exposure to asbestos found, for example, in braking bands). Hence, the Working Group considered this study to be less informative for the present evaluation.]

[Arbman et al. \(1993\)](#) conducted a case–control study on adenocarcinoma of the colon or rectum in south-eastern Sweden, in 1984–1986, that included 98 cases of colon cancer, 79 cases of rectal cancer, and 801 controls (371 hospital-based controls and 430 population controls). Self-administered questionnaires included questions on occupational history, exposure to specific agents at work and during leisure time, and specific job activities. The risk (OR) of colon cancer for male gas station workers was 2.2 (90% CI, 0.8–6.1) when adjusted for age and physical activity at work. There were no cases of colon cancer among female gas station workers, nor any rectal cancer among male and female gas station workers. [The Working Group noted the imprecision in the estimates and the lack of adjustment for other lifestyle factors and potential co-exposure to carcinogenic agents, although



adjustment for physical activity in the workplace was considered to be useful.]

### 2.5.2 Cancer of the respiratory system

#### (a) Cohort studies

In a cohort study in 16 524 male and 2445 female service station attendants in four Nordic countries who were identified from the 1970 censuses and followed for about 20 years, ([Lynge et al., 1997](#)) reported an excess risk among men for cancers of the nose (SIR, 3.1; 95% CI, 1.5–5.7; 10 cases), lung (SIR, 1.3; 95% CI, 1.1–1.4; 208 cases), and larynx (SIR 1.5; 95% CI, 0.9–2.2; 25 cases). Among women, the SIR for cancer of the nose was 8.0 (95% CI, 1.0–28.9; 2 cases). [The Working Group noted as a strength that this was a large cohort of service station attendants who were most probably exposed to gasoline during car refuelling before self-service by the customer became normal practice in the Nordic countries in the 1970s and 1980s. Limitations included the probable nondifferential misclassification of exposure status because the information on occupation was available only at a single point in time (the 1970 census), and the lack of adjustment for potential confounders such as smoking. The exposure misclassification, lack of information on duration of exposure, and failure to identify the most highly exposed participants may have resulted in SIR estimates that were biased towards the null.]

[Schnatter et al. \(1993\)](#) (an update of [Schnatter et al., 1992](#)) conducted an industry-funded cohort study in 4889 active or living retired male workers between 1964 and 1983 who had worked for  $\geq 1$  year in the marketing or distribution operating sector of a petroleum company in Canada and who were also classified as ever exposed to hydrocarbons (see Section 2.1.3). The SMR for lung cancer in marketing or distribution workers exposed to hydrocarbons was 0.79 (95% CI, 0.57–1.06). [The Working Group noted the imprecise estimates. Even with restriction of

the analyses to workers who were ever exposed to hydrocarbons, which was a strength of the study, co-exposure to other potential occupational carcinogens was likely for petroleum distribution workers, which could induce upwards bias in the estimates. Also, the Working Group noted the risk of HWSB because of the inclusion of living retired workers only, excluding those who may have died from occupational cancers before retirement, potentially causing an underestimation of risk.]

[Sorahan \(2007\)](#) conducted an industry-sponsored cohort study of male petroleum distribution workers in the UK that included 16 477 men first employed in the period 1946–1974 for  $\geq 12$  months and with some employment after 1950 (see Section 2.1.3). Follow-up was conducted for cancer incidence between 1971 and 2003. There was a deficit of mesothelioma (pleura), with an SRR of [0.33] (95% CI, [0.15–0.63]), and SRRs for the estimates for respiratory cancers were: [0.61; 95% CI, 0.17–1.55] for the nose and sinuses; [1.17; 95% CI, 0.89–1.51] for the larynx, and [1.05; 95% CI, 0.98–1.13] for the lung and bronchus. When stratified by job title, drivers assumed to handle gasoline when distributing the refinery products (on- and offloading) did not have excess mortality from cancer of the pleura (SMR, [0.65]; 95% CI, [0.21–1.52]). [The Working Group noted that the cohort study was relatively large, but co-exposure to other carcinogenic refinery products and other agents may have acted as a potential confounder, resulting in a potential bias upwards. Limiting the cohort to those with  $\geq 12$  months employment may have introduced some HWE bias.]

[Wong et al. \(1993\)](#) performed an industry-funded retrospective cohort study in land-based terminal petroleum workers (employed  $\geq 1$  year in 1946–1985) who were primarily exposed to gasoline vapour ( $n=9026$ ). They assessed mortality from a range of cancers until June 1989 (see Section 2.1.3), including cancers of the respiratory system (173 cases) and lung (165

cases) as secondary outcomes. Crude SMRs for cancer of the respiratory system ([0.658; 95% CI, 0.565–0.762]) and cancer of the lung ([0.662; 95% CI, 0.568–0.766]) were both well below unity. No elevated lung SMRs were found for any category of duration of exposure (1–4 years, 5–9 years, 10–19 years, 20–29 years, and  $\geq 30$  years), or time since first exposure or latency. [The Working Group noted that the crude SMRs for overall mortality and all cancers combined were considerably below unity ([0.513 and 0.664], respectively), indicating HWE bias in this cohort.] In a later analysis from an overlapping cohort with extended follow-up, [Wong et al. \(1999\)](#) assessed cancer incidence in combined land- and marine-based petroleum distribution workers who were exposed to gasoline. No excess risk was found for cancers of the larynx (SMR, 0.74) or lung (SMR, 0.84). [The Working Group noted the reporting of detailed exposure information as a strength. Limitations included the use of only mortality data. Co-exposure to other potential occupational carcinogens was likely for petroleum distribution workers, and no occupational or lifestyle-related confounders were considered in the analysis. A substantial HWE bias was noted, some of which was probably explained by the limiting of the cohort to those with  $\geq 12$  months of employment, suggesting cohort selection bias on the basis of health status at the time of hire. The need to take the healthy-worker bias into account in cohorts of petroleum workers in general, and in this study in particular, has been emphasized previously ([Dement et al., 1997](#)).]

In the cohort study of service station attendants in the Latium region of Italy in which causes of mortality from non-malignant and malignant diseases were assessed, [Lagorio et al. \(1994a\)](#) did not find an excess of respiratory cancer (overall or separately for lung and larynx) for men or women, or for service station attendants in small stations where exposure to gasoline was expected to be higher than in large stations. [The Working Group noted that

exposure to gasoline was probably high among service station attendants in Italy because refueling activities were common practice at the time of the study. However, the study was of small size with a short follow-up period, and the outcome was based on mortality data. Lack of information on lifestyle factors known to be risk factors for oesophageal cancer, such as smoking and alcohol consumption habits, did not allow for any confounder control.]

In the study by [Patel et al. \(2004\)](#) of residents in the area affected by a gasoline spill (see Section 2.1.6), 5 cases of cancer of the trachea, bronchus, lung, and pleura were observed, with an SIR of 0.67 (95% CI, 0.22–1.56) compared with the state of Pennsylvania referent. [The Working Group noted the small number of outcomes and imprecise confidence intervals. It was also possible that the retrospective study design based on self-reported incident cancers resulted in incomplete ascertainment of cancer cases, resulting in downward bias of the SIR. Finally, latency was not considered in the analysis, possibly diluting the estimates.]

#### (b) Case-control studies

In a hospital-based, multicancer site case-control study (described in Section 2.1.10), [Siemiatycki et al. \(1987a\)](#) included estimations of the association between cancer of the lung ( $n = 857$ ) and occupational exposure to automotive gasoline among men aged 35–70 years. There was no evidence on an association between occupational exposure to gasoline (ever versus never) and risk of lung cancer (ORs ranged between 0.8 and 1.1 for the four subtypes investigated) (see Table S2.5 for details; Annex 2, Supplementary tables for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>). [The Working Group noted that the study involved the assessment of exposure to automotive gasoline using expert judgement, which was a strength compared with studies based on self-report; however, this may cover a

broad range of occupations, and the main ones coded included mechanics, farmers, forestry workers, and motor transport. No adjustment for co-exposure to other carcinogenic agents or exposures prevalent in this group (e.g. asbestos, PAH, diesel exhaust, welding) was conducted, which could induce upward bias in the estimates. The Working Group noted the adjustment for smoking as a strength.]

In a case-control study on cancer of the larynx based on hospital records in the USA, [Brown et al. \(1988\)](#) identified a total of 303 cases during the period from July 1975 through June 1980 (5 years). Using self-reported information on employer, job title, and duties for jobs held for 6 months or longer after 1939, 16 industrial and 24 occupational categories were formed. An industrial hygienist classified job titles for potential exposure to 12 chemical and physical agents, including gasoline or diesel fumes. The estimates were adjusted for smoking and alcohol consumption. Risk of laryngeal cancer when occupationally exposed to gasoline or diesel fumes was increased (RR, 1.50; 95% CI, 1.00–2.26), but no exposure-response relation was found. [The Working Group noted that despite the strength of the detailed occupational history for the patients and controls, gasoline exposure was combined with diesel, and it was not possible to assess how much gasoline contributed to the increased risk. Information was available on exposure to 11 known carcinogens; however, these were not adjusted for in the analysis. There was no adjustment for co-exposure to other carcinogenic agents that have been linked to laryngeal cancer and for which information was available (e.g. asbestos, acid mist), which could lead to upward bias.]

In a hospital-based case-control study in Germany on the association between occupational exposures and laryngeal cancer, 30 cases of prevalent (1984–1985) and 55 cases (1986) of incident laryngeal cancer were compared with 100 male hospital controls with diseases not

considered to be related to smoking, alcohol consumption, or occupational exposures ([Ahrens et al., 1991](#)). Self-reported information on lifetime occupational history and lifestyle (smoking and drinking habits) was collected. Data on job titles and industry were grouped into 31 occupations and 21 branches of industry, respectively. An exposure checklist of 19 occupational exposures, including gasoline, was used for each job. Self-reported exposure to gasoline was associated with an increased risk of laryngeal cancer (OR, 2.8; 95% CI, 1.0–7.7). [The Working Group noted as a strength that the exposure assessment also included information on specific occupational agents in addition to industry and occupation. In addition, the estimates were adjusted for smoking and alcohol consumption, important risk factors for laryngeal cancer. However, limitations included the potential for recall bias, because the information on job held was self-reported; and interviewer bias, because the interviewer was not blinded to case status. Both these biases could lead to overestimation of the association between occupational exposure and laryngeal cancer. Survival bias may have been induced by the inclusion of prevalent cases (35% of the cases), although this may have been modest because of the high 1-year survival rate, reported by the authors as 85%.]

In a case-control study using data from the Surveillance, Epidemiology and End Results (SEER) programme, [Wortley et al. \(1992\)](#) included 235 cases of laryngeal cancer diagnosed between September 1983 and February 1987 and 547 controls selected using random-digit dialling and frequency-matched on age and sex. Self-reported information on lifetime occupational history (industry, job title, tasks) and smoking and alcohol consumption habits was collected during an interview. Seven per cent of the case interviews were with the closest next of kin. Case participants reporting being ever employed in the occupation “garage and service station related” had an odds ratio for

laryngeal cancer of 0.8 (95% CI, 0.4–1.8), with no increase if employed for > 10 years compared with < 10 years. Allowing for possible induction and latency effect (excluding exposures in the 10 years immediately before diagnosis) did not change the estimates. [The Working Group noted as a strength that the cases and controls were drawn from a large and comprehensive population-based database with a relatively long follow-up time with respect to occupational history. The Working Group noted as a limitation that a JEM was used for other exposures but not for gasoline, and that the assessment of gasoline relied on reports of working in a garage and service station, introducing probable nondifferential misclassification of exposure, biasing the estimates towards the null.]

[Teschke et al. \(1997\)](#) conducted a case–control study in British Columbia, Canada, to investigate cancer of the nasal cavity and sinuses. The study included 48 cases of histologically confirmed nasal cancer registered by the British Columbia Cancer Agency between 1 September 1990 and 31 May 1991 and 159 population controls (shared) selected from provincial voter lists. The controls were frequency-matched on the age and sex distribution of cases for both cancer sites. Data collection involved face-to-face or telephone interviews using a standardized questionnaire that covered occupational, residential, smoking, and medical histories. The risk (OR) of nasal cancer associated with employment as service station attendant and managers was 0.8 (95% CI, 0.2–2.8) for the nasal cavity and sinuses, an estimate that was only marginally affected when removing the most recent 20 years before diagnosis in the latency analysis. [The Working Group noted that the sample size for service station attendants was small. Also, in the grouping of occupations, workers with varying work responsibilities (e.g. managers) and exposure were placed in the same occupation group, which may not have adequately captured gasoline exposure. This nondifferential misclassification

of exposure would be expected to bias the risk estimates towards the null, potentially underestimating the true associations. A strength was the adjustment for smoking, as well as adjustment and stratification for sex.]

[De Stefani et al. \(1998\)](#) carried out a hospital-based case–control study in Uruguay that included 112 cases of incident laryngeal cancer and 509 controls with cancers not related to tobacco and alcohol exposures. On the basis of self-reported occupational history and use of chemicals at work, information on duration of exposure to 14 selected substances, including gasoline, was provided. The case and control participants were also asked questions on tobacco smoking and alcohol consumption. The risk (OR) of laryngeal cancer for ever being occupationally exposed to gasoline versus never was 1.4 (95% CI, 0.8–2.6; 22 cases). The risk of laryngeal cancer increased with substantial (> 20 years) occupational exposure to gasoline (OR, 1.7; 95% CI, 0.9–3.5; 16 cases). In the same case–control population as that described above by [De Stefani et al. \(1998\)](#), [De Stefani et al. \(2005\)](#) conducted similar analyses for adenocarcinoma of the lung as an outcome. This superseded a previous version based in a partially overlapping cohort and that contained less specific histological detail on lung cancer ([De Stefani et al., 1996](#)). For risk of adenocarcinoma of the lung, the ORs for occupational gasoline exposures were 1.1 (95% CI, 0.7–1.8; 31 cases) for ever-versus-never exposure, 2.4 (95% CI, 1.3–4.5; 22 cases) for exposure of 1–20 years, and 0.4 (95% CI, 0.2–0.9; 9 cases) for substantial exposure (> 20 years). [The Working Group noted that the estimates were adjusted for potential confounding from lifestyle factors. However, the numbers of exposed cases were low, in particular when stratifying into exposure groups, and no adjustment for co-exposure to other occupational carcinogens was conducted.]

[Shangina et al. \(2006\)](#) conducted a multi-centre case–control study in four European countries (Poland, Romania, Russian Federation,



and Slovakia) that included 316 cases of incident laryngeal cancer and 34 cases of incident hypopharyngeal cancer in men (1999–2002). There were 728 hospital controls without cancer or diseases associated with tobacco or alcohol, who were matched on age. Information on socioeconomic and demographics, general health characteristics, lifestyle factors (tobacco, alcohol, diet, oral hygiene), and occupational history were collected by trained interviewers. Occupational exposure to 73 agents, including gasoline/petroleum, was assessed. An OR of 1.31 (95% CI, 0.88–1.95; 53 cases) was reported for risk of laryngeal cancer after ever exposure to gasoline/petroleum. For gasoline/petroleum, no analyses by frequency, intensity, or confidence of exposure were reported. [The Working Group noted the crude exposure measure for occupational exposure to gasoline/petroleum (never/ever). The number of cases of hypopharyngeal cancer did not allow estimation of risk for ever being exposed to gasoline/petroleum. A strength was the adjustment for smoking and alcohol consumption.]

In the population-based Environment And Genetics in Lung cancer Etiology (EAGLE) case-control study in the Lombardy region of northern Italy, [Consonni et al. \(2010\)](#) enrolled 2100 cases of incident lung cancer and 2120 randomly selected population controls matched on age, sex, and region of residence (April 2002 through June 2005). Information on occupational history, including both industry and occupation for jobs held for  $\geq 6$  months, and on lifestyle factors, including smoking behaviour, was collected through self-administered questionnaires. The included occupation that was relevant for the evaluation of automotive gasoline was having ever worked as a filling station attendant under the industry code “trade” (ISIC). For men, filling station attendants were at an increased odds of lung cancer (OR, 7.41; 95% CI, 1.76–31.17; 16 cases), after adjusting for area of residence, age, smoking, and numbers of jobs

held. After adjusting for educational level, the estimate decreased somewhat (OR, 6.64; 95% CI, 1.58–27.94). [The Working Group noted that, although the study included a large number of cases of incident lung cancer, there was a small sample size for service station attendants. When using service station attendants as a proxy for gasoline exposure, nondifferential misclassification of exposure was expected that would bias the risk estimates towards the null. The estimates reported were high but imprecise, with rather wide confidence intervals.]

[Wang et al. \(2014\)](#) conducted a hospital-based case-control study that included 1000 participants (500 patients with lung cancer and 500 cancer-free controls matched on age, gender, and place of residence) in China (2010 and 2012); the aim was to investigate the association between lung cancer risk and a specific genotype. Information on sociodemographic details and occupational and lifestyle history was collected using a standardized interview conducted by trained interviewers. Occupational exposure to gasoline/diesel was reported to be a risk factor for lung cancer (OR, 4.65; 95% CI, 1.40–15.45). [The Working Group noted that the association between occupational exposure to gasoline/diesel and lung cancer was not the main focus of the study, and hence no assessment of exposure specifically to gasoline was conducted. Also, it was not possible to distinguish gasoline from diesel, and no information on potential co-exposure to other carcinogenic agents was provided. Hence, the study was considered to be of limited value for the present evaluation.]

In the multicentre population-based ICARE study in France ([Luce et al., 2011](#)), associations were investigated between petroleum-based solvents, including gasoline, and incident primary cancers identified from cancer registries in 10 geographical areas, including cancer of the lung among men ([Mattei et al., 2016](#)) and cancers of the neck and head among women ([Carton et al., 2017](#)) and men ([Barul et al., 2018, 2019](#)).

All cases of incident cancer diagnosed between 2001 and 2007 were captured (the response rate for contacted patients was 82.5% for cases of head and neck cancer and 87.1% for cases of lung cancer). Cases were histologically confirmed and aged 18–75 years at diagnosis. Control participants were a random sample of the population from the same area and were frequency-matched according to the sex- and age-group distribution of the cancer cases. An additional stratification of the control group according to the socioeconomic status of the general population was also performed (response rate among contacted control participants, 80.6%).

For ICARE, lifetime occupational history information, including each job held for  $\geq 1$  month, was captured during in-person interviews conducted as close as possible to diagnosis, and coded by a combination of International Standard Classification of Occupations (ISCO 68) and French classification of activities (NAF) 1999 edition. These codes were linked with JEMs, developed for the French population ([Févotte et al., 2011](#)), that included five petroleum-based solvents such as gasoline, assigning estimates of probability of exposure (percentage of exposed workers), intensity of exposure, and frequency of exposure for different calendar periods between 1947 and 2005 to account for changes in exposure over time. Lifetime indices included ever-versus-never occupational exposure (with ever exposure generally defined as having a probability level of  $>0\%$ ), cumulative occupational exposure (product of probability, frequency, intensity, and duration of each job period), average occupational exposure intensity, and duration. The reference category consisted of those never occupationally exposed to a specific solvent.

Among men within ICARE, [Mattei et al. \(2016\)](#) examined 2260 cases of lung cancer and 2780 controls without any history of respiratory cancer. Ever exposure was defined as having an exposure probability of  $>50\%$ , with a sensitivity

analysis performed based on  $>10\%$ . All analyses were systematically adjusted for smoking and asbestos exposure (on the basis of a JEM for asbestos; probability of exposure,  $>30\%$ ). The analysis was performed using unconditional logistic regression models examining tertiles of exposure to gasoline alone as well as to each other type of solvent. Bayesian profile regression was also used to further examine clusters of solvent exposures. A total of 67 cases and 72 controls were in the tertile of highest exposure to gasoline. There were strong correlations between exposure to gasoline and exposure to ethylene glycol ( $r = 0.78$ ) or benzene ( $r = 0.62$ ). Among men in ICARE, no association was found between gasoline exposure and lung cancer (OR in the highest exposed group, 1.11; 95% CI, 0.73–1.70) ([Mattei et al., 2016](#)). [The Working Group noted that the strengths of this study included that gasoline exposure was examined alone and in combination with exposure to other solvents, a high specificity of gasoline exposure was assigned, and that detailed adjustment for smoking and asbestos exposure was performed. Limitations included the moderate number of highly exposed participants.]

Additionally, within ICARE, there were 383 cases of squamous cell carcinoma (SCC) of the hypopharynx, 454 cases of SCC of the larynx, and 2780 controls in men ([Barul et al., 2018](#)). Adjusted unconditional logistic regression models were used to estimate associations. The analysis also examined risks of combined solvent exposures where there were at least 10 exposed cases, including among participants with exposure to gasoline, white spirit, and diesel, as well participants with exposure to these three agents plus benzene. There was a strong correlation between exposure to gasoline and exposure to diesel/fuels/kerosene ( $r = 0.69$ ), as well as moderate correlations for other agents, including white spirit ( $r = 0.34$ ), ketones and esters ( $r = 0.39$ ), and ethylene glycol ( $r = 0.58$ ). No association was found for any of the head and neck cancers,



including hypopharyngeal cancer (OR for ever versus never, 1.11; 95% CI, 0.83–1.50) and laryngeal cancer (OR for ever versus never, 0.93; 95% CI, 0.70–1.23) (Barul et al., 2018). [The Working Group noted that gasoline exposures alone and in combination with other solvents were examined where possible. Although outcome data on site-specific incident cancers were examined, the number of exposed participants in the analysis was small, and there was potential for chance findings.]

Barul et al. (2019) examined associations between occupational exposure to gasoline among 350 cases of SCC of the oral cavity, 543 cases of SCC of the oropharynx, and 2780 controls (all men). Adjusted unconditional logistic regression models were used to estimate associations. In an additional analysis, further adjustment was also made for exposure to all solvents. A strong correlation ( $r = 0.69$ ) was observed for exposure to gasoline and diesel/fuels/kerosene. A deficit in risk was noted for oral cancer (OR, 0.69; 95% CI, 0.48–1.00) and oropharyngeal cancer (OR, 0.68; 95% CI, 0.51–0.92) in men (Barul et al., 2019), and the risk estimates decreased with increasing duration of exposure for both the oral cavity ( $P$  for trend, 0.03) and oropharynx ( $P$  for trend,  $<0.01$ ) (Barul et al., 2018). [The Working Group noted that, although there was an attempt to adjust for exposure to all solvents, and there were outcome data on site-specific incident cancers, there were also small numbers of exposed participants.]

Within ICARE, data were captured on 296 cases of SCC of the head and neck in women, and 775 controls (Carton et al., 2017). Adjusted unconditional logistic regression models were used to estimate associations. Because of the low number of exposed women, analyses using exposure metrics for gasoline other than ever versus never for SCCs of the head and neck (OR, 1.54; 95% CI, 0.36–6.63) were hampered (Carton et al., 2017). [The Working Group noted as a strength the focus on analysis of incident cancer among women in relation to specific solvents,

adding to the body of literature. However, the small number of exposed cases did not allow for detailed analysis.]

[Overall, for ICARE, the Working Group considered that exposure assessment using JEMs specifically designed for the French population was a strength. The country-specific JEM could be applied using a combination of occupation and activity data, making it more dynamic in terms of both time period and variation between occupations across industries. However, the JEM was based on broad occupational categories that did not account for the diversity of jobs and tasks within a single occupation. As a result, nondifferential misclassification of exposure was expected, which could bias effect estimates towards the null. This issue was particularly relevant for the exposure metric “ever versus never exposed”. The low number of exposed women did not allow for detailed analyses, and using JEMs may have led to greater misclassification of exposure for women.]

## 2.6 Cancer of the breast

See Table S2.6 (Annex 2, Supplementary tables for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>).

### 2.6.1 Female breast cancer

There were three retrospective cohort analyses of incident or fatal female breast cancer among workers in specific occupations or industries in Europe (Lagorio et al., 1994a; Lynge et al., 1997; Weiderpass et al., 1999). There was one prospective cohort study in the USA (Ekenga et al., 2015). There was one study on environmental exposure, in a retrospective cohort of residents exposed to gasoline vapour in the USA (Patel et al., 2004). In Denmark, there was one register-based nested case-control study on parental occupational exposure and breast cancer risk among female

offspring ([Pedersen and Hansen, 2023](#)). There was one population-based case-control study in Canada ([Band et al., 2000](#)).

Findings from [Bulliard et al. \(2022\)](#) were considered to be uninformative as the exposure category comprised those working in the broad economic activity branch of trade, repair of motor vehicles and of domestic articles.

#### (a) Cohort studies

In a cohort of gas station workers in the Nordic countries, there were 2445 included women aged 20–64 years, with a total of 42 889 person-years of follow-up accrued over the 15–20 years of follow-up time since 1970 ([Lynge et al., 1997](#)) (see Section 2.1.1). Based on 61 observed cases of incident breast cancer, the SIR was 1.2 (95% CI, 0.9–1.5). [The Working Group noted as strengths the use of high-quality registers encompassing national populations of four Nordic countries and the use of incident rather than mortality outcomes in this study. Limitations included the crude exposure assessment on the basis of occupation and industry from the 1970 census and the lack of information on duration of exposure, although the authors indicated relative occupational stability of the workers over time, which reduced the Working Group's concerns. There was also a lack of data on personal lifestyle or reproductive factors.]

In a study on breast cancer incidence, [Weiderpass et al. \(1999\)](#) used job title data from the 1970 census in Finland, including 892 591 women who reported having an occupation, who were followed between 1971 and 1995 (see Section 2.1.1). In total, there were 15 481 680 person-years of follow-up and 23 638 cases of breast cancer. Job title data from 1970 were linked to FINJEM to provide estimates of probability and mean level of exposure to a range of agents, including gasoline. Among premenopausal women (aged <50 years), there was no clear association with breast cancer for women in occupations with a  $\geq 20\%$  probability

of exposure to gasoline (SIR, 1.55; 95% CI, 0.39–6.20) compared with women in occupations with a <20% probability of exposure; or among women with a low (SIR, 0.91; 95% CI, 0.43–1.92) or medium/high (SIR, 1.55; 95% CI, 0.39–6.20) ( $P$  for trend, 0.84) mean level of exposure compared with women with no exposure. Among postmenopausal women (aged  $\geq 50$  years), there was also no clear association between breast cancer and exposure to gasoline ( $\geq 20\%$  probability) (SIR, 0.70; 95% CI, 0.34–1.47), or according to low (SIR, 0.95; 95% CI, 0.64–1.39) or medium/high (SIR, 0.70; 95% CI, 0.34–1.47) ( $P$  for trend, 0.37) mean levels of exposure. [The Working Group noted the use of high-quality population-based registers, incident outcomes, the availability of data on some personal or reproductive factors, and analysis of pre- and postmenopausal disease as strengths. Limitations included exposure assessment on the basis of a single census year, use of a non-gender-specific JEM, and imprecise findings.]

In a retrospective cohort of 2665 self-employed filling station attendants in Italy that included 357 women who were exposed to gasoline vapour, [Lagorio et al. \(1994a\)](#) (see Section 2.1.4) reported a total of 2 deaths from breast cancer in women and an SMR of [1.04] (95% CI, [0.13–3.76]). The majority of women had a duration of employment at entry of 1–10 years (63.8%). [The Working Group noted the small number of female workers included and breast cancer deaths, the use of fatal rather than incident end-points, the short follow-up period, limitations in the exposure assessment, and the lack of information on personal lifestyle and reproductive factors.]

In a study of residents with presumed community exposure to gasoline vapour because of groundwater contamination via leaking underground petroleum storage tanks, [Patel et al. \(2004\)](#) compiled a retrospective cohort of 663 people living for  $\geq 6$  months in 275 households from 1990 to 2000 with chronic low-level

exposures (see Section 2.1.5). SIRs were used to compare cancer rates for cohort members, who were defined by residence within a defined remediation area potentially exposed to gasoline fumes from the gasoline spill, to those for the Pennsylvania state population. Overall, there were a total of 12 cases of incident female breast cancer, and 7.9 cases expected. The SIR was 1.52 (95% CI, 0.79–2.66). [The Working Group noted the unique aspects of this study examining potential exposure to gasoline vapour in a community setting. Limitations included the lack of refined information on individual-level exposure to gasoline vapour, with exposure status being based on the definition of a remediation area defined by groundwater sampling performed  $\geq 5$  years after the spill and on exposure estimation models. The statistical analysis did not consider potential confounding by personal lifestyle or reproductive factors. There were few breast cancer cases, leading to some imprecision in the estimates.]

In the Sister Study, a prospective cohort composed of cancer-free women whose sisters had breast cancer who were enrolled between 2004 and 2009 in the USA, [Ekenga et al. \(2015\)](#) analysed occupational chemical exposures among 47 640 participants who reported having worked outside the home (see Section 2.1.7). Specifically, they examined self-reported workplace exposure to gasoline or other petroleum products, as one of 14 workplace chemical exposures. Information on ever use as well as on frequency and duration of use was captured. In total, 1966 cases of incident breast cancer were observed. Cox proportional hazards regression models adjusting for race/ethnicity, education, income, parity, and age at first birth were used. Among the 1998 women who reported ever use of gasoline or other petroleum products, there were 66 cases of breast cancer. Overall, there was no association with ever use of gasoline or other petroleum products, defined as having handled the agent at least once per week in any job, compared with never use for total breast cancer

(HR, 0.8; 95% CI, 0.7–1.1) or invasive breast cancer (HR, 1.0; 95% CI, 0.7–1.3). There was a significant inverse association between ever use of gasoline or other petroleum products and in situ disease (HR, 0.5; 95% CI, 0.2–0.9; 11 exposed cases). Among women in the highest quartile of cumulative use, defined as lifetime total days of use of gasoline or other petroleum products, HRs in the highest categories of use were modestly elevated (HR for total breast cancer, 1.2; 95% CI, 0.8–1.9; and HR for invasive breast cancer, 1.4; 95% CI, 0.9–2.3). Among women in the highest quartile of cumulative exposure to gasoline or other petroleum products compared with women in the lowest quartile (exposed-only analysis), there was an elevated HR for total breast cancer (HR, 2.3; 95% CI, 1.1–4.9) as well as for invasive disease (HR, 2.5; 95% CI, 1.1–5.9), and there were significant trends ( $P$  for trend, 0.03 and 0.02, respectively). There were no clear associations with ever use by hormone receptor status or for premenopausal or postmenopausal disease. For hormone receptor-positive breast cancer, HRs were increased in the highest categories of cumulative use, comparing with never users (HR, 1.4; 95% CI, 0.9–2.3), and in the analysis restricted to ever users only, comparing with women in the lowest exposure category (HR, 2.5; 95% CI, 1.0–5.8;  $P$  for trend, 0.06). [The Working Group noted the prospective study design that included cases of incident breast cancer, hormone receptor status, and a range of personal lifestyle and reproductive data as strengths. Limitations included the self-reported exposure assessment and small numbers of exposed women. The cohort was also probably at greater underlying risk of disease because of family history, and thus findings for other (occupational) agents may have been attenuated.]

In a case-control study on parental occupational exposure and breast cancer among female offspring in Denmark, [Pedersen and Hansen \(2023\)](#) matched each of 5587 cases of breast cancer for whom information was available on

maternal or paternal occupational history, identified using the Danish Cancer Registry, to 20 female cancer-free controls, using the Danish Civil Registration System. Cases born after 1965 were included in the study, because information on parental employment was accessible from 1964 through 2014, and they were diagnosed in 2016 or before. As such, cases were aged up to 51 years in the current study. Information on estrogen receptor status was available for 82% of cases. Controls were randomly selected using incidence-density sampling and matched to cases on sex and year of birth. Industry code was used to link maternal and paternal employment using the Danish version of NOCCA to obtain information on probability and mean intensity level over four different periods for gasoline, one of several specific occupational chemical exposures included in the study. The most common industries involving gasoline exposure for mothers included automobile and motorcycle businesses, manufacture of other machinery, and iron shipyards. The most common industries involving gasoline exposure for fathers included automobile and motorcycle businesses, manufacture of other machinery, and forge- and machine-repair workshops. A total of 3.4% of cases and 3.2% of controls had mothers who were ever occupationally exposed to gasoline, and 15.2% of cases and 15.3% of controls had fathers who were ever occupationally exposed. For maternal exposure to gasoline, no association was observed with ever occupational exposure until 1 year after birth or ever exposure in the perinatal period (1 year before birth until 1 year after birth). In the analysis of cumulative exposure, there was a positive although imprecise OR for women in the highest quartile of cumulative gasoline exposure (OR, 1.26; 95% CI, 0.93–1.70; 53 exposed cases) for exposure until 1 year after birth. No associations were observed for duration of exposure, with mutual adjustment for maternal or paternal chemical exposures, in the full case analysis, or among women born before 1980. For paternal

exposure, no associations were observed in the analysis by ever exposure until birth or in the perinatal period 1 year before birth until birth. There were also no clear associations observed for exposure duration or cumulative exposure, or with mutual adjustment for maternal or paternal chemical exposures, in full case analysis, or among women born before 1980. Occupational exposure to gasoline among the participants themselves was 10.9–11.1% for cases and 11.7–12.1% for controls among women for whom information on maternal and paternal employment history was available. [The Working Group noted that this was a large-scale study based on high-quality registry data, containing detailed information on parental occupational history in specific time windows of exposures, as well as data on the estrogen receptor status of the disease. Limitations included the lack of information on personal and reproductive factors, the lack of occupational information before 1964 and on parental leave or on potential changes in work tasks, and that the JEM was not gender-specific.]

#### (b) *Case-control studies*

In a population-based case-control study conducted in British Columbia, Canada, that included 995 women with incident breast cancer and 1020 controls, information on lifetime occupational history and other personal factors was captured by self-administered questionnaire ([Band et al., 2000](#)). This information included lifetime job descriptions, occupational and industry titles, duration and period of work. Analysis was performed according to usual occupation or industry, or ever occupation or industry, adjusting for potential confounding variables. For premenopausal women, no clear associations were observed for ever working in the gasoline service station industry or motor vehicle repair shop industry, and there were few exposed cases. Among postmenopausal women, a positive association was observed for ever working in the gasoline service station industry



(OR, 7.65, 90% CI, 1.29–45.3; 9 exposed cases). [The Working Group noted the population-based design of the study, with detailed information on a range of potential confounders captured for cases of incident breast cancer. Limitations included the broad nature of the occupational exposure assessment, moderate participation rates, and low numbers of exposed women.]

### 2.6.2 Male breast cancer

There was one population-based nested case-control study in Europe ([Hansen, 2000](#)) and two retrospective cohort analyses of incidence of or mortality from male breast cancer among workers in specific occupations or industries, one of which was in Europe ([Sorahan, 2007](#)) and one in the USA ([Wong et al., 1993](#)). There was one hospital-based case-control study in Europe ([Lenfant-Pejovic et al., 1990](#)), and another multi-country study in Europe, first reported by [Ville-neuve et al. \(2010\)](#) but superseded by [Laouali et al. \(2018\)](#).

The findings of the study by [Cocco et al. \(1998\)](#) were considered to be uninformative because they related to employment in industries involved in the manufacture of motor vehicles and motor vehicle equipment and were thus considered not relevant to the agent as defined in the present monograph.

#### (a) Cohort and nested case-control studies

A register-based nested case-control study in Denmark included a total of 230 cases of male breast cancer and 12880 controls, randomly selected using incidence-density sampling and matched on birth year, who were members of a compulsory national pension fund established in 1964 for all paid employees ([Hansen, 2000](#)) (see Section 2.1.1). Cases were born between 1897 and 1966 and had a first diagnosis of primary breast cancer between 1970 and 1989, according to the Danish Cancer Registry. After reconstruction of employment histories and worker status

(white-collar, blue-collar) for included participants, occupational exposure to gasoline vapour and PAHs was assigned to blue-collar workers with  $\geq 3$  months of employment in companies with the trades codes for service station, vehicle maintenance, wholesale trade of gasoline, or car repair shops. Conditional logistic regression models were used to estimate associations of potential exposure and trends with potential exposure duration, with a 10-year lag period and adjusting for birth year and socioeconomic status. Overall, there were 19 exposed cases (8.3%) and 514 exposed controls (4.0%). A positive, relatively precise OR of 2.2 (95% CI, 1.4–3.6) was observed among men who were potentially exposed to automotive gasoline and combustion products (PAHs). ORs tended to increase somewhat with application of a 10-year lag in the analysis (OR for exposed, 2.5; 95% CI, 1.3–4.5). [The Working Group noted the limited available information on the exposure assessment, which was based on company trade code and worker status, as well as the joint exposure to both gasoline vapour and combustion products, as well as the small numbers of exposed cases.]

[Sorahan \(2007\)](#) carried out a study in an industry-sponsored retrospective cohort of 16 477 petroleum distribution workers in the UK (part of a larger cohort that included oil refinery workers) with potential exposure to gasoline vapour (see Section 2.1.3). The cohort was followed from 1951 to 2003 for mortality and from 1971 to 2003 for cancer incidence. [Sorahan \(2007\)](#) reported a total of 4 deaths from male breast cancer, and 2.5 expected, among petroleum distribution workers, and an SMR of [1.57] (95% CI, [0.43–4.03]). Regarding cancer incidence among petroleum distribution workers, there were a total of 4 cases of male breast cancer, and 6.3 expected, and the SRR was [0.63] (95% CI, [0.17–1.63]). [The Working Group noted the small number of male breast cancer cases and deaths, as well as the crude exposure assessment, and multiple co-exposures. The Working Group

also noted the refinement of various aspects of the cohort related to previous duplicate entries or incomplete cancer registration data in previous work. The Working Group considered that the estimates may be affected by downward bias because of the HWE, and that there may have been some HWSB because of restriction of the analyses to employees with 1 year of employment.]

In an industry-funded retrospective cohort of 18 135 primarily White male marketing and marine distribution workers in the USA with potential exposure to gasoline vapour, [Wong et al. \(1993\)](#) (see Section 2.1.3) reported 2 deaths from breast cancer among marine distribution workers and a high but imprecise SMR of [8.389 (95% CI, 1.016–30.285)]. Both breast cancer deaths occurred among male non-deck personnel considered to have minimal exposure. No breast cancer-related deaths were reported for land-based distribution workers. [The Working Group noted the small number of male breast cancer deaths observed and the potential for downward bias from the HWE. Women represented 1.1% of the marine distribution workers.]

#### (b) *Case-control studies*

There was one largely hospital-based case-control study conducted in France and Switzerland that captured information on 91 male patients with histologically diagnosed breast cancer and 255 matched controls ([Lenfant-Pejovic et al., 1990](#)). In France, 73 cases of male breast cancer were recruited between 1975 and 1988 at the Gustave Roussy oncology centre, Villejuif, and 3 controls (patients with malignant haemolymphopathy, colorectal carcinoma, or basal cell carcinoma of the skin) from the registry of the same centre were matched on year of diagnosis and age (5-year group) to each case. In Switzerland, 18 cases were included from the Geneva tumour registry from 1970 to 1986 and 2 male controls (patients with malignant haemolymphopathy or colorectal carcinoma) treated at the same hospital as the case were matched to

each case on year of diagnosis, age, and citizenship. A range of detailed personal information was captured, including occupational exposure at work, via general practitioners, by mail in France and telephone in Switzerland. Information on patient occupation was also captured in registries at both study centres. The total number of controls by cancer site was 91 with colorectal cancer, 91 with haemolymphopathy, and 73 with basal cell carcinomas. Analysis estimated ORs (and 95% CIs) considering the matched design. An OR of 1.3 (95% CI, 0.5–3.7) for occupational exposure to gasoline and grease was reported. [The Working Group noted that the use of other cancer controls may result in a conservative study design, potentially biasing ORs towards the null. Information on occupational exposures only in the most recent job was captured via general practitioners of patients, not the patients themselves, and the extent of misclassification was difficult to ascertain. There was also a lack of information on participation rates in the published work. Information on gasoline and grease exposure was nonspecific. Finally, there was a small number of included cases, and a small number of these were exposed in their current job.]

A multicountry case-control study on rare cancers that was conducted in eight countries in Europe included 104 cases of male breast cancer and 1901 largely population-based controls, although patients with other cancers were also included ([Laouali et al., 2018](#)). Recruitment followed population-based methods in five countries (Denmark, France, Germany, Italy, and Sweden), and hospital-based methods in three countries (Latvia, Portugal, and Spain) (methodological details were described by [Lynge et al., 2005](#), and [Villeneuve et al., 2010](#)). Cases were aged 35–70 years, diagnosed in 1995–1997 in the study areas, and recruited through contact with clinical and pathology departments. As the study focused on occupational exposures, older participants were excluded because of potential difficulties in recall and exposure



assessment for earlier occupations. Population controls were frequency-matched to cases on 5-year age groups, sex, and study area and were selected from population registers, electoral lists, and municipality registers. Hospital controls, comprising patients with colon cancer who were selected because of the lack of previously identified associations between this cancer type and occupational chemical exposures, were recruited randomly from patients with incident cancers in the three countries. In Portugal, a small number of patients with stomach cancer also served as controls. The overall participation rate for cases was 85% (122 eligible, 104 recruited) and for controls was 67% (2850 eligible, 1901 recruited), and was generally higher for hospital controls than for population-based controls (Lyng et al., 2005; Villeneuve et al., 2010; Laouali et al., 2018). Information on lifetime occupational history and questions related to 27 specific work task modules was obtained during in-person or telephone interviews. In the analysis of JEM-based exposure to gasoline, there were 9 exposed cases (8.6%), and 112 exposed controls (5.8%). A positive OR (OR, 1.9; 95% CI, 0.7–5.0) was observed in the category of highest cumulative exposure score in the analysis of gasoline exposure alone, and this was attenuated in the analysis adjusting for other solvent exposures (OR, 0.7; 95% CI, 0.2–3.0). No association was observed in the analysis with a 10-year lag period. [The Working Group noted the broad geographical scale of the study, the incident-based outcome, and that information captured on lifetime occupational history. Limitations included the modest participation rates for controls, particularly for population controls, and that the controls were typically of higher educational status. The total number of included cases, as well as exposed cases, was small.]

## 2.7 Other solid tumours

Several studies examined associations of occupational or environmental automotive gasoline exposure and other solid tumours (see Table S2.7, Annex 2, Supplementary tables for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>).

### 2.7.1 Cancer of the male genital system

There were five retrospective cohort analyses of incidence of or mortality from cancers of the male genital system and prostate gland among workers in specific occupations or industries, including workers in Europe (Lagorio et al., 1994a; Lyng et al., 1997; Sorahan, 2007), Canada (Schnatter et al., 1993), and the USA (Wong et al., 1999). There was one prospective cohort analysis of prostate cancer incidence among licensed pesticide applicators (Alavanja et al., 2003). There was one study on environmental exposure in a retrospective cohort of residents exposed to gasoline vapour in the USA (Patel et al., 2004). There was one hospital-based case-control study in the USA (Kardaun et al., 1991) and three population-based case-control studies in the USA and in Canada (Siemiatycki et al., 1987a; Van den Eeden et al., 1991; Sauvé et al., 2016), including one population-based multicase case-control study (Siemiatycki et al., 1987a).

There was one retrospective cohort analysis of testicular cancer incidence in Finland (Guo et al., 2005), and two population-based case-control studies of parental occupational exposure and testicular germ cell cancer in male offspring in Europe (Olsson et al., 2018; Guth et al., 2023).

#### (a) Cohort studies

In a cohort of gas station workers in the Nordic countries, there was a total of 16 524 men aged 20–64 years, and a total of 288 533 person-years of follow-up was accrued since 1970–1971 over the 15–20 years of follow-up (Lyng et al.,

[1997](#)) (see Section 2.1.1). There were 127 observed cases of incident prostate cancer and 144.46 expected, and the SIR was 0.9 (95% CI, 0.7–1.0). [The Working Group noted the use of high-quality national registers and incident outcomes. Limitations included the crude exposure assessment, which was based on information on occupation and industry from the 1970 census.]

[Guo et al. \(2005\)](#) used job title data from the 1970 census in Finland and included 667 121 men who reported having an occupation who were followed between 1971 and 1995 (see Section 2.1.1). In total, 387 cases of incident testicular cancer were observed during 19.7 million person-years. Job title data from 1970 was linked to FINJEM to provide estimates of probability and mean level of exposure to a range of chemical and ergonomic agents, including gasoline. Testicular cancer risk was not associated with lower ( $<1$  ppm-year) (SIR, 1.17; 95% CI, 0.77–1.71) or higher ( $\geq 1$  ppm-year) (SIR, 1.19; 95% CI, 0.44–2.59) cumulative gasoline exposure, compared with no exposure, in an analysis with a 10-year lag period. [The Working Group noted the use of high-quality population-based registers. Limitations included the assessment of exposure on the basis of a single census year, leading to nondifferential exposure misclassification.]

A retrospective industry-sponsored cohort mortality study in Canada was conducted among 6672 male workers in petroleum marketing and distribution who had worked for  $\geq 1$  year between 1964 and 1983 ([Schnatter et al., 1993](#)) (see Section 2.1.3). In the SMR analysis for marketing distribution workers exposed to hydrocarbons, there were 19 observed deaths from prostate cancer, with an SMR of 1.17 (95% CI, 0.70–1.82). According to exposure frequency, an SMR of 1.77 (95% CI, 0.92–3.09) was observed among workers with estimated daily exposure assigned by industrial hygienists using work history records, largely inhalation exposure during product transfer, based on 12 deaths from

prostate cancer. [The Working Group noted the assignment of frequency of exposure categories by expert hygienists. Limitations included missing information on work history, the small number of included workers and deaths from prostate cancer, reliance on fatal end-points, and the short cohort follow-up time. Downward bias from the HWE and HWSB may also have been present.]

[Sorahan \(2007\)](#) carried out an industry-sponsored retrospective cohort in the UK of 16 477 petroleum distribution workers with potential exposure to gasoline vapour, followed from 1951 to 2003 for mortality and from 1971 to 2003 for cancer incidence (see Section 2.1.3). For prostate cancer, the authors reported 187 deaths among distribution workers and 162.7 expected, with an SMR of [1.15] (95% CI, [0.99–1.33]). There were 426 cases of prostate cancer and 421.4 expected, with an SRR of [1.01] (95% CI, [0.92–1.11]). For testicular cancer, there were 5 observed deaths among distribution workers, with an SMR of [0.92 (95% CI, 0.30–2.15)]. There were 20 cases among distribution workers, with an SRR of [1.47] (95% CI, 0.90–2.27). [The Working Group noted the crude exposure assessment and multiple co-exposures, as well as potential downward bias from the HWE and HWSB because of restriction to workers employed for  $\geq 12$  months.]

In an industry-funded retrospective cohort in the USA of 18 135 primarily White male marketing and marine petroleum distribution workers with potential exposure to gasoline vapour, [Wong et al. \(1999\)](#) reported a total of 88 deaths from prostate cancer among petroleum distribution workers, and a precise, inverse SMR of 0.77 (95% CI, 0.62–0.95) (see Section 2.1.3). There were also 2 deaths from cancer of the testes and other urinary organs, with an SMR of 0.33 (95% CI, 0.04–1.19). [The Working Group noted the small number of cancer deaths observed and reliance on fatal outcomes, as well as the potential for downward bias from the HWE.]

In a retrospective cohort of 2665 self-employed filling station attendants in Italy, including 2308 men exposed to gasoline vapour, [Lagorio et al. \(1994a\)](#) reported a total of 2 deaths from prostate cancer and an SMR of [0.38] (95% CI, [0.05–1.36]) (see Section 2.1.4). [The Working Group noted that exposure to gasoline was probably high among service station attendants in Italy because refuelling activities were common practice at the time of the study. Limitations included the small cohort size and number of prostate cancer deaths observed, leading to imprecise estimates, the crude exposure assessment, and reliance on fatal end-points.]

In the United States Agricultural Health Study (AHS), prostate cancer risk was evaluated in association with gasoline exposure among pesticide applicators in nonfarm jobs (see Section 2.1.5) ([Alavanja et al., 2003](#)). The OR for prostate cancer risk for ever exposure to gasoline was 0.53 (95% CI, 0.3–0.8). [The Working Group noted the prospective design and analysis of incident prostate cancer outcomes as strengths of this study. Limitations included the short follow-up time (average follow-up, 4.3 years), crude exposure assessment, that gasoline exposure was ascertained for nonfarm jobs in a subset of the main cohort, and the small number of exposed cases ( $n = 24$ ).]

In a study of residents with presumed community exposure to gasoline vapour because of groundwater contamination via leaking underground petroleum storage tanks, [Pate et al. \(2004\)](#) compiled a retrospective cohort of 663 people living for  $\geq 6$  months in 275 households between 1990 and 2000 who had chronic low-level exposures (see Section 2.1.5). Overall, there was a total of 9 cases of incident prostate cancer observed, with 7.12 expected. The SIR was 1.26 (95% CI, 0.58–2.40). [The Working Group noted the unique aspects of this study examining potential exposure to gasoline vapour in a community setting. Limitations included the lack of refined exposure assessment information, with exposure

status being based on the definition of a remediation area. The use of incident cancer cases rather than mortality was a strength, although few prostate cancer cases were observed.]

#### (b) Case-control studies

[Siemiatycki et al. \(1987a\)](#) conducted a population-based case-control study on 20 cancer types among men aged 35–70 years in Montreal, Canada, including 452 cases of prostate cancer and a reference group formed of 1733 cases of other cancers among the 3726 patients recruited. Overall, there were 56 prostate cancer cases with exposure to automotive gasoline (see Section 2.1.10). The Mantel-Haenszel OR for all participants for any versus never exposure to automotive gasoline, with five stratifying variables of age, socioeconomic status, ethnic group, cigarette smoking, and blue-or white-collar job industry, was 1.0 [95% CI, 0.8–1.3]. [The Working Group noted the detailed exposure assessment performed, as well as the conservative approach to analysis that used other cancer cases as controls. Limitations included the small numbers of exposed cases of prostate cancer and potential for co-exposures in those with the most exposure to gasoline.]

In a case-control study of 223 cases and 212 controls in the Washington, District of Columbia area, USA, [Kardaun et al. \(1991\)](#) assessed parental occupational exposure in the 12 months before birth and risk of testicular cancer among offspring. The cases were living, aged 18–42 years, and diagnosed between 1976 and 1981 at three participating hospitals. The controls consisted of men with other cancers (non-genital tract) recruited at the same hospital as the cases. In-person and telephone interviews were conducted with 88% of cases and 90% of controls, and queried father's usual occupation during childhood. In addition, telephone interviews were performed with 84% of case mothers and 82% of control mothers in order to capture detailed job histories of the father and mother

during the prenatal period. There were no clear associations observed with most occupations. There was a positive, although imprecise, association for fathers working in automobile service stations (OR, 4.0; 95% CI, 0.6–24.5) compared with all other occupations, and seminoma, based on 3 exposed cases. [The Working Group noted the small number of exposed cases, and the potential for selection and recall bias, and for some exposure misclassification because of the use of job title.]

In a population-based case-control study in Western Washington State, USA, [Van den Eeden et al. \(1991\)](#) included 323 cases of primary testicular germ cell tumour among noncryptorchid White men and 658 controls, assessing longest held occupation and occupation ever worked for  $\geq 6$  months in the previous 10 years. The cases were living, aged 20–69 years, and diagnosed between 1977 and 1984, from the tumour registry. Of the 535 eligible cases, 10.4% were already deceased. The controls were identified via random-digit dialling among individuals of the same age, race, and county as the cases; 66.1% of the controls were interviewed. Study participants were typically aged below 55 years. The longest-worked occupation for 0.3% of the cases was service station attendant/helper, and 2.2% of the cases had ever worked in this occupation. There were no clear associations observed with either category (RRs, 1.0; 95% CI, 0.1–10.2; and 1.6, 95% CI, 0.6–4.3; respectively). [The Working Group noted the small number of exposed cases, the crude exposure assessment based on job title, and the relatively low participation rates, particularly among controls.]

In a population-based case-control study in Montreal, Canada, [Sauvé et al. \(2016\)](#) evaluated associations with lifetime occupation and industry for 1937 cases of prostate cancer and 1994 population controls. The cases were aged  $\leq 75$  years and were diagnosed with incident disease at French-speaking hospitals in Montreal (representing 80% of all new cases)

between September 2005 and December 2009. The controls were recruited from electoral lists and were frequency-matched on 5-year age group. Participation rates were 79.4% for cases and 55.5% for controls. Detailed information on lifetime occupational history for each job held for  $\geq 1$  year was obtained from in-person interviews. In the analyses, associations were assessed for 696 occupations and 613 industries, with the reference category for each analysis comprising people who had never been employed in the occupation or industry of interest. Analysis evaluated total and grade-specific prostate cancer incidence, using multinomial logistic regression models. Semi-Bayes adjustment was used in an attempt to account for multiple comparisons. More than 76% of control participants reported having been screened by prostate-specific antigen and/or digital rectal examination in the 2 years before the date of diagnosis or the reference date. During the period 1943–2012, a total of 19 373 unique jobs were reported, and the mean number of jobs per participant was 4.9. A significant positive association was observed for total prostate cancer incidence among men who had ever worked as a service station attendant (OR, 2.30; 95% CI, 1.04–5.08; 25 exposed cases), and this was attenuated somewhat with semi-Bayes adjustment (OR, 1.67; 95% CI, 0.90–3.09). For high-grade disease (524 cases), associations with ever working as a service station attendant were observed before (OR, 4.35; 95% CI, 1.82–10.44) and after semi-Bayes adjustment (OR, 2.36; 95% CI, 1.23–4.52). [The Working Group noted as a strength that this was a large, population-based study of incident disease. The study was unique in examining risk according to disease grade. There was also an attempt to adjust for multiple comparisons performed. Limitations included the small numbers of exposed prostate cancer cases for some occupations and industries. The response rate for controls was moderate, although there was no evidence of selection bias according to sociodemographic factors.]



In a study in Denmark, [Olsson et al. \(2018\)](#) evaluated parental occupational exposures, including to gasoline and other solvents, and risk of testicular germ cell tumours in sons. Overall, there was a total of 3421 cases aged 14–49 years, diagnosed from 1918–2014 and 14 024 controls matched (5:1) on birth year. Cases were selected from the Danish Cancer Registry and controls from the central population registry. Information on paternal and maternal occupational information was obtained from the Danish Supplementary Pension Fund, with information on company classified into national branch or industry codes, and the Danish version of the Nordic Occupational Cancer Study JEM (based on the FINJEM and complementary measurement data) was used to assign parental exposure estimates in four time periods. Cases and controls were born in Denmark and had at least one parent working until the birth of the child. Exposure was defined as a job in which a proportion of workers regularly inhale the agent at a level exceeding a non-occupational background level. Maternal exposure was considered in the year of the child's birth, and paternal exposure in the year before the child's birth. Conditional logistic regression models were run for maternal and paternal exposure separately, overall and for seminoma (49%) and non-seminoma (51%) separately, and by birth decade. Models were adjusted for any metal exposure. Overall there was no clear association observed for maternal (OR, 1.02; 95% CI, 0.63–1.65; 34 exposed cases) or paternal (OR, 0.95; 95% CI, 0.77–1.17; 222 exposed cases) occupational exposure to “gasoline and other solvents” overall, or by subtype or birth year, with the exception of ORs for paternal exposure of 1.20 (95% CI, 0.91–1.59) for non-seminoma and 0.73 (95% CI, 0.53–0.99) for seminoma ( $P$  for heterogeneity, 0.02). [The Working Group noted that this was a large-scale study based on high-quality registry data. Limitations included the crude and nonspecific nature of the exposure assessment, with assessment of “gasoline and other solvents”

and small numbers of exposed cases. Strong correlations were observed between paternal occupational exposure to gasoline and a range of other solvents, including benzene ( $r=0.86$ ), trichloroethylene ( $r=0.75$ ), and toluene ( $r=0.70$ ), for example. Similar correlations, although more moderate, were observed for maternal occupational exposure to gasoline and other solvents, including benzene ( $r=0.67$ ), toluene ( $r=0.53$ ), and trichloroethylene ( $r=0.49$ ).]

In a nationwide multicentre population-based case–control study in France, [Guth et al. \(2023\)](#) examined parental occupational exposure to solvents, including automotive gasoline, and incidence of testicular germ cell cancer. The study included 454 patients (aged 18–45 years) with histologically confirmed testicular germ cell tumours who were referred for sperm cryopreservation from 2015–2018. Both seminoma and non-seminoma tumours were considered. There were 670 controls with no previous history of testicular cancer or cryptorchidism who were frequency-matched on 5-year age group and hospital centre region (2:1) in two groups: (i) partners of women consulting for fertility disorders with normal sperm production (group A); and (ii) partners of women treated for a pathological pregnancy in specialized maternity clinics (group B). The response rate overall was 93.4%. Participants were interviewed by telephone to capture paternal and maternal occupation and industry at year of birth, and a French JEM, based on the Matgéné programme, was applied to assign exposure to a range of solvents, including fuels and petroleum-based solvents. Overall, 5.8% of case fathers and 4.8% in group A of controls and 8.1% in group B of control fathers were exposed to automotive gasoline. Paternal exposure to automotive gasoline was correlated with exposure to ethylene glycol (Spearman correlation coefficient,  $r=0.59$ ) benzene ( $r=0.56$ ), and kerosene-diesel oil/fuel oil ( $r=0.51$ ). No association was observed with ever exposure and risk of all testicular germ cell tumours (OR, 0.74; 95% CI,

0.43–1.28; 24 exposed case fathers) or of non-seminoma or seminoma specifically, compared with fathers who had never been exposed to automotive gasoline. There was also no association with high exposure to automotive gasoline. An insufficient number of mothers exposed to automotive gasoline was available for an analysis to be performed. In an analysis using solvent principal components for fuels and petroleum-based solvents, no associations were observed. For men born in the 1970s, there was a strong positive association with maternal occupational exposure to the more general category of fuels and petroleum-based solvents in the period 1969–1980 (OR, 2.74; 95% CI, 1.11–6.76; 14 exposed case mothers), although exposure to automotive gasoline specifically was not evaluated. [The Working Group noted the large, nationwide, population-based study design, availability of information on both paternal and maternal occupation, the national JEM, information on disease subtypes, information on a range of potential confounding variables, and the high response rates. Limitations included the small number of exposed parents, the non-gender-specific JEM, and lack of information on specific tasks or potential changes in tasks during the preconception period.]

### 2.7.2 Cancer of the female genital system

There were two retrospective cohort studies in Europe on associations with incident ([Lynge et al., 1997](#)) or fatal ([Lagorio et al., 1994a](#)) female genital cancers among workers in specific occupations or industries, and one registry-based study using exposure estimates from a JEM ([Vasama-Neuvonen et al., 1999](#)). There was also one study on environmental exposure in a retrospective cohort of residents exposed to gasoline vapour in the USA ([Patel et al., 2004](#)).

### Cohort studies

In a study in a cohort of gas station workers in the Nordic countries, 2445 women were included with 41 889 person-years of follow-up accrued since 1970–1971 over the 15–20-year follow-up period ([Lynge et al., 1997](#)) (see Section 2.1.1). A total of 17, 12, and 7 cases of incident cancer of the cervix uteri, corpus uteri, and ovary, respectively, were observed, with SIRs of 1.3 (95% CI, 0.8–2.1), 1.0 (95% CI, 0.5–1.8), and 0.6 (95% CI, 0.2–1.1). [The Working Group noted the use of high-quality registers encompassing national populations of four Nordic countries, and the use of incident rather than mortality outcomes. Limitations included the crude exposure assessment that was based on occupation and industry from a single time point (the 1970 census) and the lack of information on lifestyle and reproductive factors.]

[Vasama-Neuvonen et al. \(1999\)](#) used job title data from the 1970 census in Finland, including 892 591 women who reported having an occupation and who were followed during 1971–1995 (see Section 2.1.1). In total, there were 15 481 680 person-years of follow-up and 5072 observed cases of incident ovarian cancer. Job title data from 1970 was linked to FINJEM to provide estimates of probability and mean level of exposure to a range of chemical and ergonomic agents, including gasoline. There was no excess incidence of ovarian cancer in women in jobs with exposure to gasoline (defined as jobs whereby  $\geq 20\%$  of women were exposed) (SIR, 0.8; 95% CI, 0.2–3.4) compared with women working in jobs with a lower proportion of exposed ( $< 20\%$ ), or in women with a low (SIR, 0.8; 95% CI, 0.4–1.8) or medium/high (SIR, 0.8; 95% CI, 0.2–3.4) ( $P$  for trend, 0.63) mean level of exposure compared with those with no exposure. [The Working Group noted the overlap with the study by Lynge et al., but considered the use of FINJEM instead of service station work to be useful for the present evaluation. Strengths included the



use of high-quality population-based registers and the availability of data on some personal or reproductive factors. Limitations included the exposure assessment based on a single census year and a non-gender-specific JEM.]

In a retrospective cohort of 2665 self-employed filling station attendants in Italy that included 357 women who were exposed to gasoline vapour, [Lagorio et al. \(1994a\)](#) (see Section 2.1.4) reported 1 death from cancer of the uterus and an SMR of [1.54] (95% CI, [0.04–8.58]). [The Working Group noted that exposure to gasoline was probably high among service station attendants in Italy as refuelling activities were common practice at the time of the study. A limitation was the small number of included workers and cancer deaths observed, leading to imprecise estimates.]

In a study of residents with presumed community exposure to gasoline vapour because of groundwater contamination from leaking underground petroleum storage tanks, [Patel et al. \(2004\)](#) (see Section 2.1.5) compiled a retrospective cohort of 663 people living for  $\geq 6$  months in 1990–2000 in 275 households with chronic low-level exposures. Overall, there was 1 observed case of incident cervical cancer and 0.38 expected, with an SIR of 2.63 (95% CI, 0.08–14.66). [The Working Group noted the unique aspects of this study on potential exposure to gasoline vapour in a community setting. Limitations included the lack of refined exposure assessment information on personal lifestyle and reproductive factors. Only 1 case of cervical cancer was observed, which was noted as a considerable limitation.]

### 2.7.3 Melanoma of the skin

There was one retrospective cohort study in Canada ([Schnatter et al., 1993](#)) and three retrospective cohort studies in Europe in which associations were examined with either incident or fatal melanoma among workers in specific

occupations or industries ([Lagorio et al., 1994a](#); [Lyngge et al., 1997](#); [Sorahan, 2007](#)). There was one multicase–control study on incident cutaneous melanoma in Canada ([Fritschi and Siemiatycki, 1996](#)).

#### (a) Cohort studies

In a cohort of gas station workers in the Nordic countries ([Lyngge et al., 1997](#)) (see Section 2.1.1), there was a total of 33 cases of incident melanoma among men and 42.82 expected, with an SIR of 0.8 (95% CI, 0.5–1.1). Among women, there were 6 cases of incident melanoma and 7.11 expected, with an SIR of 0.8 (95% CI, 0.3–1.8). [The Working Group noted the use of high-quality national registers and incident outcomes. Limitations included the crude exposure assessment, based on occupation and industry from the 1970 census, and lack of information on solar UV exposure.]

In an industry-sponsored retrospective cohort mortality study of 6672 male petroleum marketing and distribution workers in Canada, [Schnatter et al. \(1993\)](#) (see Section 2.1.3) reported an SMR of 2.49 (95% CI, 0.67–6.36) for melanoma on the basis of 4 observed deaths among marketing distribution workers exposed to hydrocarbons. In an analysis based on categories of exposure frequency, findings were imprecise. [The Working Group noted limitations including missing work history information, the small number of included workers and melanoma deaths, reliance on fatal end-points, and the short cohort follow-up time. Some downward bias from the HWE and HWSB because of restriction to workers employed for  $\geq 12$  months may be present.]

In an industry-sponsored retrospective cohort in the UK of 28 555 oil refinery workers and 16 477 petroleum distribution workers with potential exposure to gasoline vapour, who were followed from 1951 to 2003 for mortality and from 1971 to 2003 for cancer incidence, [Sorahan \(2007\)](#) (see Section 2.1.3) reported

15 deaths from melanoma and 17.6 expected among distribution workers (SMR, [0.85]; 95% [0.48–1.41]). For cancer incidence, there were 48 cases of melanoma and 40.2 expected (SRR, [1.19]; 95% CI, [0.88–1.58]). [The Working Group noted the small number of melanoma cases and deaths, as well as the crude exposure assessment and multiple co-exposures. Some downward bias from the HWE and HWSB because of restriction to workers employed for  $\geq 12$  months may be present.]

In a retrospective cohort of 2665 self-employed filling station attendants in Italy, including of 2308 men and 357 women, exposed to gasoline vapour, [Lagorio et al. \(1994a\)](#) (see Section 2.1.3) reported one death from melanoma and an SMR of [1.23] (95% CI, [0.03–6.85]). [The Working Group noted that exposure to gasoline was probably high among service station attendants in Italy as refuelling activities were common practice at the time of the study. A limitation noted was the small number of included workers and cancer deaths observed, and lack of information on solar UV exposure.]

#### (b) Case-control studies

[Fritschi and Siemiatycki \(1996\)](#), in a multi-case-control study in Montreal, examined 103 cases of cutaneous melanoma among men, a control group of 533 cases of other cancers, and 533 population controls (see Section 2.1.10). There were 6 cases exposed to leaded petrol, and findings in multivariable analyses were reported as finding no association, without reporting any effect estimates. [The Working Group noted the detailed exposure assessment performed overall and analysis of incident cancer cases. Limitations included the small numbers of included and exposed cases of cutaneous melanoma. Information on adult solar UV exposure was not captured.]

### 2.7.4 Cancer of the nervous system and thyroid gland

There were eight retrospective cohort analyses of incidence or mortality of cancers of the nervous system, thyroid gland, or other solid tumour sites among workers in specific occupations or industries in Europe and North America ([Preston-Martin, 1989](#); [Schnatter et al., 1993](#); [Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Wong et al., 1999](#); [Navas-Acién et al., 2002](#); [Wesseling et al., 2002](#); [Sorahan, 2007](#)). There was also one study on environmental exposure in a retrospective cohort of residents exposed to gasoline vapour in the USA ([Patel et al., 2004](#)). There was one hospital-based case-control study in China ([Hu et al., 1998](#)) and a multicentre hospital-based case-control study in the USA ([Zheng et al., 2001](#)). There was one population-based case-control study in the USA ([De Roos et al., 2003](#); [Rajaraman et al., 2004](#)) and another multi-country population-based case-control study ([McLean et al., 2014](#); [Benke et al., 2017](#)).

#### (a) Cohort studies

In a study in a cohort of gas station workers in the Nordic countries, there were a total of 16 524 men aged 20–64 years, with a total of 288 533 person-years of follow-up accrued since 1970–1971 over the 15–20 year follow-up time, and 2445 women with 41 889 person-years of follow-up ([Lynge et al., 1997](#)) (see Section 2.1.1). SIRs of 1.0 or below were reported in both men and women for cancers of the thyroid and brain. [The Working Group noted the use of high-quality national registers and the use of incident outcomes. Limitations included the crude exposure assessment from a single census year (1970).]

[Navas-Acién et al. \(2002\)](#) used job title data from the 1960 and 1970 censuses in Sweden to evaluate potential associations for risk of glioma and meningioma during 1971–1989 (see Section 2.1.1). In total, 1 779 646 men aged 25–64 years were included in the study. There

were 3363 gliomas and 1166 meningiomas observed in men. The overall cohort included both men and women, although the analysis for glioma and meningioma was reported only for men because of the small number of observed cases in women. Adjusted Poisson regression models were used to estimate associations between occupation and risk of glioma and meningioma in men. In men with the occupation of filling station attendant in the 1970 census, in comparison with all other occupations, 15 cases of glioma were observed, with 12.1 expected (RR, 1.25; 95% CI, 0.75–2.08). Three men reported this sector and occupational code in both the 1960 and 1970 censuses (RR, 0.73; 95% CI, 0.24–2.27). [The Working Group noted the use of high-quality national population-based registers, incident outcomes, and a long follow-up period. Limitations included the exposure assessment based on one or two census years and the imprecise findings.]

[Wesseling et al. \(2002\)](#) used job title data from the 1970 census in Finland, including 413 877 women who reported having a blue-collar occupation who were followed between 1971 and 1995 (see Section 2.1.1). In total there were 6 905 913 person-years of follow-up and 693 cases of incident cancer of the brain and nervous system (meningioma, 43%; glioma, 29%; other cancer types, 28%). Job title data from 1970 were linked to FINJEM to provide estimates of probability and mean level of exposure to a range of chemical and ergonomic agents, including gasoline, as well as the product of probability and mean level. In adjusted Poisson regression models, there was no clear association for low exposure, compared with never exposure, to occupational exposure to gasoline and brain cancer incidence (SIR, 1.47; 95% CI, 0.47–4.56). [The Working Group noted the use of high-quality population-based registers, incident outcomes, and a long follow-up period. Limitations included the exposure assessment based on a single census year, the non-gender-specific JEM, participants

with low-level exposure only, imprecise findings, and that brain tumour subtypes were not examined separately.]

[Preston-Martin \(1989\)](#) examined occupation and industry groups among all male cases (White, aged 25–64 years) of primary tumours of the brain, cranial nerves, and meninges in Los Angeles County, USA, in 1972–1985 based on the Cancer Surveillance Program register. Proportionate incidence ratios (PIRs) were calculated, the expected number being based on rates per 10-year age groups according to the total population of the USA. Overall, there was 1 case of glioma among garage and gas station attendants and a PIR of [0.719], and there were 2 cases of meningioma and a PIR of [6.295] ( $P \leq 0.05$ ). [The Working Group noted the broad nature of the occupational data, captured at time of diagnosis only, although garage work was considered to carry modest co-exposures. There were few numbers of exposed cancer cases, and multiple comparisons were performed.]

In an industry-sponsored retrospective cohort mortality study of 6672 male petroleum marketing and distribution workers in Canada, [Schnatter et al. \(1993\)](#) (see Section 2.1.3) reported an SMR of 0.83 [95% CI, 0.22–2.12] for brain cancer on the basis of 4 observed deaths among marketing distribution workers exposed to hydrocarbons. [The Working Group noted that the limitations of the study included missing work history information, the small number of included workers and deaths from brain cancer, reliance on fatal end-points, and the short cohort follow-up time. Some downward bias from the HWE and HWSB because of restriction to workers employed for  $\geq 12$  months may have been present.]

In an industry-sponsored retrospective cohort in the UK that included 16 477 petroleum distribution workers with potential exposure to gasoline vapour, who were followed from 1951 to 2003 for mortality and from 1971 to 2003 for cancer incidence, [Sorahan \(2007\)](#) (see

Section 2.1.3) reported 45 deaths from brain cancer and 56.8 expected deaths among distribution workers, SMR, [0.79] (95% CI, [0.58–1.06]). For cancer incidence, there were 46 cases of brain cancer and 54.4 expected cases, with an SRR of [0.85] (95% CI, [0.62–1.13]) among distribution workers. [The Working Group noted the small number of brain cancer cases and deaths, as well as the crude exposure assessment and multiple co-exposures. Some downward bias from the HWE and HWSB because of restriction to workers employed for  $\geq 12$  months may have been present.]

In an industry-funded retrospective cohort in the USA of 18 135 primarily White male marketing and marine distribution workers with potential exposure to gasoline vapour, [Wong et al. \(1999\)](#) (see Section 2.1.3) reported a total of 26 deaths from cancer of the brain and CNS among petroleum distribution workers, and an SMR of 0.76 (95% CI, 0.50–1.11). There were also 2 deaths from cancer of the thyroid and other endocrine glands, with an SMR of 0.80 (95% CI, 0.10–2.90). [The Working Group noted the small number of cancer deaths observed, reliance on fatal outcomes, and the broad nature of the outcome classification.]

In a retrospective cohort in Italy of 2665 self-employed filling station attendants (2308 men and 357 women) who were exposed to gasoline vapour, [Lagorio et al. \(1994a\)](#) (see Section 2.1.4) reported a total of 6 deaths from cancer of the nervous system and an SMR of [2.14] (95% CI, [0.79–4.66]). [The Working Group noted the small number of workers included and cancer deaths observed, limitations in the exposure assessment, and the broad outcome classification.]

In a study of residents with presumed community exposure to gasoline vapour because of groundwater contamination from leaking underground petroleum storage tanks, [Patel et al. \(2004\)](#) (see Section 2.1.3) compiled a retrospective cohort of 663 people living for  $\geq 6$  months in

275 households with chronic low-level exposures in 1990–2000. Overall, there were 2 observed cases of incident brain cancer, and 0.55 expected. The SIR was 3.64 (95% CI, 0.44–13.13). [The Working Group noted the unique aspects of this study on potential exposure to gasoline vapour in a community setting. Limitations included the lack of refined exposure assessment information. There were few observed cases of brain cancer, leading to imprecision in the estimates.]

#### (b) Case-control studies

[Hu et al. \(1998, 1999\)](#) conducted a hospital-based case-control study on incident glioma and meningioma in 1989–1996 in China that included 218 cases of glioma and 436 controls, and 183 cases of meningioma and 366 controls. Cases presenting for surgery were recruited from six hospitals. Two controls were matched to each case on sex, age, and area of residence; the controls were patients diagnosed with non-neoplastic and non-neurological disease from hospital surgical departments, most commonly for general surgery. Information regarding occupational exposures was captured in an in-hospital interview. Response rates were 100%. In total, there were 6 cases of glioma in patients with occupational exposure to petrol or diesel oil (adjusted OR, 1.65; 95% CI, 0.45–6.06), and 8 exposed cases of meningioma in men (OR, 2.67; 95% CI, 0.67–10.59). [The Working Group noted the small number of exposed cancer cases and imprecise findings, as well as the crude exposure assessment. The high response rate was noted as a strength.]

In a population-based case-control study on 375 cases of incident brain glioma and 2434 controls in Iowa, USA, [Zheng et al. \(2001\)](#) reported a positive association between work in the gasoline service station industry and brain glioma among men (OR, 2.4; 95% CI, 0.9–6.3; 6 exposed cases). The association was especially elevated, although imprecise, among men who had worked for  $< 10$  years in the industry (OR,



4.8; 95% CI, 1.2–19.3). In the study, histologically confirmed glioma cases in men aged 40–85 years with no previous cancer were identified through the Iowa state health registry. The case participation rate was 91%. Population controls with no history of cancer, matched on sex and age group, were selected from driver license records (controls aged <65 years) and from US Health Care Financing Administration listings (controls aged ≥65 years). The participation rate was 82% for controls aged <65 years and 80% for controls aged ≥65 years. Detailed occupational information for all jobs held for ≥5 years was captured via postal questionnaires completed by participants or proxies (for cases, 74.4% of respondents were proxies). [The Working Group noted as strengths the high participation rates among cases and controls, and information on lifelong occupational history, as well as other personal factors. Limitations included the large proportion of proxy respondents for cases and the small number of exposed incident cancer cases, leading to imprecise estimates.]

In a multicentre hospital-based case-control study on incident glioma, meningioma, and acoustic neuroma in the USA, 489 cases of glioma, 197 cases of meningioma, 96 cases of acoustic neuroma, and 799 controls were recruited between 1994 and 1998 ([De Roos et al., 2003](#); [Rajaraman et al., 2004](#)). Cases, aged ≥18 years, were recruited from three specialized hospitals and typically interviewed within 1 year of symptom onset. The participation rates were 92% for glioma cases, 94% for meningioma cases, and 93% for acoustic neuroma cases. Control participants were patients admitted to the same hospital as the cases for non-neoplastic conditions including injuries, circulatory system disorders, musculoskeletal disorders, and digestive disorders; they were frequency-matched (1:1) on hospital, sex, race/ethnicity, age, and proximity of residence to the hospital. The participation rate for controls was 86%. Participants completed an in-person interview

that captured information on lifetime occupational history for each job held for ≥6 months, recorded according to occupation and industry codes. Unconditional logistic regression models adjusting for matching factors were used to estimate associations for ever working in an occupation, or ever having worked in the occupation for >5 years, compared with never working in the occupation of interest. Among gas station attendants, there was a reduced incidence of glioma (OR, 0.5; 95% CI, 0.3–0.9) and meningioma (OR, 0.3; 95% CI, 0.1–1.5), although the latter reduction was less precise. In contrast, for acoustic neuroma, there was a positive association with ever working as a gas station attendant (OR, 2.4; 95% CI, 1.0–6.0; 8 exposed cases). Findings were similar, although less precise, when using conditional logistic regression in the analysis (OR, 2.5; 95% CI, 0.8–7.3). [The Working Group noted the incident-cancer-based study and the high participation rates as strengths. Limitations included small numbers of exposed cases, leading to estimate imprecision.]

[McLean et al. \(2014\)](#) investigated associations between occupational exposure to solvents, including gasoline, and the incidence of meningioma in the multicentre case-control INTEROCC study, a substudy of the INTERPHONE study on mobile phone use. The INTEROCC study included 10 study centres in seven countries: Australia, Canada, France, Germany, Israel, New Zealand, and the UK. Cases and controls were recruited between 2000 and 2004, and generally included participants aged 30–59 years, although expanded age criteria were applied in some centres. Case diagnosis was verified histologically or by unequivocal diagnostic imaging. Population controls were randomly selected and matched to cases on year of birth, sex, and study region, according to centre-specific approaches. Participants were interviewed, in some cases using a proxy respondent, and information was captured on lifetime occupational history, including job title, tasks, start and

end dates, company name, and activity for each job held for  $\geq 6$  months. The INTEROCC-JEM, an adaptation of FINJEM ([van Tongeren et al., 2013](#)) was applied to the study data to provide estimates of exposure probability and intensity for gasoline, as well as other specific solvents or groups of solvents. Stratified conditional logistic regression models were used to estimate ORs, with strata for sex, age (5-year groups), country-region, and adjustment for education. In total, there were 1906 cases and 5565 controls. The overall participation rate was 81% for cases and 50% for controls. There were 5 cases of meningioma (0.3%) and 25 controls who were ever exposed ( $\geq 1$  year, 5-year lag, probability  $\geq 25\%$ ) for a mean duration of 4.1 and 4.2 years, respectively. The overall OR for ever exposure to gasoline was 0.73 (95% CI, 0.26–2.01) compared with the reference category of never exposure to any solvent. Participants with an exposure probability of  $\geq 5\%$  but  $< 25\%$  were excluded from the analysis because of uncertain exposure status, as were participants exposed for  $< 1$  year. Sensitivity analysis was performed using different probabilities of exposure and lag periods. [The Working Group noted that this was a large, geographically broad, population-based study that included detailed occupational information and examined a range of solvent exposures, and correlated exposures, and had an incidence-based study design. There were, however, few exposed cases, limiting the informativeness of the study for the present evaluation, and the participation rate among controls was low.]

[Benke et al. \(2017\)](#) investigated associations between occupational exposure to solvents, including gasoline, and glioma incidence in the INTEROCC study (see [McLean et al., 2014](#), above). In total, there were 2000 cases of glioma and 5565 controls. The overall participation rate was 68% for cases and 50% for controls. The OR for ever exposure to gasoline was 0.36 (95% CI, 0.12–1.05) overall – 0.44 (95% CI, 0.12–1.62) for men and 0.24 (95% CI, 0.03–1.84) for women

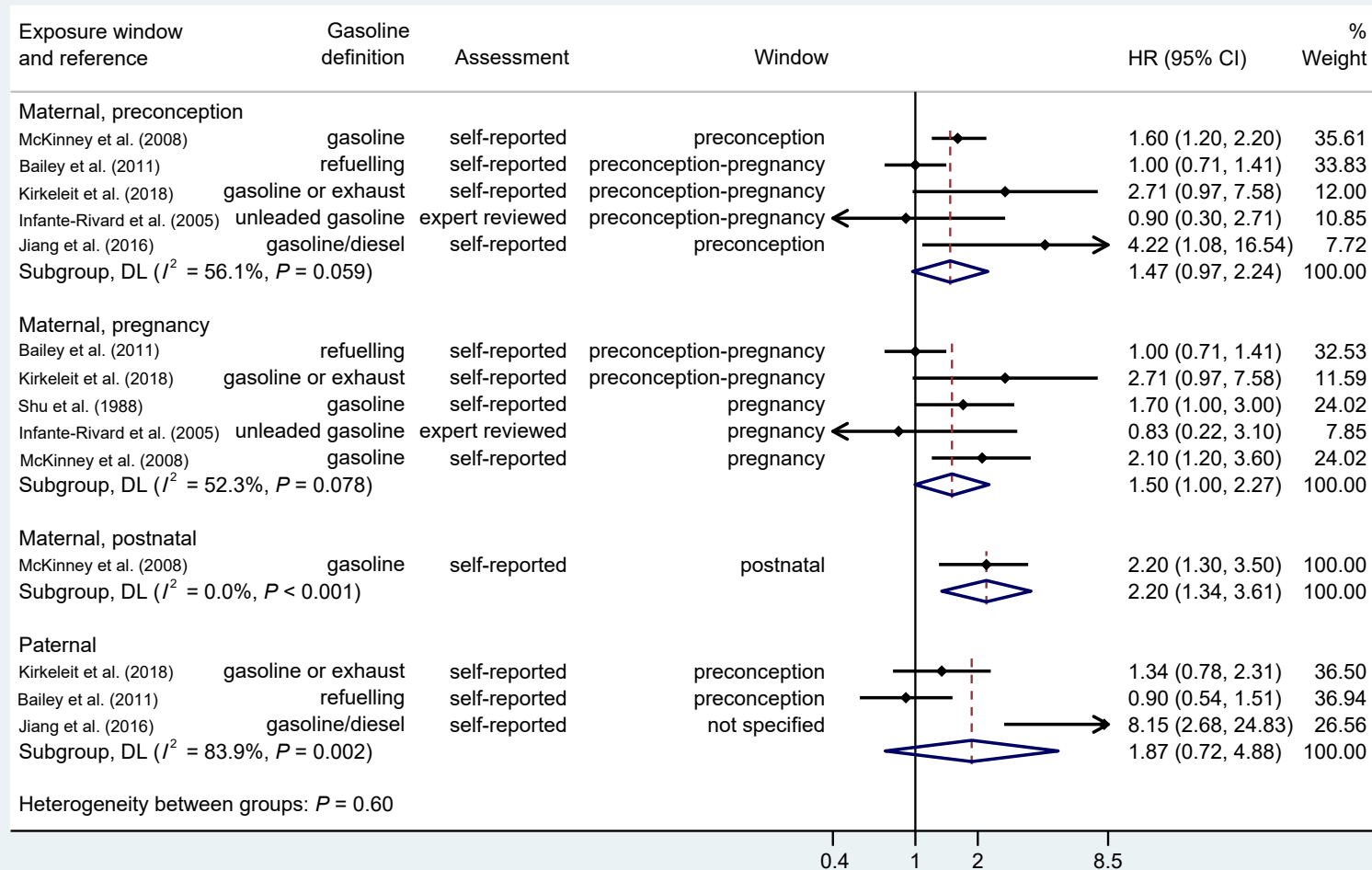
– compared with the reference category of never exposure to any solvent. Participants with an exposure probability of  $\geq 5\%$  but  $< 25\%$  were excluded from the analysis because of uncertain exposure status. Sensitivity analysis was performed using different probabilities of exposure and lag periods. [The Working Group noted as strengths that this was a large, geographically broad, population-based study that included detailed occupational information and examined a range of solvent exposures and correlated exposures, and that it had an incidence-based study design. There were, however, few exposed cases, leading to imprecise estimates, and the low participation rate among controls could have led to selection bias.]

## 2.8 Meta-analyses performed by the Working Group

The Working Group conducted random-effects meta-analyses of epidemiological studies on the association between gasoline exposure and childhood ALL, adult lymphatic and haematopoietic malignancies (NHL, multiple myeloma, CLL, AML and MDS, and Hodgkin lymphoma), and cancers of the kidney, urinary bladder, lung, larynx, nasal cavity, stomach, and female breast.

The results (including  $P$  estimates) for the selected cancer sites are illustrated using forest plots in [Fig. 2.1](#) and Figs S2.2 to S2.5 for childhood ALL; [Fig. 2.6](#) and Fig. S2.7 for AML and MDS; [Fig. 2.8](#) and [Fig. 2.9](#) for NHL; [Fig. 2.10](#) and Fig. S2.11 for CLL; [Fig. 2.12](#) and Fig. S2.13 for multiple myeloma; Fig. S2.14 for Hodgkin lymphoma; [Fig. 2.15](#), [Fig. 2.16](#), Fig. S2.17, Fig. S2.18 for kidney cancer; [Fig. 2.19](#), [Fig. 2.20](#), and Fig. S2.21 for cancer of the urinary bladder; Fig. S2.22 for lung cancer; Fig. S2.23 for nasal cancer; Fig. S2.24 for laryngeal cancer; [Fig. 2.25](#) and [Fig. 2.26](#) for stomach cancer; and Fig. S2.27 for female breast cancer. Results are described and discussed in Section 2.9. The supplementary

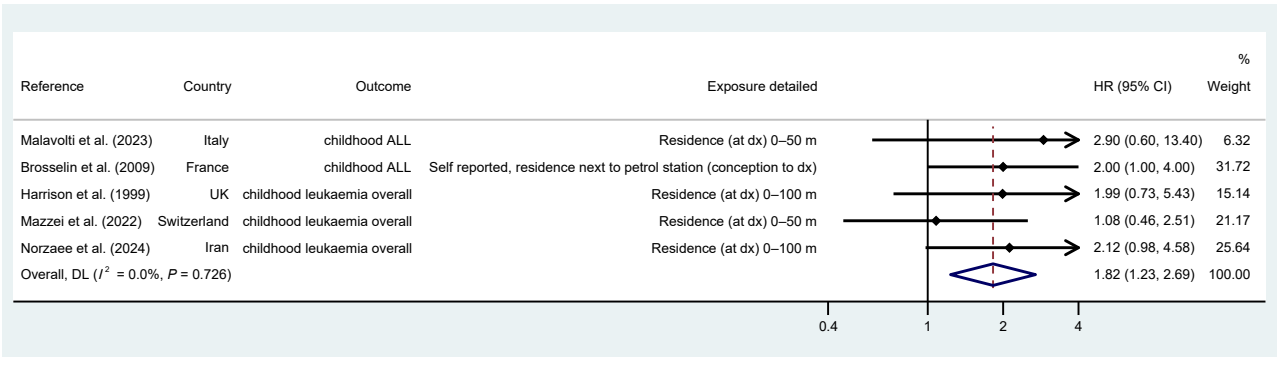


**Fig. 2.1 Meta-analysis for childhood acute lymphoblastic leukaemia (using self-reported exposure in [McKinney et al., 2008](#))**

Weights are from random-effects model. For [McKinney et al. \(2008\)](#), the Working Group used self-reported exposure before expert review. The results from [Kirkeleit et al. \(2018\)](#) and [Bailey et al. \(2011\)](#) are repeated under “Maternal, preconception” and “Maternal, pregnancy” windows because the original estimates were for the entire preconception–pregnancy period.

CI, confidence interval; DL, DerSimonian–Laird estimate of tau<sup>2</sup>; HR, hazard ratio.

**Fig. 2.4 Meta-analysis for childhood acute lymphoblastic leukaemia including only studies evaluating risk by distance from service stations**



Weights are from random-effects model.  
ALL, acute lymphoblastic leukaemia; CI, confidence interval; DL, DerSimonian–Laird estimate of  $\tau^2$ ; dx, diagnosis; HR, hazard ratio; UK, United Kingdom.

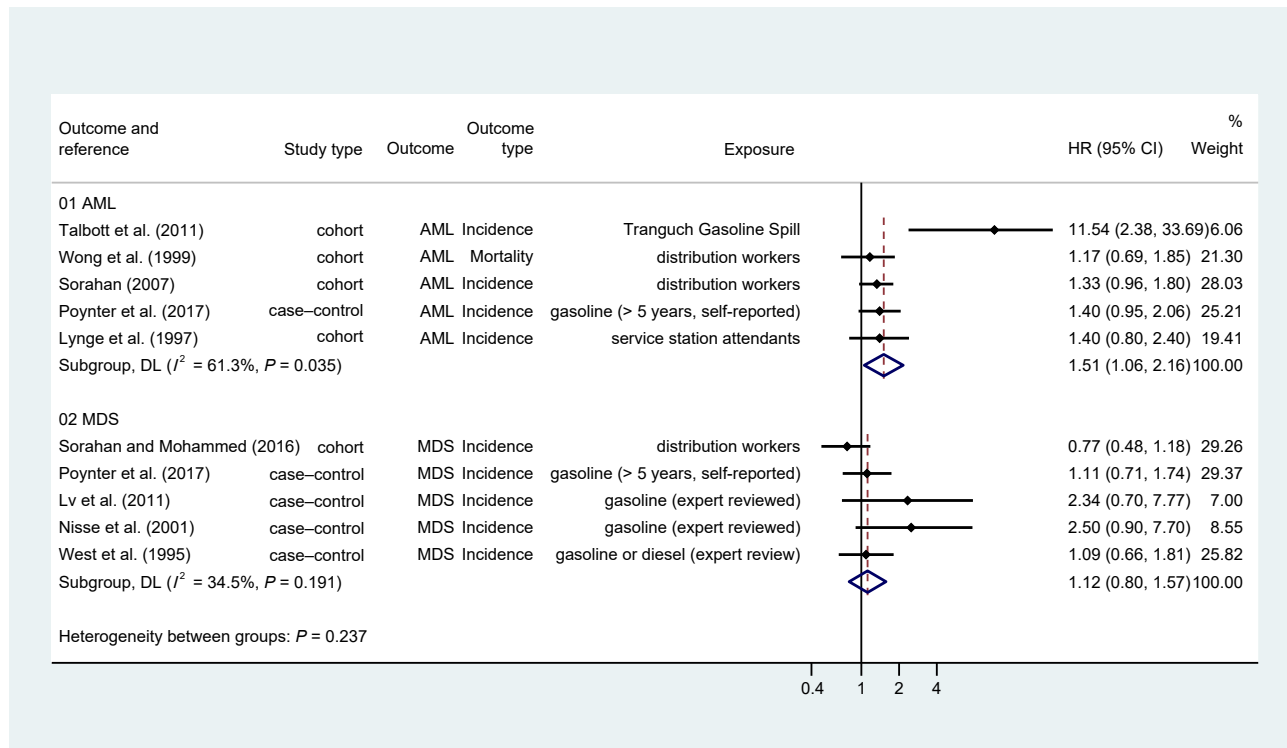
figures are included in Annex 3 (Supplementary figures for Section 2, Cancer in humans, online only, available from: <https://publications.iarc.who.int/652>).

The Working Group meta-analyses were designed to facilitate additional appraisal of the evidence relevant to the present evaluation. For example, for some cancer sites, the meta-analyses included only incidence data (Fig. S2.7, Fig. S2.11, Fig. S2.13, Fig. 2.16, Fig. 2.20, and Fig. 2.26). As mortality studies were noted to be particularly prone to healthy-worker biases, incidence-only meta-analyses were considered to allow exploration of healthy-worker bias effects. Sensitivity analyses were done by excluding studies considered to be less informative, such as the study by Harrison et al. (1999), who used patients with other cancers as controls, and that by Brosselin et al. (2009), because of the crude measurement of distance from petrol station, in the meta-analysis for risk of childhood leukaemia in relation to distance to petrol station (Fig. S2.5). To indirectly assess the effect of confounding by smoking on the meta-estimate for cancer of the urinary bladder, the Working Group compared the bladder cancer meta-estimates with the meta-estimate for lung cancer, including only studies that reported on both cancer sites (Fig. S2.22).

Where possible, analyses were stratified on the basis of the exposure group (estimated gasoline exposure if exposure was based on self-reported measurement, expert review, or JEM; occupation-based, or environment-based).

To minimize the possibility of individuals being counted twice in a meta-analysis, study results known to be from overlapping populations were not pooled, or the most relevant result was chosen: incidence results were selected before mortality results (e.g. in Sorahan, 2007); results for estimated gasoline exposure were selected before those for broad occupational categories (e.g. in Dryver et al., 2004); and, when ever-versus-never analysis was not reported, the results for the highest exposure category were used (e.g. Poynter et al., 2017).

Funnel plots were produced for end-points for which at least five studies were included, to allow visual inspection of potential reporting or publication biases. Funnel plots for childhood ALL, adult lymphatic and haematopoietic malignancies, and kidney, urinary bladder, lung, laryngeal, nasal, stomach, and female breast cancers for studies on ever-versus-never exposure to gasoline (including occupations used as proxies) are included in Annex 3 (Figs S2.28–S2.40; available from: <https://publications.iarc.who.int/652>).

**Fig. 2.6 Meta-analysis for adult acute myeloid leukaemia or myelodysplastic syndrome**

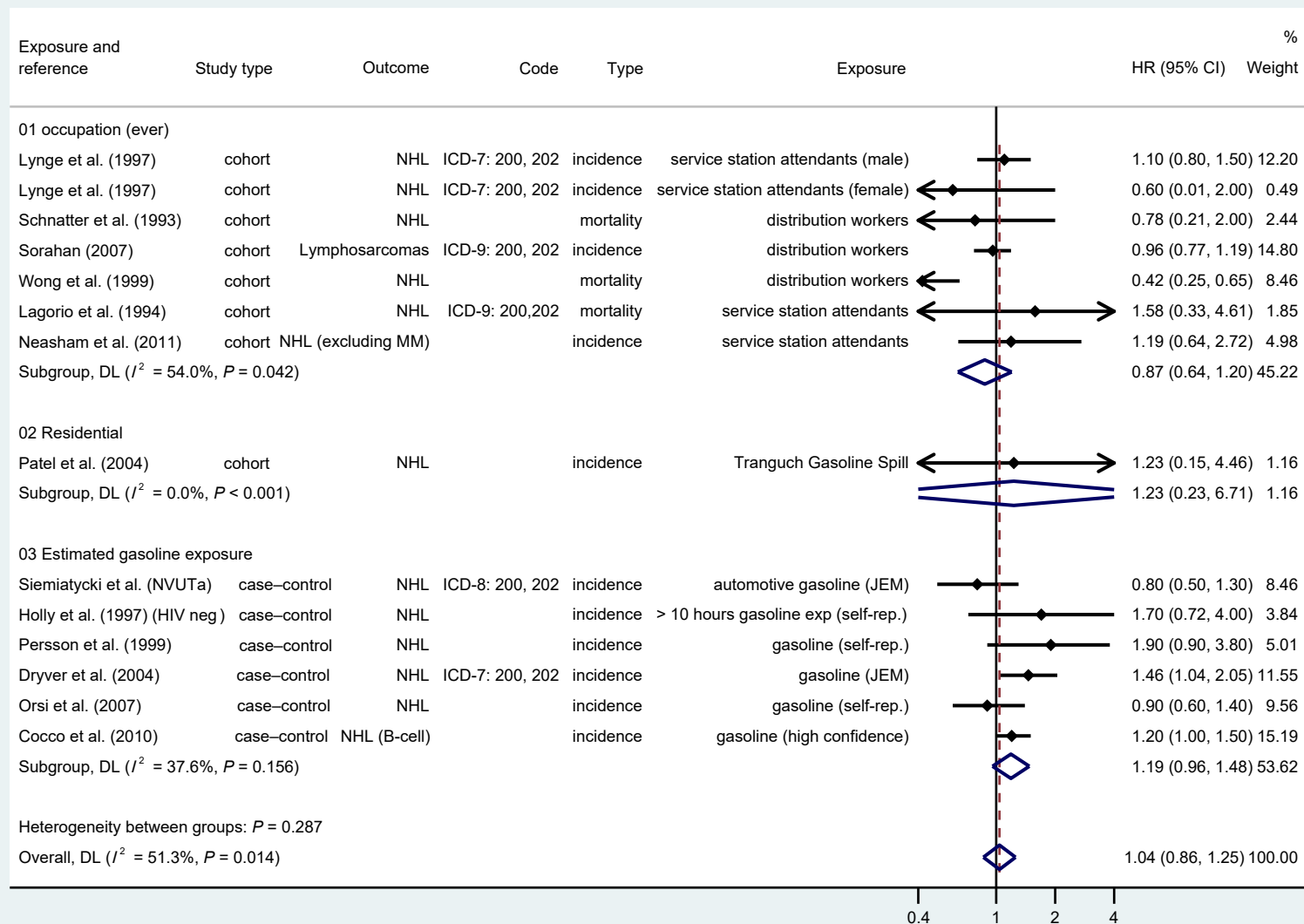
Weights are from random-effects model. For [Sorahan and Mohammed \(2016\)](#), the incidence of myeloid leukaemia, which includes AML, was used, in the absence of an estimation for AML incidence.

AML, acute myeloid leukaemia; CI, confidence interval; DL, DerSimonian–Laird estimate of  $\tau^2$ ; HR, hazard ratio; MDS, myelodysplastic syndrome.

[The Working Group did not observe any major indication of publication bias within the funnel plots for any of the cancer outcomes explored.]

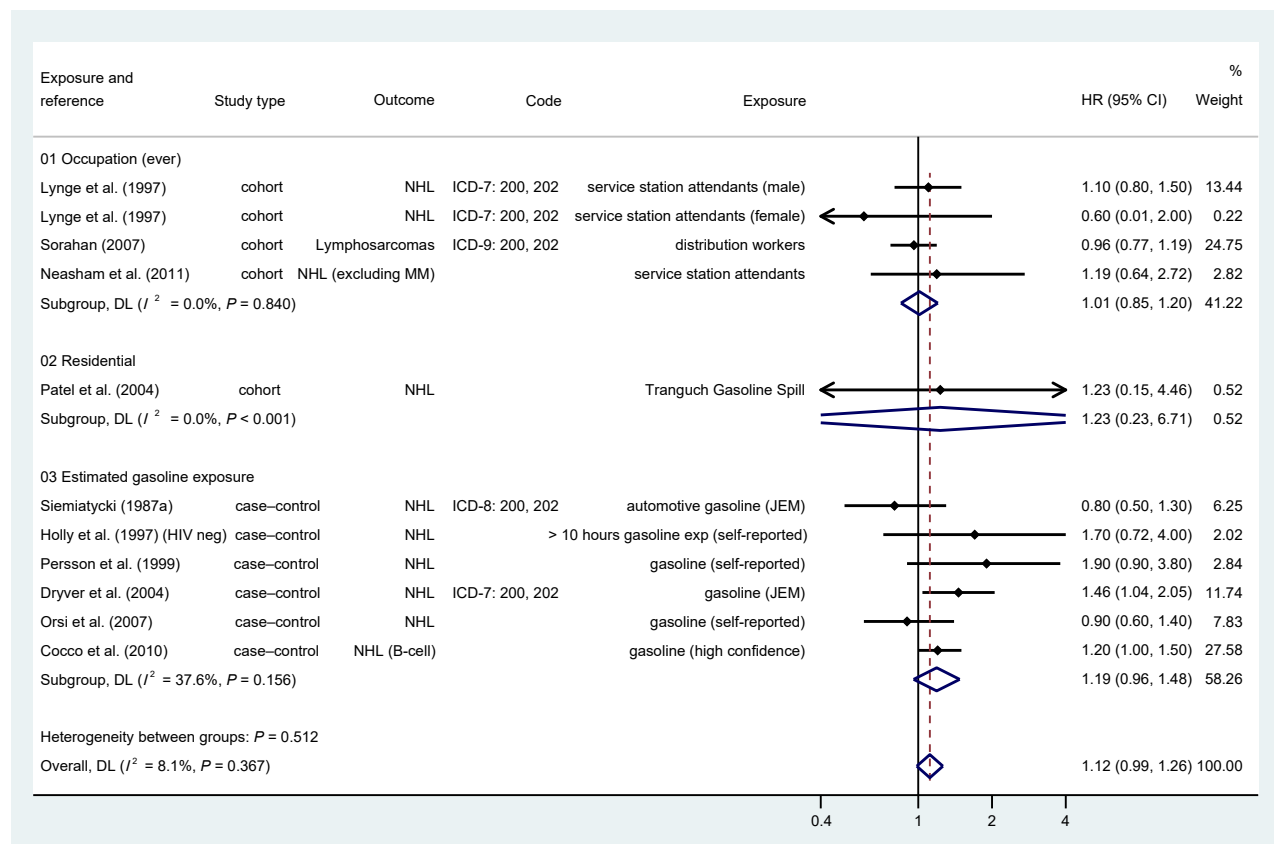
The meta-estimate for childhood ALL was calculated separated by time window and parental type of exposure (maternal exposure during preconception, pregnancy, or postnatally; and paternal exposure during preconception). There were three studies that provided an estimation for maternal exposure covering the preconception and pregnancy windows ([Bailey et al., 2011](#); [Kirkeleit et al., 2018](#); and [Infante-Rivard et al., 2005](#), which provided, additionally, a separate estimation for maternal exposure during pregnancy). These studies were included in both the maternal preconception and pregnancy windows ([Fig. 2.1](#), Fig. S2.2, Fig. S2.3). For the study by [McKinney et al. \(2008\)](#), who presented

the estimation for self-reported exposure before and after review by an industrial hygienist, in its main analysis the Working Group used the self-reported exposure before industrial hygienist review ([Fig. 2.1](#), Fig. S2.3), as the industrial hygienist review excluded partially exposed individuals which was expected to lead to bias towards the null, and in the sensitivity analysis the Working Group used the self-reported exposure after the industrial hygienist had reviewed exposure (Fig. S2.2), because of concerns about differential misclassification from self-report (see Section 2.2 and Section 1.6.1).

**Fig. 2.8 Meta-analysis for adult non-Hodgkin lymphoma**

Weights are from random-effects model. For [Cocco et al. \(2010\)](#), exposure characterized as “high confidence” was used. Multiple myeloma cases are excluded from the NHL definition for Lynge, Sorahan, Lagorio, Neasham, Siemiatycki, Dryver, and Cocco. For the others, inclusion of multiple myeloma cases in the NHL estimation cannot be excluded.

CI, confidence interval; DL, DerSimonian–Laird estimate of  $\tau^2$ ; HR, hazard ratio; ICD, International Classification of Diseases; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

**Fig. 2.9 Meta-analysis for non-Hodgkin lymphoma (incidence only)**

Weights are from random-effects model. For [Cocco et al. \(2010\)](#), exposure characterized as “high confidence” was used.

CI, confidence interval; DL, DerSimonian–Laird estimate of  $\tau^2$ ; HR, hazard ratio; ICD, International Classification of Diseases; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

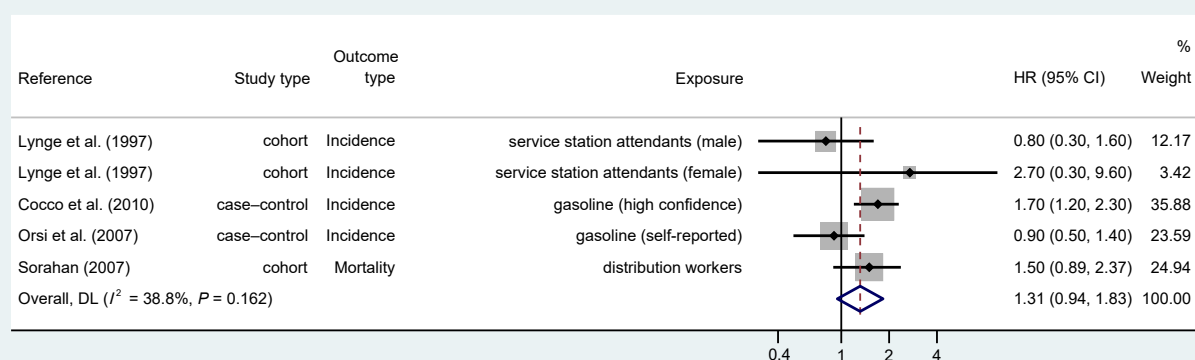
## 2.9 Evidence synthesis for cancer in humans

This section contains a synthesis of epidemiological studies on exposure to automotive gasoline in relation to the risk of cancer at various organ sites and tissue types in humans. Since the previous evaluation of gasoline in 1988, summarized in *IARC Monographs Volume 45* ([IARC, 1989](#)), numerous epidemiological studies have been conducted that provide information on cancer in relation to automotive gasoline exposure.

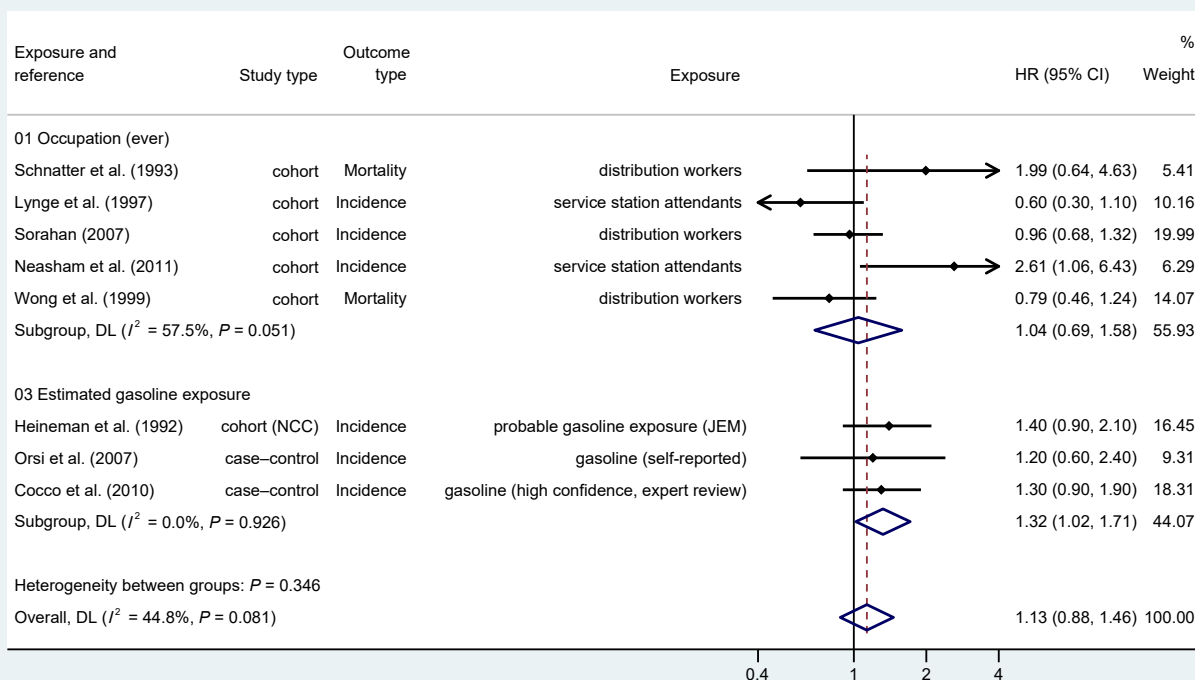
### 2.9.1 Studies evaluated

To assess the association between automotive gasoline and cancer, the Working Group relied mostly on epidemiological studies involving occupationally exposed individuals, except for one study of residents in an area where groundwater was contaminated and some studies of childhood cancer related to residential proximity to service stations.

The epidemiological studies evaluated covered a wide range of cancer types, both childhood cancers (17 studies) and cancers in adults, including cancers of lymphatic and haematopoietic tissue (27 studies), the urinary tract (25 studies), digestive system (19 studies), and

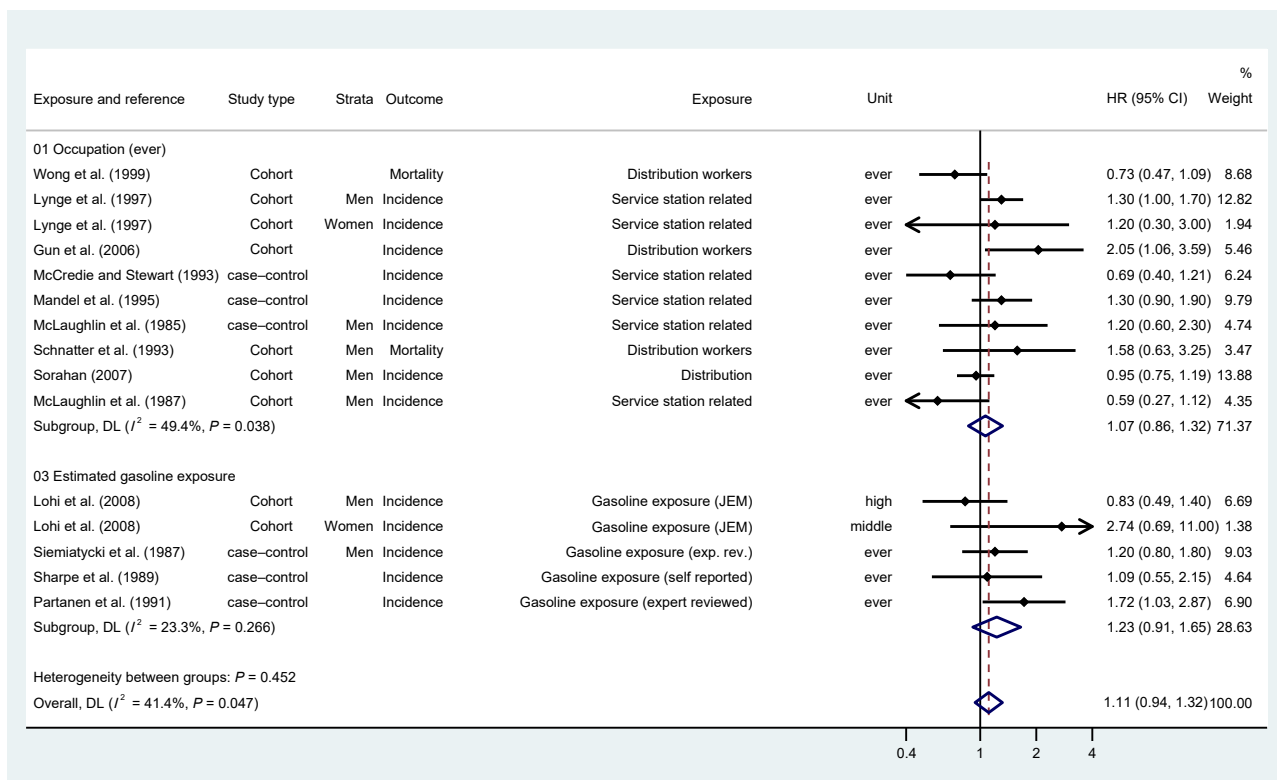
**Fig. 2.10 Meta-analysis for adult chronic lymphocytic leukaemia**

Weights are from random-effects model. For [Cocco et al. \(2010\)](#), exposure characterized as “high confidence” was used. CI, confidence interval; CLL, chronic lymphocytic leukaemia; DL, DerSimonian–Laird estimate of tau<sup>2</sup>; HR, hazard ratio.

**Fig. 2.12 Meta-analysis for adult multiple myeloma**

Weights are from random-effects model. For [Cocco et al. \(2010\)](#), exposure characterized as “high confidence” was used. CI, confidence interval; DL, DerSimonian–Laird estimate of tau<sup>2</sup>; HR, hazard ratio; JEM, job-exposure matrix; NCC, nested case-control.



**Fig. 2.15 Meta-analysis for kidney cancer**

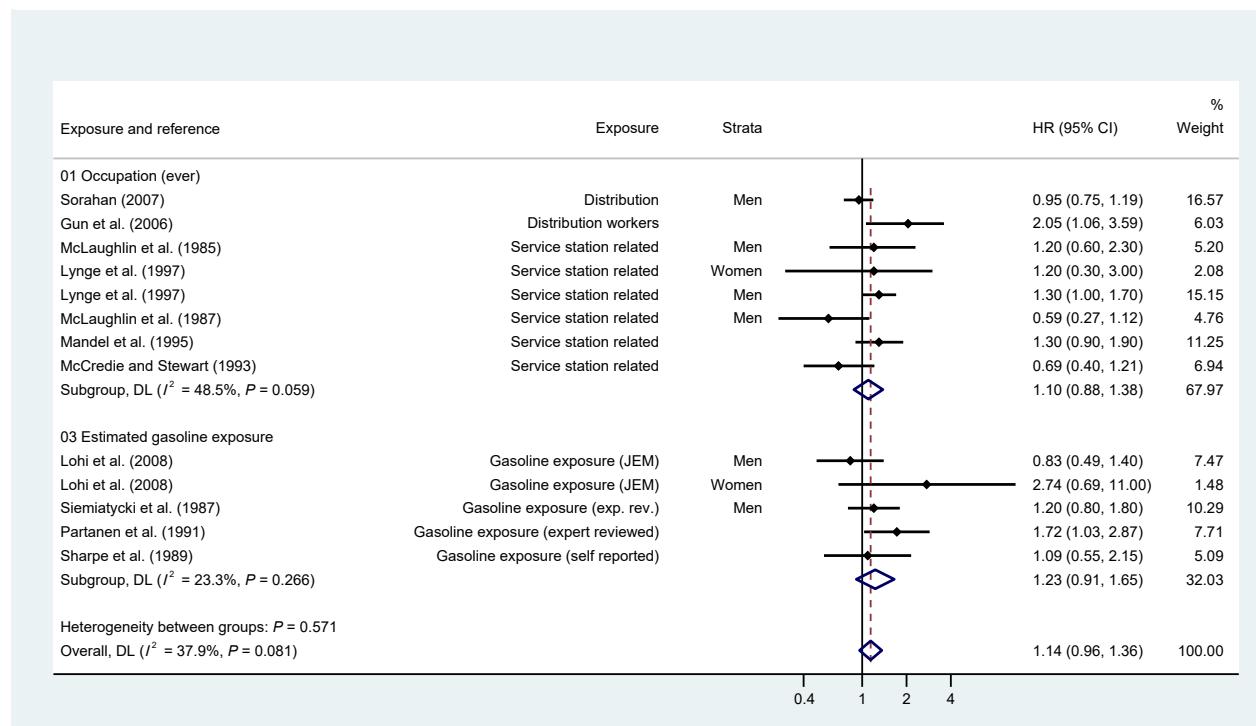
Studies reporting on hydrocarbons distillation range were not included. For [Sorahan \(2007\)](#), incidence was used. Weights are from random-effects model.

CI, confidence interval; DL, DerSimonian-Laird estimate of  $\tau^2$ ; HR, hazard ratio; JEM, job-exposure matrix.

respiratory tract (21 studies). Cancers of the breast, including male breast, and brain, and other solid tumour types were also investigated. The end-points were either cancer incidence or mortality. The studies evaluated were conducted in North America (Canada, USA), Europe (mostly in Nordic countries), Australia, China, Uruguay, and Iran. Several studies were conducted in multiple countries, for example, the two case-control studies from INTEROCC involved Australia, Canada, France, Germany, Israel, New Zealand, and the UK.

The Working Group reviewed 18 record-linkage studies relating national records of occupation to cancer incidence data; one cohort of environmentally exposed participants; three population-based cohorts; one cohort

of agricultural workers; four cohorts of petroleum distribution workers; one cohort of service station attendants; and 78 case-control studies on specific cancer types. The case-control study in Montreal, Canada included several cancer types ([Siemiatycki et al., 1987a](#)); and the ICARE study included cancers of the lung and aero-digestive tract ([Mattei et al., 2016](#); [Carton et al., 2017](#); [Barul et al., 2018, 2019](#)). Regarding childhood cancers, there were five studies on residential distance from service stations of which four used residential-based measurements and one used self-report (based on a questionnaire on whether participants were living near a service station).

**Fig. 2.16 Meta-analysis for kidney cancer (incidence only)**

Studies reporting on hydrocarbons distillation range were not included. Weights are from random-effects model. CI, confidence interval; DL, DerSimonian–Laird estimate of  $\tau^2$ ; HR, hazard ratio; JEM, job-exposure matrix.

Methods of exposure assessment were varied and included the use of job titles as a surrogate for gasoline exposure, self-declaration of exposure by the study participants, JEMs, and expert assessment on the basis of detailed information about workplace exposure or job tasks. None of the study results were based on individual exposure measurements at individual work sites.

The informativeness of these studies was considered on the basis of the exposure assessment methodology (Section 2.9.2) and the potential for bias and control of confounding, which were crucial in determining the weight given to each study when interpreting the evidence.

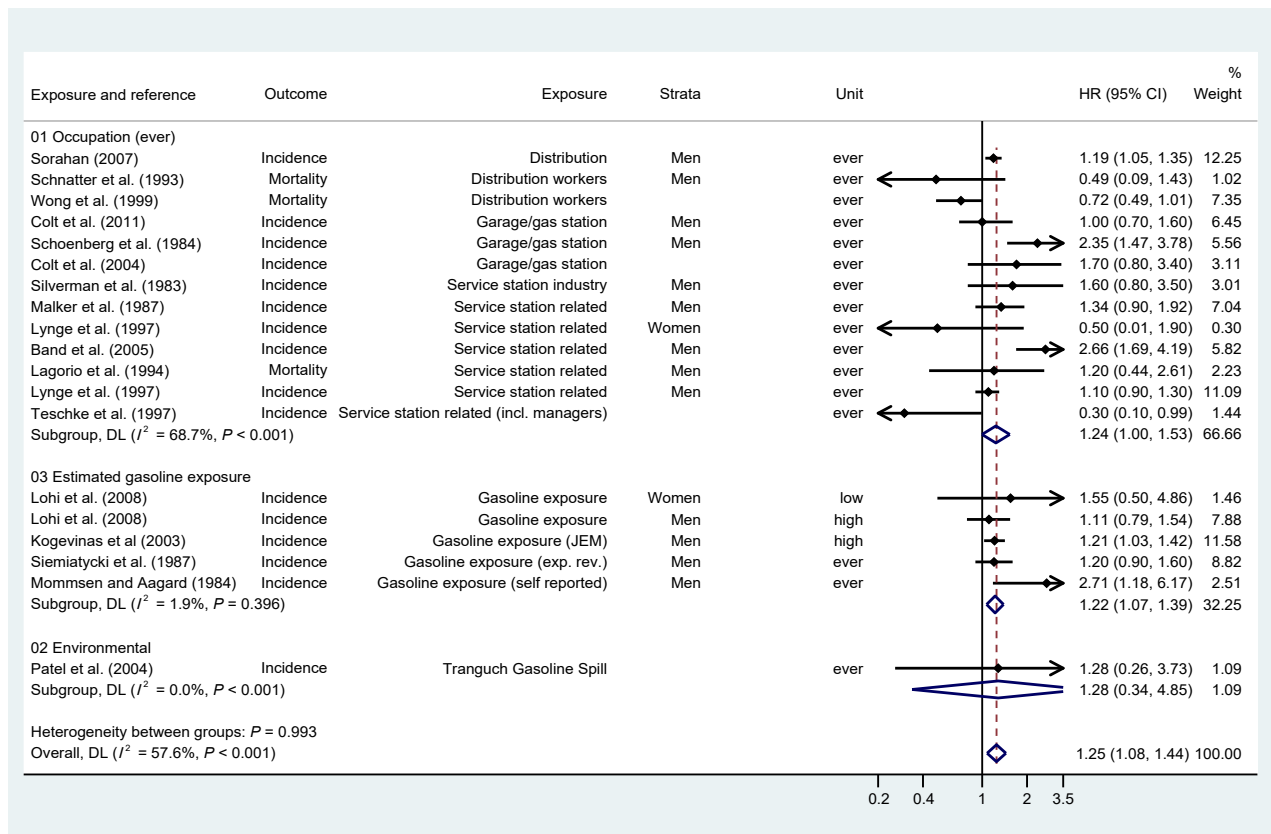
### 2.9.2 Exposure assessment

The Working Group considered the quality of the exposure assessment when determining the informativeness of each study. A critical review

of the exposure assessment for key epidemiological studies is summarized in Section 1.6.1, and details for individual studies are provided in Table S1.15 (Annex 1, Supplementary material for Section 1, Exposure characterization, online only; available from: <https://publications.iarc.who.int/652>).

#### (a) Methods of exposure assessment

The present evaluation included several cohort studies defined by occupations that were considered by the Working Group to be a good proxy for gasoline exposure, including land-based distribution workers and service station attendants. In other population-based studies, exposure was assessed using JEM or evaluation by an industrial hygienist from details of a description of work tasks obtained by questionnaire.

**Fig. 2.19 Meta-analysis for cancer of the urinary bladder**

For [Sorahan \(2007\)](#), incidence was used. Weights are from random-effects model.

CI, confidence interval; DL, DerSimonian–Laird estimate of tau<sup>2</sup>; HR, hazard ratio; JEM, job-exposure matrix.

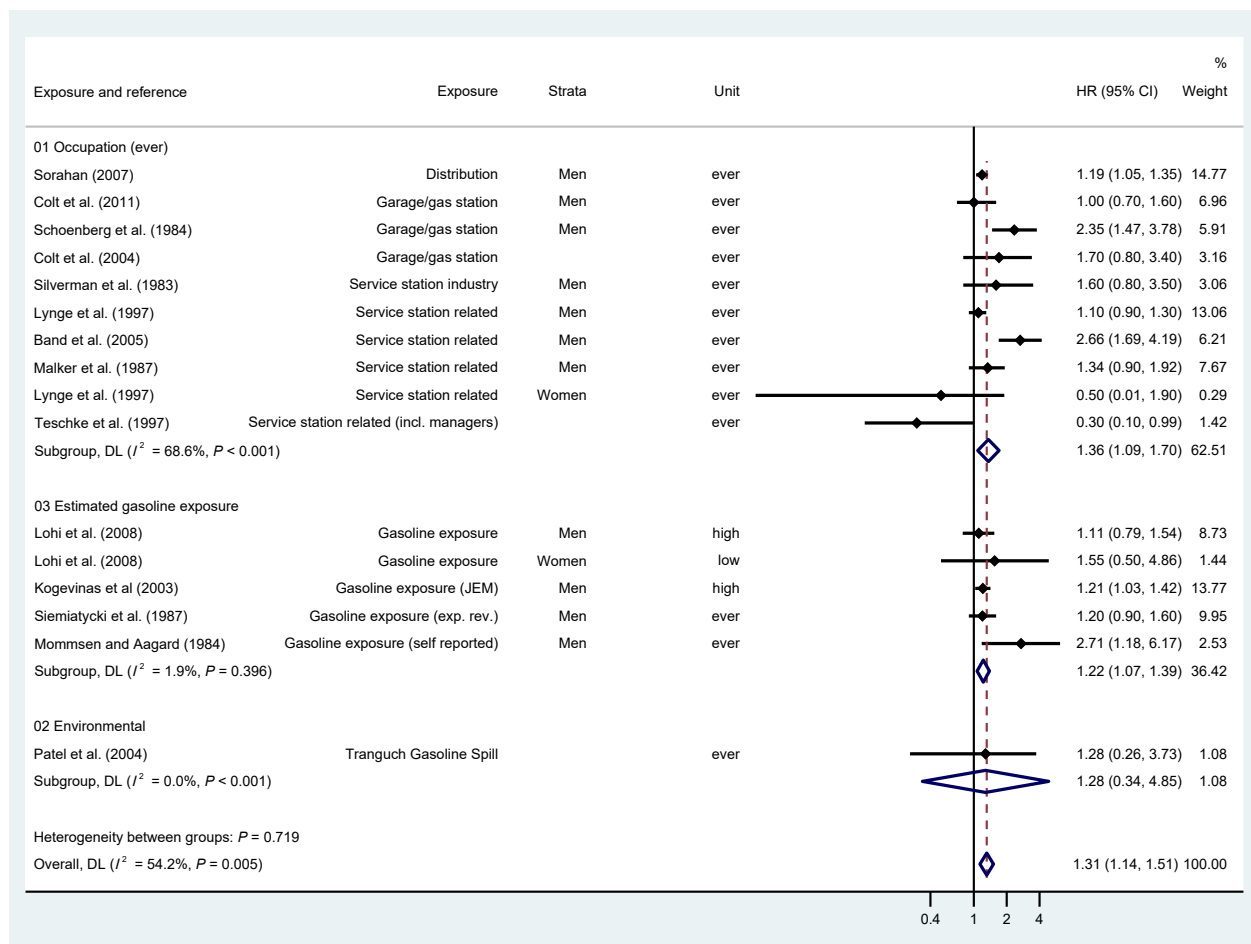
Cohort studies in which exposure was defined by occupation were conducted either through census (e.g. [Lynge et al., 1997](#); [Lohi et al., 2008](#)) or employers' records (e.g. [Wong et al., 1999](#); [Sorahan, 2007](#)). The lack of a full job history, quantitative measures of exposure, and information on co-exposures limited the informativeness of these studies for the present evaluation.

Case-control studies in which questionnaires were used to collect information on job or exposure covered a longer time period (e.g. [Siemiatycki et al., 1987a](#); [Kogevinas et al., 2003](#); [Dryver et al., 2004](#)). Self-reports and job codes based on average exposures were subject to similar concerns regarding exposure misclassification error.

Studies on exposure to gasoline in residential areas relied on measures of geographical distance from the source of gasoline (service station, distance from gasoline spill) as surrogate measures of exposure (e.g. [Patel et al., 2004](#); [Mazzei et al., 2022](#); [Norzaee et al., 2024](#)). Exposure estimates were enhanced when coupled with measured or modelled estimates of gasoline dispersion ([Talbot et al., 2011](#)).

#### (b) Differential and nondifferential exposure misclassification

When information about gasoline exposures was collected prospectively, before the occurrence of cancer, or retrospectively but blinded to cancer occurrence, exposure misclassification was probably nondifferential with respect to the

**Fig. 2.20 Meta-analysis for cancer of the urinary bladder (incidence only)**

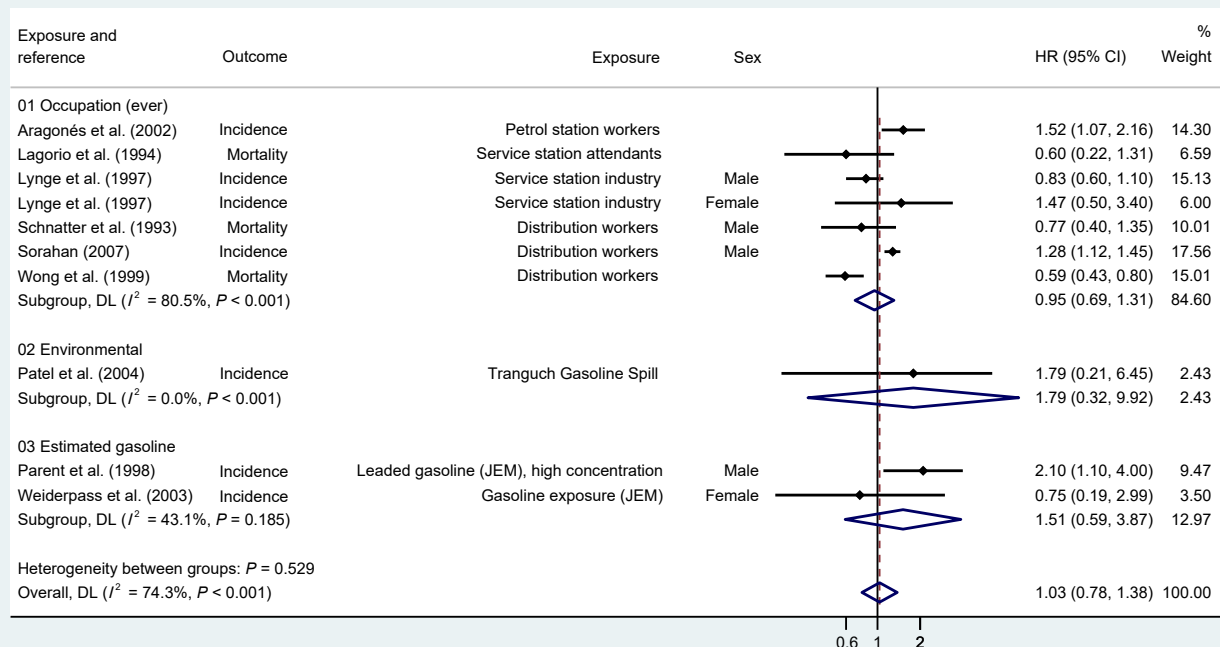
For Sorahan (2007), incidence was used. Weights are from random-effects model.

CI, confidence interval; DL, DerSimonian–Laird estimate of  $\tau^2$ ; HR, hazard ratio; JEM, job-exposure matrix.

cancer outcome. Nondifferential misclassification error would be expected to bias the results towards the null, or towards an underestimate of the association between gasoline exposure and cancer risk.

In situations where information was collected retrospectively, after cancer had occurred, the possibility exists that the quality of the information on exposure differed according to the cancer outcome. This differential misclassification could bias the result either towards or away from the null, depending on the nature of the misclassification error. Sometimes steps were taken to reduce or check for recall bias and the

potential for differential misclassification of exposure. Recall bias was of particular concern in studies that relied on self-reported exposure to gasoline, e.g. leading to risk overestimates if the cases tended to report exposure more frequently and more accurately than did the controls (e.g. Mandel et al., 1995). In this situation, the risk estimate could be overestimated. Some studies evaluated in the present monograph that relied in part on the next of kin of the deceased case to obtain exposure information may be particularly prone to recall bias (Björk et al., 2001), unless the proportion of next-of-kin interviews was the same in the case and in the control groups

**Fig. 2.25 Meta-analysis for stomach cancer**

Weights are from random-effects model. [Lyngé et al. \(1997\)](#) (for the portion in Sweden) was not included because of overlap with the study by [Aragonés et al. \(2002\)](#). For the Montreal case-control study, the study by [Parent et al. \(1998\)](#) was used. CI, confidence interval; DL, DerSimonian-Laird estimate of tau<sup>2</sup>; HR, hazard ratio; JEM, job-exposure matrix.

([Stemhagen et al., 1983](#); [McLaughlin et al., 1985](#); [Partanen et al., 1991](#)).

In several studies, such as those based on censuses (e.g. [Patel et al., 2004](#); [Talibov et al., 2019](#)), latency was not considered, and this may introduce a bias towards the null, particularly for cancers with longer latencies.

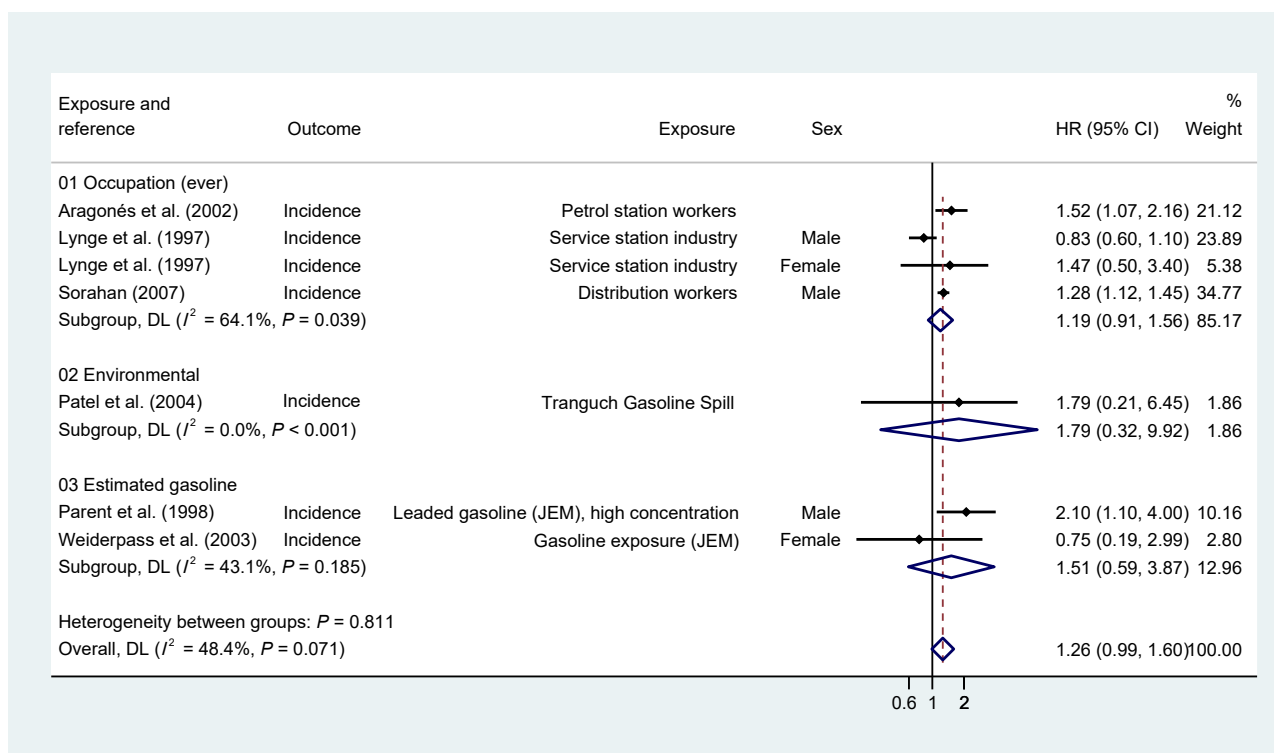
(c) *Exposure duration, frequency, and intensity in relation to exposure-response trends*

Studies in which only dichotomous measures of exposure to gasoline were provided (e.g. [Sharpe et al., 1989](#); [Nisse et al., 2001](#)) lacked the ability to examine risk by level of exposure, defined by duration, frequency, or intensity of exposure. In addition, workers with greater exposure were grouped with workers with less exposure into

one exposed category, which could diminish estimates of cancer risk.

Estimates of cumulative exposure over time are more informative than estimates based on one point in time. In some instances, a cumulative measure of gasoline exposure combined the number of years in a job with the estimated intensity of exposure to gasoline in that job (e.g. [Partanen et al., 1991](#); [Cocco et al., 2010](#)).

Attempts to measure risk of cancer by multiple levels of gasoline exposure were probably hampered by misclassification error, and a linear increase in risk by increasing exposure could have been masked.

**Fig. 2.26 Meta-analysis for stomach cancer (incidence only)**

Weights are from random-effects model. The study by [Lynge et al. \(1997\)](#) (for the portion in Sweden) was not included because of overlap with the study by [Aragonés et al. \(2002\)](#). For the Montreal case-control study, the study by [Parent et al. \(1998\)](#) was used. CI, confidence interval; DL, DerSimonian-Laird estimate of tau<sup>2</sup>; HR, hazard ratio; JEM, job-exposure matrix.

#### (d) Exposure assessment by time period

Gasoline exposures, composition, emission controls, and exposure limits have changed over time; this information is summarized in Sections 1.4.1 and 1.5.1.

### 2.9.3 Key considerations regarding confounding and bias

#### (a) Confounding

Potential confounding from lifestyle-related factors or from co-exposures at the workplace depends on the cancer under consideration. Uncontrolled confounding may overestimate or underestimate the true association between gasoline exposure and cancer.

Whereas in case-control studies information is typically collected by questionnaires on the major potential lifestyle-related confounders of the cancer of interest, studies on retrospective occupational cohorts and record linkage generally lack information on non-occupational risk factors, as this is usually not available in employers' records or national databases. Potential confounders are cancer-specific. For example, smoking can confound the association between an occupational factor and lung cancer, but has no effect in studies in which smoking is not or is only weakly associated with the cancer under investigation. In the absence of data on potentially important confounders at an individual level, [Lohi et al. \(2008\)](#) adjusted for tobacco smoking and other risk factors for kidney cancer (socioeconomic status, BMI) aggregated at the occupational group level, available from



FINJEM. Using aggregated data available for a potential confounder, however, does not rule out residual confounding and may induce overadjustment. The Working Group considered lung cancer as a negative control outcome to gauge the expected magnitude of confounding from tobacco smoking for other cancer types. The lack of an increase in risk of lung cancer associated with gasoline exposure suggested that substantial confounding by tobacco smoking was unlikely for other cancer types, which have smaller associations with tobacco smoking than does lung cancer.

Confounding by occupational exposures other than gasoline is another, potentially more important, issue that should be considered when interpreting study results. In studies on occupational groups in which exposure to gasoline is not assessed specifically, a role of gasoline cannot be isolated from that of co-exposures to carcinogens (see [Table 1.11](#)). Although the Working Group restricted the present review to studies of cancer risk in occupations in which the exposure to gasoline was predominant, such as service station attendants, co-exposures to carcinogens may occur. For example, the Canadian Job-Exposure Matrix (CANJEM) indicated that 14% of the service station attendants are exposed to diesel engine emissions in addition to gasoline (data available from: [https://lavoue.shinyapps.io/Shiny\\_canjem\\_v3/](https://lavoue.shinyapps.io/Shiny_canjem_v3/); [Siemiatycki and Lavoué, 2018](#)). However, the available literature suggested that the intensity of exposure to exhausts from diesel and gasoline in service station workers is similar to that of ambient environmental levels (see Section 1.4.2). When exposures are highly correlated, adjusting for multiple exposures is not always feasible. The degree of confounding would be expected to vary by cancer type and is discussed within the relevant sections below.

#### (b) *Selection bias*

Both cohort studies and case-control studies are subject to selection bias, but the way in which this bias occurs differs.

A classical selection bias in occupational cohort studies, which is sometimes seen as confounding, is the HWE, which occurs when mortality or morbidity rates are compared with those of a reference population, generally the population of the region or country. This effect arises because individuals who are employed are generally healthier than those who are unemployed, disabled, or otherwise unable to work. As a result, the observed health outcomes in a working population may be biased, leading to an underestimation of the SMRs or SIRs associated with exposures or occupations ([Schnatter et al., 1993](#); [Wong et al., 1999](#); [Sorahan, 2007](#)). Such bias may have occurred in the retrospective occupational cohort studies evaluated in the present monograph, particularly those studies with a short length of follow-up, because the HWE is often more pronounced at the start of employment and may diminish over time as the workers get older, although HWSB, whereby only healthier workers remain in employment, may become stronger over time. To avoid such bias, the authors of several retrospective cohort studies (e.g. [Aragónés et al., 2002](#); [Lohi et al., 2008](#); [Schnatter et al., 2012](#)) conducted internal analyses without reference to an external population, e.g. using a nested case-control study design with controls selected from cancer-free individuals within the cohort, or conducting a Poisson regression analysis within the cohort. HWSB may occur if there is exposure-related dropout from the workforce among a susceptible population. This was considered to be a concern in some of the studies on gasoline distribution workers for which a minimum duration of employment of 1 year was required (e.g. [Schnatter et al., 1993](#); [Wong et al., 1993, 1999](#); [Sorahan, 2007](#)).

Another source of selection bias in retrospective or prospective cohort studies may arise because participants who withdraw during follow-up may differ systematically from those who remain. For example, if those who eventually develop cancer are less likely to continue participation, the risk would be underestimated. Accounting for the number of participants lost to follow-up was considered by the Working Group when evaluating study quality.

Another source of potential selection bias in studies on occupational exposure during pregnancy and childhood cancer risk in the offspring is live birth bias. A downward live birth bias occurs when exposure to environmental agents during pregnancy results in spontaneous abortion or stillbirth ([Leung et al., 2021](#)). If gasoline exposure were to induce spontaneous abortion, it is possible that some of the available studies were affected by live birth bias, especially those on maternal occupational exposures occurring during pregnancy ([Shu et al., 1988](#); [De Roos et al., 2001](#); [Infante-Rivard et al., 2005](#); [Tsai et al., 2006](#); [McKinney et al., 2008](#); [Kirkeleit et al., 2018](#); [Hall et al., 2021](#)).

Case-control studies are also prone to selection bias, which may occur when the cases and controls are not representative of the same target population. Selection bias can also occur if the participation of cases and/or controls is low (e.g. [Partanen et al., 1991](#) and [McKinney et al., 2008](#)), and if the reasons for non-participation differ between cases and controls. If the response rate is low, adjustment for socioeconomic status may reduce the magnitude of the bias (e.g. [Poynter et al., 2017](#)). In cancer studies, case participants who die before the data are collected may not be captured in the study sample, introducing a selection bias. To overcome this problem, investigators may collect information from the relatives of deceased patients, but this can lead to differential recall and therefore bias if the quality of the information collected differs from that from the controls, and if the proportion of next-of-kin

interviews is higher than for the cases (e.g. [Björk et al., 2001](#)). The participation rate and the proportion of next-of-kin interviews were considered by the Working Group in its assessment of study quality.

In the multicancer case-control study in Montreal, Canada, ([Siemiatycki et al., 1987a](#)), the controls were selected from among cases of cancer types other than the one under investigation and that did not share the same risk factors. This strategy may introduce a downward selection bias, which was seen as “conservative” if cases and controls were to share some unknown occupational risk factors. A similar concern applied to the study on childhood leukaemia by [Harrison et al. \(1999\)](#).

#### 2.9.4 *Childhood acute lymphoblastic leukaemia and other childhood cancer*

Childhood ALL is believed to originate in utero, with evidence for a two-hit mechanism; the second hit appears to occur postnatally ([de Smith and Spector, 2024](#)). Hence, the Working Group was interested in exposures both during pregnancy and postnatally. The Working Group also recognized that some mothers hold the same job across the perinatal period, which may result in increased risk estimates associated with preconception exposures.

In one cohort study ([Kirkeleit et al., 2018](#)) and several case-control studies ([Shu et al., 1988](#); [Infante-Rivard et al., 2005](#); [McKinney et al., 2008](#); [Brosselin et al., 2009](#); [Bailey et al., 2011](#); [Jiang et al., 2016](#); [Malavolti et al., 2023](#)), the authors investigated the relation between parental exposure to automotive gasoline and risk of paediatric ALL. Studies on the risk of childhood leukaemia (not providing risk separately for childhood ALL) were also considered to inform the present evaluation ([Harrison et al., 1999](#); [Shi et al., 2013](#); [Mazzei et al., 2022](#); [Norzaee et al., 2024](#)), because ALL accounts for 68–80% of childhood leukaemia ([Daltveit et al., 2025](#)).

In the Working Group's meta-analysis of all five studies on residential proximity to service stations ([Harrison et al., 1999](#); [Brosselin et al., 2009](#); [Mazzei et al., 2022](#); [Malavolti et al., 2023](#); [Norzaee et al., 2024](#)), an elevated risk of childhood ALL or leukaemia was observed in individuals living in closer proximity, within 100 m versus greater distances (meta-OR, 1.82; 95% CI, 1.23–2.69) ([Fig. 2.4](#)). After excluding two studies considered to be less informative, one of which used patients with other cancer types as controls ([Harrison et al., 1999](#)) and one study that relied on self-reported proximity to service stations ([Brosselin et al., 2009](#)), the meta-OR remained elevated (1.68; 95% CI, 0.98–2.87) ([Fig. S2.5](#)). The two most informative studies of residential proximity ([Malavolti et al., 2023](#); [Norzaee et al., 2024](#)) used GIS records of home addresses for all children that were linked to nearby stations ([Malavolti et al., 2023](#); [Norzaee et al., 2024](#)), which reduced concerns over selection bias and recall bias. In both studies, increases in risk of ALL or leukaemia were reported with residence within 50 m or 100 m of a service station.

The MoBa cohort was considered to be informative because its prospective study design lessened concern regarding recall errors ([Kirkeleit et al., 2018](#)). However, MoBa was designed as a pilot study, had small numbers of exposed cases, and the maternal exposure measure did not differentiate between gasoline and exhaust. Nonetheless, risk increases were found to be associated with maternal exposure (>30 days, defined by authors to represent occupational exposure; [Kirkeleit et al., 2018](#)). In another informative study, detailed questionnaires were used to determine maternal occupational exposures and industrial hygienist review was also employed ([Infante-Rivard et al., 2005](#)). This study included 790 cases of ALL and an equal number of matched controls, with high participation rates. The results showed elevations in ALL risk with exposure to leaded gasoline (OR,

4.14; 95% CI, 0.46–37.16), but no association for unleaded gasoline (OR, 0.83; 95% CI, 0.22–3.10).

In the United Kingdom Childhood Cancer Study (UKCCS), industrial hygienist review was also used, and a blind external validation of that review was also conducted ([McKinney et al., 2008](#)). However, participation rates were modest among controls, potentially biasing results away from the null. The Working Group raised questions about the exclusion of mothers considered to be partially exposed after the industrial hygienist review, for which there was evidence of differential reclassification between cases and controls, which could bias results downward.

The Working Group's meta-analysis, including studies by [Shu et al. \(1988\)](#), [Infante-Rivard et al. \(2005\)](#), [McKinney et al. \(2008\)](#), [Bailey et al. \(2011\)](#), [Jiang et al. \(2016\)](#), [Kirkeleit et al. \(2018\)](#) (although not [Shi et al., 2013](#), because this was not specific to childhood ALL), stratified by parental exposure period, showed increases in risk of ALL associated with maternal gasoline exposures during pregnancy, both when using self-reported estimation for the study by [McKinney et al. \(2008\)](#) (meta-OR, 1.50; 95% CI, 1.00–2.27; [Fig. 2.1](#)) and when using the industrial hygienist review of exposure for [McKinney et al. \(2008\)](#) (meta-OR, 1.28; 95% CI, 0.88–1.87; [Fig. S2.2](#)). These estimates were calculated using the estimate for unleaded gasoline exposure in the study by [Infante-Rivard et al. \(2005\)](#). When using the estimates for “leaded” exposure and using self-reported estimation for [McKinney et al.](#), the meta-OR for childhood ALL and maternal exposure during pregnancy was 1.65 (95% CI, 1.06–2.55).

An exposure–response relation was investigated in three of the most informative studies, and evidence of an exposure–response effect was observed in all three studies ([Kirkeleit et al., 2018](#); [Malavolti et al., 2023](#); [Norzaee et al., 2024](#)), supporting the validity of the association.

The other case-control studies relied on self-reported exposures. In one case-control study in China ([Jiang et al., 2016](#)), the point estimate was high, but detailed information on how exposure was assessed was lacking, and no participation rates were reported. A separate study, also in China, had a very high participation rate but lacked detailed information on how exposure was assessed ([Shu et al., 1988](#)). The authors found positive associations between ALL and maternal occupational exposure during pregnancy. Finally, in a case-control study focusing on frequency of gasoline refuelling, no association was found; however, the intensity of exposure was expected to be much lower than that in the occupational settings of other studies ([Bailey et al., 2011](#)). Several of the case-control studies had the potential for recall bias, given the self-reported nature of the exposure information. Higher point estimates were seen for self-reported exposures than for exposures determined by industrial hygienist review. The potential for recall bias was also seen in another study that, similarly to that by [McKinney et al. \(2008\)](#), provided risk estimates for before and after industrial hygienist review, for a different childhood cancer, neuroblastoma ([De Roos et al., 2001](#)). These observations tempered the Working Group's confidence in eliminating recall bias as an explanation for the results.

The Working Group also considered the potential role of live birth bias, which causes effect estimates to skew downward ([Leung et al., 2021](#)), although this bias was considered to be modest.

Overall, the Working Group considered that the body of evidence suggested a credible positive association between automotive gasoline exposure and risk of childhood ALL. However, the small number of studies, potential for selection and recall biases, and conflicting results in studies using industrial hygienist review, meant that chance and bias could not be ruled out with reasonable confidence.

Additional case-control studies reported on other childhood cancers, including CNS tumours ([Mazzei et al., 2022](#)), neuroblastoma ([De Roos et al., 2001](#)), childhood AML ([Shu et al., 1988](#); [Brosselin et al., 2009](#)), Wilms tumour ([Kantor et al., 1979](#); [Tsai et al., 2006](#)), NHL and Hodgkin lymphoma ([McKinney et al., 2008](#)), hepatoblastoma ([Janitz et al., 2017](#)), and germ cell tumours ([Hall et al., 2021](#)). There were too few studies for the Working Group to determine whether exposure to automotive gasoline was associated with these other childhood cancer types.

### *2.9.5 Adult lymphatic and haematopoietic malignancies*

There were 13 cohort and 14 case-control studies on the association between adult lymphatic and haematopoietic cancers and exposure to gasoline, having worked as a service station attendant, or having worked as a petroleum distribution worker. Most of the studies focused on broad categories or subcategories of lymphatic and haematopoietic malignancies, and therefore the evidence is synthesized below separately for NHL, multiple myeloma, AML, MDS, and Hodgkin lymphoma. However, the Working Group noted that the definitions of these entities varied between studies, with different classifications and subcategorizations used depending on the time when the study was conducted and its sample size. As there were few contemporary studies, most of the evidence came from studies using old classification systems. Therefore, in the following text, multiple myeloma is discussed separately from NHL; CLL is discussed together with NHL; and leukaemia as a broad category and other leukaemia subtypes (monocytic leukaemia, CML, and lymphoid leukaemia) are discussed briefly in the AML section. As MDS is a haematopoietic stem cell neoplasm that often progresses to AML and may share common risk factors, the evidence for MDS was determined



to contribute to the AML evaluation and also to warrant evaluation as a separate category.

The Working Group also noted that most of the studies were based on men or included a small proportion of women. Only two studies reported specific results for women, both with fewer than 5 exposed cases ([Clavel et al., 1995](#); [Lynge et al., 1997](#)).

(a) *Acute myeloid leukaemia and myelodysplastic syndrome*

There were three occupational cohort studies ([Lynge et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)), one cohort of environmentally exposed individuals ([Talbott et al., 2011](#)), and one case-control study ([Poynter et al., 2017](#)) on exposures related to automotive gasoline and AML risk. All studies observed an increased risk (mortality or incidence) associated with exposures related to automotive gasoline, with an overall pooled estimate of 1.51 (95% CI, 1.06–2.16) ([Fig. 2.6](#)). In addition to appraising the meta-estimate, the Working Group considered the individual studies. The only mortality study available ([Wong et al., 1999](#)), in which an SMR of 1.17 (95% CI, 0.69–1.85) was estimated, despite being affected by a strong HWE, was kept in the meta-analysis because AML was associated with very poor survival at the time of the study. The authors of this study also found an OR of 0.97 (95% CI, 0.91–1.04) for AML mortality per year of duration of employment. When this study, which was deemed less informative by the Working Group, was excluded from the meta-analysis, the overall estimate was 1.67 (95% CI, 1.06–2.64) for incident AML ([Fig. S2.7](#)). In the other two occupational cohort studies, which may have been affected by exposure misclassification resulting in a downward bias, an SIR of 1.40 (95% CI, 0.80–2.40) was observed for service station attendants ([Lynge et al., 1997](#)) and an SIR of 1.33 (95% CI, 0.96–1.80) was observed for distribution workers in the UK ([Sorahan, 2007](#)). An earlier case-control study by [Rushton and Romaniuk \(1997\)](#) that was

nested within the cohort later used by [Sorahan \(2007\)](#) provided some evidence of an exposure-response relation, with an OR of 1.03 (95% CI, 0.99–1.07) per year of employment when treated as a continuous variable, although this analysis included one case of acute monocytic leukaemia along with AML. In addition, in the large, pooled case-control study nested in cohorts of petroleum workers in the UK, Canada, and Australia ([Schnatter et al., 2012](#)), which included cases from [Rushton and Romaniuk \(1997\)](#), the OR was 2.02 (95% CI, 1.08–3.78) for having ever versus never worked as a tanker driver for  $\geq 1$  year.

In the cohort study of residents in the area affected by the gasoline spill in Pennsylvania, USA, the authors estimated a very high SIR of 11.5, on the basis of 3 exposed cases, when the analysis took into account latency from the beginning of the spill ([Talbott et al., 2011](#)). The Working Group noted that this SIR estimate was an outlier and did not focus on its magnitude, but rather on the fact that no AML cases occurred among the exposed in the first 5 years of follow-up after the start of the spill and all 3 cases were diagnosed in the subsequent 5 years, which was consistent with the expected latency of AML.

The case-control study by [Poynter et al. \(2017\)](#) was informative, although the exposure assessment was based on self-report and there was a low response rate of 58% among cases and 64% among controls. However, the study had a relatively large sample size, and the analyses were adjusted for socioeconomic status, which may reduce concerns over differential participation of cases and controls. This was the only study on AML in which adjustment was made for other potential confounders, including smoking, exposure to radiation, and having lived on a farm or in a rural area. The study provided some evidence of an exposure-response relation; when compared with no occupational and/or residential gasoline exposure, there was an OR of 0.96 (95% CI, 0.50–1.83; 14 exposed cases) for exposure of  $< 5$  years and an OR of 1.40 (95% CI, 0.95–2.06,

46 exposed cases) for exposure of  $\geq 5$  years ( $P$  for trend, 0.22). From the information provided in the paper, the Working Group calculated a crude OR estimate of 1.30 for the higher exposure level, which suggested a small effect of confounding and may be used to infer a lack of confounding in the other studies on AML.

For the evaluation of MDS, which was deemed relevant to the evaluation of AML, there was one cohort study of petroleum distribution workers (Sorahan and Mohammed, 2016) and four case-control studies (West et al., 1995; Nisse et al., 2001; Lv et al., 2011; Poynter et al., 2017). Overall, the results were in the same direction as those for AML; the meta-estimate was of a lower magnitude (1.12; 95% CI, 0.80–1.56) and there were positive findings in four out of five studies (Fig. 2.6). The largest study was that by Poynter et al. (2017), who reported an OR of 1.11 (95% CI, 0.71–1.74; 37 exposed cases) for MDS and occupational and/or residential exposure to gasoline of  $\geq 5$  years, a result that was in the same direction but of smaller magnitude and lower precision than that reported for AML in the same study. There were only 4 exposed cases of MDS with an exposure duration of  $<5$  years, and although the OR for this category was not estimated in the publication, the lower prevalence of cases (2%) than controls (3%) in the lower exposure category ( $<5$  years) provided some support for a possible exposure-response relation. For comparison, the prevalence was 13% in cases and 9% in controls for exposure of  $\geq 5$  years. Two case-control studies, both based on expert review of occupational exposure to gasoline, focused only on MDS: in a study in China, Lv et al. (2011) found an OR of 2.34 (95% CI, 0.70–7.77) that was adjusted for several risk factors including benzene (which may be inappropriate), smoking, and education; and in a study in France, Nisse et al. (2001) found an OR for ever exposure of 2.5 (95% CI, 0.9–7.7), with no evidence ( $P = 0.8$ ) of differences between cases and controls in lifetime mean cumulative gasoline exposure.

The two remaining studies were less informative. The case-control study by West et al. (1995) had a crude exposure assessment (self-reported information on occupational or non-occupation exposure to gasoline and diesel), probably with strong nondifferential misclassification and bias towards the null. In the cohort of distribution workers in the UK, Sorahan and Mohammed (2016) found an SRR of [0.77 (95% CI, 0.48–1.18)] for MDS – thus in the opposite direction to that for AML in the same cohort – and no evidence of an association with duration of employment. However, the analysis for MDS was only possible starting from 1995 in a cohort of workers first employed between 1946 and 1974, resulting in missing information for a relevant risk period and potential bias of the estimates towards the null. In the pooled case-control study of petroleum workers by Schnatter et al. (2012), which included data from the UK cohort described by Sorahan and Mohammed (2016), the OR was 2.16 (95% CI, 0.79–5.88) for having ever worked as a tanker driver for  $\geq 1$  year versus never working as a tanker driver for 1 year. Some evidence of an exposure-response relation was also observed in the study by Schnatter et al. (2012); the association was stronger when restricting to tanker drivers with a higher exposure certainty.

Leukaemia was analysed as a broad group in six studies (Schnatter et al., 1993; Lagorio et al., 1994a; Lynge et al., 1997; Wong et al., 1999; Sorahan, 2007; Talbott et al., 2011), with no clear evidence of a positive association. In all studies that reported results both for AML and for leukaemia as a broad category, the estimates for AML were higher than those for leukaemia (Lynge et al., 1997; Wong et al., 1999; Sorahan, 2007; Talbott et al., 2011), suggesting a specific association between gasoline exposure and AML. The analyses of the environmentally exposed cohort affected by the gasoline spill in Pennsylvania, USA, resulted in an SIR of 6.49 of leukaemia, with 5 cases, and there was a suggestion of an exposure-response relation when



SIRs for all leukaemias were stratified according to low, medium, and high levels of exposure (Talbot et al., 2011). With just one study on each leukaemia subtype, no clear evidence of a positive association was found for monocytic leukaemia (Sorahan, 2007), lymphoid leukaemia (Sorahan, 2007), or CML (Björk et al., 2001). For CML, Rushton and Romaniuk (1997) found some evidence of an association with years of duration of employment within the cohort of distribution workers in the UK, reporting an estimated OR of 1.04 (95% CI, 0.96–1.12) per year of employment duration. In addition, a study by Schnatter et al. (2012) that contained cases overlapping with those in Rushton and Romaniuk (1997) found an OR for CML of 1.26 (95% CI, 0.51–3.11) for having ever versus never worked as a tanker driver in the pooled nested case–control study in the cohorts of petroleum workers in the UK, Canada, and Australia.

The Working Group concluded that a positive association between gasoline exposure and AML was observed. Chance, bias, and confounding could be ruled out with reasonable confidence. This conclusion was supported by the high consistency across the results, the consistency of the results for AML and MDS, and some evidence of a positive exposure–response relation from two studies. Positive associations were also observed in occupational cohort studies, where non-differential exposure misclassification and the HWE are likely. There was minimal evidence of confounding in the study in which the authors adjusted for socioeconomic status and smoking. The Working Group also considered the contribution of heterogeneous sources of evidence, including occupational cohort studies of different types of workers, a cohort of an environmentally exposed population and a case–control study.

For MDS, the Working Group concluded that the presence of a credible positive association between exposure to gasoline and the risk of MDS was supported by the consistency of the

results of the three most informative studies and some evidence of higher risk in tanker drivers compared with other workers in the pooled nested case–control study in petroleum workers. However, null or negative results were reported in two studies, and the meta-estimate was weak, making it less robust to the role of chance, bias, or confounding, which could not be ruled out.

#### (b) *Non-Hodgkin lymphoma*

Five occupational cohorts (Schnatter et al., 1993; Lagorio et al., 1994a; Lynge et al., 1997; Wong et al., 1999; Sorahan, 2007), one cohort study in the general population (Neasham et al., 2011a), one residential cohort study in an environmentally exposed population (Patel et al., 2004), and six case–control studies (Siemiatycki et al., 1987a; Holly et al., 1997; Persson and Fredrikson, 1999; Dryver et al., 2004; Orsi et al., 2007; Cocco et al., 2010) contributed to the present evaluation for NHL.

In the Working Group meta-analysis on NHL incidence or mortality, a meta-estimate of 1.04 (95% CI, 0.86–1.25) was observed (Fig. 2.8). After exclusion of mortality studies (Schnatter et al., 1993; Lagorio et al., 1994a; Wong et al., 1999), there was a positive pooled association of 1.12 (95% CI, 0.99–1.26) (Fig. 2.9). Overall, the association was stronger in case–control studies in which gasoline exposure was assessed than in cohort studies in which employment as a service station attendant or distribution worker was assessed, the latter being more likely to be affected by nondifferential exposure misclassification and not considering latency in the analysis, methodological aspects that could introduce bias towards the null.

In some studies, the authors adjusted for socioeconomic status or education (Siemiatycki et al., 1987a; Orsi et al., 2007; Cocco et al., 2010; Neasham et al., 2011a) and also for smoking (Siemiatycki et al., 1987a; Neasham et al., 2011a). Confounding from known risk factors was not expected to play a major role in the analysis

of gasoline exposure and NHL risk, although co-exposure to unknown occupational carcinogens involved in NHL risk was possible.

Two large case-control studies ([Dryver et al., 2004](#); [Cocco et al., 2010](#)) both found an increased risk of NHL, with some evidence of an exposure-response relation. The most informative to the Working Group, because of the use of expert-based assessment of gasoline exposure, was by [Cocco et al. \(2010\)](#), who found an OR of 1.20 (95% CI, 1.0–1.5; 248 exposed cases of B-cell NHL) for ever occupational exposure to gasoline assessed with high confidence compared with never occupational exposure to any solvent, and an increasing risk with increasing cumulative exposure (*P* for continuous trend, 0.01). In the large case-control study by [Dryver et al. \(2004\)](#), the OR estimate for NHL was 1.46 (95% CI, 1.04–2.05; 78 exposed cases), for ever-versus-never occupational exposure to gasoline, which increased to 1.92 (95% CI, 1.20–3.08) for  $\geq 5$  years versus  $< 5$  years of exposure. Despite these strengths, the Working Group noted that the response rates in both studies were relatively low, particularly for the controls, with the potential for selection bias. To understand the potential impact of this bias on the risk estimates, the Working Group considered the study by [Cocco et al. \(2010\)](#), which reported a response rate of 88% for cases, 81% for hospital controls, and 51% for population controls (the rates for the two control groups were used to calculate an overall response rate for controls of 65%). Analyses stratified by type of control, which would have provided an indirect assessment of selection bias, were not conducted. The OR estimates were adjusted for education, which is a common driver of selection. However, the bias estimates produced by the Working Group showed that slightly differential response rates for the exposed and the unexposed participants and in the opposite direction for cases and controls (e.g. 92% and 85% in the exposed and unexposed cases, and 60% and 67% in the exposed and unexposed controls) could

be responsible for the modestly elevated OR (adjusted OR, 1.20).

Results were inconsistent in the other four case-control studies, which were considered to be less informative because the exposure assessment was based on self-report, with the possibility of both upward recall bias and exposure misclassification bias towards the null in three of these studies ([Holly et al., 1997](#); [Persson and Fredrikson, 1999](#); [Orsi et al., 2007](#)), and the fourth study used cancer controls, leading to a possible downward bias ([Siemiatycki et al., 1987a](#)).

In some studies, results were reported for B-cell NHL subtypes, including CLL, hairy cell leukaemia, DLBCL, and follicular NHL, as well as for T-cell NHL and mycosis fungoides. Overall, there was no clear evidence of effects for particular subtypes.

Specifically, in three of the studies mentioned above ([Lynge et al., 1997](#); [Orsi et al., 2007](#); [Cocco et al., 2010](#)) the results reported for CLL were consistent with those for NHL, i.e. no association in the studies by [Lynge et al. \(1997\)](#) and [Orsi et al. \(2007\)](#), and a positive association in the study by [Cocco et al. \(2010\)](#). In addition, in the UK cohort of distribution workers, [Sorahan \(2007\)](#) reported an increased risk of CLL mortality (SMR, 1.50; 95% CI, 0.89–2.37), and the results of the case-control study nested in the cohort showed an increasing risk of CLL with increasing years of employment (OR per year, 1.06; 95% CI, 1.01–1.11) ([Rushton and Romaniuk, 1997](#)). A pooled case-control study nested in cohorts of petroleum workers in the UK, Canada, and Australia, which included cases from [Rushton and Romaniuk \(1997\)](#), found an OR of 0.64 (95% CI, 0.36–1.14) for having ever versus never worked as a tanker driver for  $\geq 1$  year ([Schnatter et al., 2012](#)). In the meta-analysis conducted by the Working Group, the meta-estimate for CLL was 1.31 (95% CI, 0.94–1.83) for incidence and mortality, and 1.23 (95% CI, 0.77–1.98) when restricting to incidence only ([Fig. 2.10](#) and [Fig. S2.11](#)). The study on hairy cell leukaemia was

considered to be less informative as it focused on survivors and assessed exposure to gasoline and diesel ([Clavel et al., 1995, 1996](#)). [Cocco et al. \(2010\)](#) found no evidence of an association between gasoline exposure and DLBCL, follicular NHL, or T-cell NHL, and no evidence of an association was found in the study by [Morales-Suárez-Varela et al. \(2013\)](#) on mycosis fungoides.

The Working Group concluded that the presence of a credible positive association between exposure to gasoline and the risk of NHL (including CLL) was supported by the consistency of the results of the two largest studies, which were further supported by the results of some, but not all, other studies on NHL included in the present evaluation. However, results were inconsistent between cohort and case-control studies, and the role of chance in the overall estimate, and selection bias in the informative case-control studies, could not be ruled out.

### (c) *Multiple myeloma*

Four occupational cohorts ([Schnatter et al., 1993](#); [Lyngé et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)), two cohorts or nested case-control studies in the general population ([Heineman et al., 1992](#); [Neasham et al., 2011a](#)), and two case-control studies ([Orsi et al., 2007](#); [Cocco et al., 2010](#)) contributed to the evaluation for multiple myeloma. Although the results on multiple myeloma are discussed in the present section separately from those on NHL and NHL subtypes, multiple myeloma is a disease of the B-cell lineage, and the body of evidence should be considered in the light of the findings for NHL, presented in Section 2.9.5(b).

In the overall meta-analysis conducted by the Working Group, the meta-estimate for incidence or mortality of multiple myeloma was 1.13 (95% CI, 0.88–1.46) ([Fig. 2.12](#)). After exclusion of the mortality studies, a positive meta-estimate for multiple myeloma of 1.16 (95% CI, 0.88–1.54) ([Fig. S2.13](#)) was observed. Specifically, there was no association in the two occupational cohorts

([Lyngé et al., 1997](#); [Sorahan, 2007](#)) and a positive association in both the two general population cohort or nested case-control studies ([Heineman et al., 1992](#); [Neasham et al., 2011a](#)) and in the two case-control studies ([Orsi et al., 2007](#); [Cocco et al., 2010](#)). In the mortality cohort study in the USA of distribution workers exposed to gasoline, the SMR was 0.79 (95% CI, 0.46–1.24) ([Wong et al., 1999](#)), and in the case-control study nested in that cohort the OR was 1.00 (95% CI, 0.93–1.08) per year of duration of employment ([Wong et al., 1999](#)).

In some studies, the authors adjusted for socioeconomic status or education ([Orsi et al., 2007](#); [Cocco et al., 2010](#); [Neasham et al., 2011a](#)), and [Neasham et al. \(2011a\)](#) also adjusted for smoking. Some analyses in the study by [Heineman et al. \(1992\)](#) were also adjusted for engine exhaust, phthalates, and vinyl chloride. As discussed in the section on NHL, even if confounding from known factors was not expected to play a major role, co-exposure to unknown occupational carcinogens involved in multiple myeloma risk was possible.

Two studies were considered to be the most informative by the Working Group. In the nested case-control study by [Heineman et al. \(1992\)](#), which was registry-based and thus minimally affected by selection bias, the OR was 1.4 (95% CI, 0.9–2.1) for probable occupational exposure to gasoline and 1.2 (95% CI, 1.0–1.5) for possible occupational exposure compared with never occupational exposure. In the study by [Cocco et al. \(2010\)](#), which may be affected to a certain extent by selection bias, the OR was 1.3 (95% CI, 0.9–1.9) for ever occupational exposure to gasoline with high confidence compared with never exposure to any solvents. In both studies there was suggestive evidence of an exposure-response relation. However, when [Heineman et al. \(1992\)](#) restricted the analyses to probable exposure and considered a latency period of 10 years, the association for exposure to gasoline for  $\geq 5$  years (OR, 0.7) was lower than that for a duration of 1 month

to 5 years (OR, 1.6), although the confidence intervals were wide and largely overlapping.

The results of the case-control study by [Orsi et al. \(2007\)](#), which was based on self-reported exposure information, were consistent with those of the two most informative studies. In the two occupational cohort studies, the SIRs were 0.60 (95% CI, 0.3–1.1) for service station attendants ([Lyng et al., 1997](#)) and 0.96 (95% CI, 0.68–1.32) for distribution workers ([Sorahan, 2007](#)), whereas in the study in a general population cohort the HR was 2.61 (95% CI, 1.06–6.43) for having worked as a service station attendant ([Neasham et al., 2011a](#)).

In line with the evaluation for NHL, the Working Group concluded that a causal interpretation of the positive association between gasoline exposure and multiple myeloma was credible, considering the results of the most informative studies, but that chance and bias could not be ruled out with reasonable confidence. Specifically, the number of available studies was not large, the pooled positive association was weak, no association was found in the occupational cohort studies, and a role for selection bias could not be ruled out in the case-control studies.

#### (d) *Hodgkin lymphoma*

Three occupational cohort studies ([Lyng et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)), a cohort study in the general population ([Neasham et al., 2011a](#)), and two case-control studies ([Orsi et al., 2007](#); [Cocco et al., 2010](#)) contributed to the evaluation for Hodgkin lymphoma. Except for the excess risk of Hodgkin lymphoma found among gasoline station attendants in the EPIC cohort study on the basis of only 2 exposed cases of Hodgkin lymphoma ([Neasham et al., 2011a](#)), all other studies found a null or inverse association with the risk of Hodgkin lymphoma, and the meta-OR was 0.86 (95% CI, 0.60–1.22) (Fig. S2.14).

The Working Group concluded that the available studies were of insufficient number and consistency to permit a conclusion to be drawn about the presence or absence of a causal association between exposure to gasoline and the risk of Hodgkin lymphoma.

#### 2.9.6 *Cancer of the kidney*

The studies on kidney cancer included six occupational cohorts defined by occupation or industry ([McLaughlin et al., 1987](#); [Schnatter et al., 1993](#); [Lyng et al., 1997](#); [Wong et al., 1999](#); [Gun et al., 2006](#); [Sorahan, 2007](#)) and a nationwide cohort of active workers in Finland ([Lohi et al., 2008](#)). There were also six case-control studies ([McLaughlin et al., 1985](#); [Siemiatycki et al., 1987a](#); [Sharpe et al., 1989](#); [Partanen et al., 1991](#); [McCredie and Stewart, 1993](#); [Mandel et al., 1995](#)). These studies were conducted in Australia, Canada, Finland, the UK, and the USA. One cohort study was a record-linkage study conducted in four Nordic countries (Denmark, Finland, Norway, and Sweden) ([Lyng et al., 1997](#)) and one international case-control study was conducted in Australia, Denmark, Germany, Sweden, and the USA ([Mandel et al., 1995](#)).

In the Working Group's meta-analysis for kidney cancer (including studies on incidence and mortality), a meta-estimate of 1.11 (95% CI, 0.94–1.32) was observed (Fig. 2.15). For studies on kidney cancer incidence, a meta-estimate of 1.14 (95% CI, 0.96–1.36) was observed (Fig. 2.16).

The cohort studies using definitions by occupation were conducted in petroleum distribution workers ([Schnatter et al., 1993](#); [Wong et al., 1993](#); [Sorahan, 2007](#)), service station attendants ([McLaughlin et al., 1987](#); [Lyng et al., 1997](#)), and tanker drivers ([Gun et al., 2006](#)). The main limitations in the studies by Sorahan et al., Lyng et al., and Gun et al. were the lack of adjustment for potential confounders or co-exposures, the absence of information on exposure-response trends, and the potential for HWEs that may have



caused underestimation of the true risk. Using the information collected from employment records, semiquantitative exposure estimates were developed in the two small occupational studies ([Schnatter et al., 1993](#); [Wong et al., 1999](#)). No exposure–response relation for exposure duration was observed. In the very large population-based cohort study of 1.6 million Finnish people active in 1970 and followed for cancer incidence until 1995, exposure to gasoline was assessed for the longest held occupation using FINJEM ([Lohi et al., 2008](#)), and risk estimates were adjusted for tobacco smoking, BMI, and socioeconomic status available at an occupational group level. Overall, the Working Group meta-analysis showed inconsistent results across the cohort studies, with risk estimates above or below unity, and a meta-estimate of 1.07 (95% CI, 0.84–1.37) (Fig. S2.17).

Out of the six population-based case–control studies, exposure to gasoline was inferred from employment as a service station attendant in three studies ([McLaughlin et al., 1985](#); [McCredie and Stewart, 1993](#); [Mandel et al., 1995](#)), was self-reported by study participants in two studies ([Sharpe et al., 1989](#); [Mandel et al., 1995](#)), and was assessed by industrial hygienists on the basis of a detailed description of the work task in two studies ([Siemiatycki et al., 1987a](#); [Partanen et al., 1991](#)). The use of industrial hygienist reviews by [Siemiatycki et al. \(1987a\)](#) and [Partanen et al. \(1991\)](#) was considered to be a strength. The risk estimates associated with exposure to gasoline were above unity in all case–control studies except that by [McCredie and Stewart \(1993\)](#). The meta-analysis of all case–control studies (including estimated gasoline exposure and exposure on the basis of occupation) produced a meta-estimate of 1.19 (95% CI, 0.95–1.49) (Fig. S2.18). When limited to three studies of self-reported gasoline exposure (with or without expert review) ([Siemiatycki et al., 1987a](#); [Sharpe et al., 1989](#); [Partanen et al., 1991](#)), the meta-estimate increased to 1.32 (95% CI, 0.99–1.76).

However, all studies had limitations, such as low participation rates in [Partanen et al. \(1991\)](#) and [McCredie and Stewart \(1993\)](#) that could induce selection bias in an upwards bias, and differential misclassification bias because of self-reporting or next-of-kin interviews ([McLaughlin et al., 1985](#); [Partanen et al., 1991](#)).

The body of evidence available to assess the association between automotive gasoline and kidney cancer risk included large-scale cohort studies with poor exposure assessment methodology and case–control studies that used more advanced exposure assessment methods but had small sample sizes. Overall, the cohort studies showed inconsistent results. Conversely, positive associations were generally reported in case–control studies, particularly those in which exposure to gasoline was assessed by an expert, reducing the potential for exposure misclassification. Combining case–control and cohort studies in the meta-analysis produced slightly elevated meta-estimates. The Working Group considered that the positive associations between exposure to gasoline and kidney cancer observed in several studies were credible, but that chance, confounding, or bias could not be ruled out with reasonable confidence.

### 2.9.7 Cancer of the urinary bladder

The studies on cancer of the urinary bladder included cohorts of petroleum distribution industry workers or service station attendants exposed to gasoline ([Howe and Lindsay 1983](#); [Malker et al., 1987](#); [Schnatter et al., 1993](#); [Lagorio et al., 1994a](#); [Lyng et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)), a cohort of workers in the general population ([Lohi et al., 2008](#)), and a community-based cohort of individuals exposed to gasoline leaking from underground storage tanks ([Patel et al., 2004](#)). There were also multiple case–control studies ([Silverman et al., 1983](#); [Mommensen and Aagard, 1984](#); [Schoenberg et al., 1984](#); [Siemiatycki et al., 1987a](#); [Teschke](#)

[et al., 1997](#); [Kogevinas et al., 2003](#); [Colt et al., 2004, 2011](#); [Band et al., 2005](#)). One cohort study was a record-linkage study conducted in four Nordic countries (Denmark, Finland, Norway, and Sweden) ([Lynge et al., 1997](#)), and another was a multicentre case-control study conducted in six European countries (Denmark, France, Germany, Greece, Italy, and Spain) ([Kogevinas et al., 2003](#)).

The risk estimates for bladder cancer for most studies were above unity, and in the Working Group's meta-analysis the overall meta-estimate for mortality and incidence was 1.25 (95% CI, 1.08–1.45) ([Fig. 2.19](#)), and the meta-estimate for incidence-based studies was 1.31 (95% CI, 1.14–1.51) ([Fig. 2.20](#)).

In three large-scale cohort studies, incidence rather than mortality was assessed as the study end-point, which was a notable strength ([Lynge et al., 1997](#); [Sorahan, 2007](#); [Lohi et al., 2008](#)). These studies covered service station attendants, distribution workers, and estimated gasoline exposure. There were concerns over downward bias from the HWE in studies by [Lynge et al. \(1997\)](#) and [Sorahan \(2007\)](#) and upward bias from co-exposures in the study by [Sorahan \(2007\)](#). Two case-control studies were also considered of high informativeness ([Siemiatycki et al., 1987a](#); [Kogevinas et al., 2003](#)) because exposure to gasoline specifically was assessed using a JEM or expert judgement. The use of patients with other cancers (excluding lung and kidney cancer) as controls for the bladder cancer cases by [Siemiatycki et al. \(1987a\)](#) may have led to an underestimation of risk, if the cancer sites in the control group and bladder cancer shared the same risk factors. It is worth noting that in these informative studies ([Siemiatycki et al., 1987a](#); [Lynge et al., 1997](#); [Kogevinas et al., 2003](#); [Sorahan, 2007](#); [Lohi et al., 2008](#)), small but consistent elevated risk estimates were reported for bladder cancer, ranging from 1.10 to 1.20 (with the exception of an imprecise estimate in women, based on only 2 cases, reported by [Lynge et al., 1997](#)). The

Working Group noted exposure-response trends in the studies by [Lohi et al. \(2008\)](#) and [Kogevinas et al. \(2003\)](#).

The results were less consistent across mortality studies in distribution workers ([Schnatter et al., 1993](#); [Wong et al., 1999](#)) and service station attendants ([Howe and Lindsay, 1983](#)), but these studies were considered to be less informative for the bladder cancer evaluation because of the high survival rates for bladder cancer and downward bias from healthy-worker biases. In addition, [Howe and Lindsay \(1983\)](#) included other urinary organs, which could exacerbate heterogeneity in results.

The only community-based cohort study of residents exposed to gasoline leaking from underground storage tanks had limitations because of the study's very small size and the low participation rate ([Patel et al., 2004](#)), although the elevation in bladder cancer risk was consistent with the overall meta-estimate for incidence-based studies. An elevated OR for bladder cancer incidence was also noted among workers with jobs reporting "work with oil or gasoline" ([Mommensen and Aagard, 1984](#)).

In five out of seven case-control studies, an increased incidence of bladder cancer was found among service station attendants ([Silverman et al., 1983](#); [Schoenberg et al., 1984](#); [Malker et al., 1987](#); [Teschke et al., 1997](#); [Colt et al., 2004, 2011](#); [Band et al., 2005](#)). Selection bias because of low response rates could not be dismissed as an explanation in the studies by [Band et al. \(2005\)](#) and [Colt et al. \(2004\)](#), although elevated incidence in the census-based study by [Malker et al. \(1987\)](#) reduced concerns about selection bias being an explanation across the body of evidence.

The Working Group considered tobacco smoking and co-exposure to diesel exhaust to be the main potential confounders. However, the exposure studies indicated that the intensity of exposure to diesel exhaust (see Section 1.4.2(a)) within service station attendants would be lower than the intensity of exposure to automotive



gasoline. The Working Group also considered that the meta-analysis for lung cancer, which did not show an increased meta-estimate (Fig. S2.21), offered reassurance regarding the likely magnitude of confounding from tobacco and diesel exhaust. A Working Group meta-analysis of studies on both lung and bladder cancer also indicated that the association was stronger for bladder cancer (Fig. S2.22).

The evidence for a possible association between exposure to automotive gasoline and risk of urinary bladder cancer was mainly based on the results of large-scale cohort studies of workers exposed to gasoline and two case-control studies in which exposure to gasoline was specifically assessed. The small but consistent increases in risk estimates for bladder cancer observed independently in these studies were striking, and additionally two of the studies showed evidence of a positive exposure-response relation. The Working Group also noted that chance, bias, and confounding by co-exposure to occupational hazards could be excluded as explanations for the observed associations.

### 2.9.8 Cancer of the digestive and respiratory systems

#### (a) Cancer of the respiratory system

##### (i) Cancer of the lung

For lung cancer, the meta-analyses conducted by the Working Group were based on seven cohort studies and four case-control studies and found only a slightly elevated risk (meta-estimate of 1.04; 95% CI, 0.89–1.90). Except for an excess risk in male service station workers in the Nordic countries (Lynge et al., 1997) and a case-control study in Italy (Consonni et al., 2010), the findings of five cohort studies (Schnatter et al., 1993; Wong et al., 1993, 1999; Lagorio et al., 1994a; Patel et al., 2004; Sorahan, 2007) and two case-control studies (Siemiatycki et al., 1987a; Mattei et al., 2016) were null or showed deficits in lung cancer

in groups exposed to gasoline. With regard to confounding, none of the cohort studies adjusted for smoking or co-exposure to other carcinogenic agents, reducing the informativeness of these studies for the present evaluation because of the likelihood of upward bias in the estimates. However, the lack of information on duration of exposure and failure to identify the most highly exposed participants in the exposure assessment may have resulted in biased risk estimates towards the null.

In two case-control studies in which smoking was taken into account, there was some evidence of an increased risk of lung cancer (De Stefani et al., 2005; Consonni et al., 2010). However, because of the low number of cases after stratifying into the exposure groups (De Stefani et al., 2005; Consonni et al., 2010), there was a lack of precision in the estimates. In the hospital-based study, Wang et al. (2014) aimed to investigate the association between a specific genotype and risk of lung cancer and reported that occupational exposure to gasoline or diesel was a risk factor for lung cancer. There was no exposure assessment, or adjustment for smoking and co-exposure to other carcinogenic agents, and the study was considered to be less informative for the present evaluation for lung cancer.

Overall, the Working Group found little evidence that risk of lung cancer is associated with exposure to automotive gasoline.

##### (ii) Cancer of other parts of the respiratory system

#### Nasal cancer

For cancer of the nose, the meta-analyses conducted by the Working Group were based on two cohort studies and one case-control study and found an imprecise elevated risk (meta-estimate, 1.77; 95% CI, 0.62–5.12) (Fig. S2.23). In a cohort study in gasoline-filling attendants, an excess risk of nasal cancer was reported both for men (SIR, 3.1; 95% CI, 1.5–5.7; 10 cases) and for

women (SIR, 8.0; 95% CI, 1.0–28.9; 2 cases) ([Lyngge et al., 1997](#)), but this finding was not confirmed in the two other studies available ([Teschke et al., 1997](#); [Sorahan, 2007](#)). The Working Group concluded that the small number of available studies, and some inconsistencies between their results, did not permit a conclusion to be drawn about the presence or the absence of a causal association between automotive gasoline exposure and nasal cancer.

### *Laryngeal cancer*

For cancer of the larynx, four cohort studies ([Lagorio et al., 1994a](#); [Lyngge et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)) and six case-control studies ([Brown et al., 1988](#); [Ahrens et al., 1991](#); [Wortley et al., 1992](#); [De Stefani et al., 1998](#); [Shangina et al., 2006](#); [Barul et al., 2018](#)) were available. The meta-analysis carried out by the Working Group suggested that there was an imprecise, positive association between exposure to automotive gasoline and risk of laryngeal cancer (meta-estimate, 1.18; 95% CI, 0.99–1.40), with inconsistencies noted between studies. The estimations in the study by [Ahrens et al. \(1991\)](#) were difficult to interpret and were considered less informative because of the inclusion of prevalent and incident cancer cases, which introduces potential survival bias and a downward bias, and the exposure assessment that was based on self-reporting of both occupational history and lifestyle factors, introducing probable recall bias that could lead to bias away from the null. [Brown et al. \(1988\)](#) estimated the effect for “gasoline or diesel”, and hence this study was considered less informative for the present evaluation. For gas station attendants from the study by [Lyngge et al. \(1997\)](#) and those reporting ever exposure to gasoline or petroleum in the case-control study by [Shangina et al. \(2006\)](#), there was an imprecise excess of laryngeal cancer cases of 1.5 (95% CI, 0.9–2.2) and 1.3 (95% CI, 0.88–1.95), respectively. The case-control studies by [De Stefani et al. \(1998\)](#) indicated an exposure-response

relation by duration, whereas those by [Brown et al. \(1988\)](#) and [Barul et al. \(2018\)](#) did not. In all the case-control studies, there was adjustment for smoking, and [Shangina et al. \(2006\)](#) also adjusted for alcohol. The elevated risk observed in the study by [Lyngge et al. \(1997\)](#) (SIR, 1.5) could not be explained solely by an excess of smoking because of the lower risk estimate for lung cancer in that study (SIR, 1.3).

Overall, the Working Group considered that the small body of available studies with few positive associations, mostly in studies considered to be less informative; inconsistency with respect to both risk estimates and exposure-response relations; and limitations including crude characterization of exposure to automotive gasoline (e.g. occupation, gasoline/petroleum, gasoline/diesel fumes) did not permit a conclusion to be drawn on the presence or absence of a causal association between exposure to automotive gasoline and cancer of the larynx.

### *(b) Cancer of the digestive system*

#### *(i) Cancer of the stomach*

Nine studies on stomach cancer were included in the present evaluation ([Schnatter et al., 1993](#); [Lagorio et al., 1994a](#); [Lyngge et al., 1997](#); [Parent et al., 1998](#); [Wong et al., 1999](#); [Aragonés et al., 2002](#); [Weiderpass et al., 2003](#); [Patel et al., 2004](#); [Sorahan, 2007](#)). In its meta-analysis of these studies, the Working Group found that the meta-estimate for stomach cancer incidence or mortality was close to unity (meta-estimate, 1.03; 95% CI, 0.78–1.38) ([Fig. 2.25](#)). The risk estimates for stomach cancer reported for the distribution workers were likely to be biased downwards because of the HWE ([Schnatter et al., 1993](#); [Wong et al., 1999](#); [Sorahan, 2007](#)). When the analysis was restricted to six studies on incidence, an elevated risk of stomach cancer with exposure to automotive gasoline was noted (meta-estimate, 1.26; 95% CI, 0.99–1.60) ([Fig. 2.26](#)).

The studies found to be most informative for the present evaluation for stomach cancer were the large, population-based studies based on census data from Sweden ([Aragonés et al., 2002](#)) and economically active people in Finland ([Weiderpass et al., 2003](#)) (the latter using FINJEM), as well as a hospital-based case–control study in which the risk of stomach cancer was assessed according to several exposure metrics for gasoline ([Parent et al., 1998](#)). Although some of these most informative studies identified an excess risk of stomach cancer ([Parent et al., 1998](#); [Aragonés et al., 2002](#)), the findings were inconsistent between studies, and an imprecise but reduced risk of stomach cancer was noted by [Weiderpass et al. \(2003\)](#). No excess mortality from cancer of the stomach (SMR, 0.60; 95% CI, 0.22–2.31) was found in a study of filling station attendants in Italy ([Lagorio et al., 1994a](#)), although imprecision limited the informativeness of this study.

Evidence for an exposure–response relation was supported by a population-based case–control study in which [Parent et al. \(1998\)](#) reported an elevated OR for stomach cancer and substantial exposure to leaded gasoline (OR, 2.0; 95% CI, 1.2–3.3), high concentration exposure (OR, 2.1; 95% CI, 1.1–4.0), and longer duration of exposure (OR for  $\geq 20$  years, 1.6; 95% CI, 1.0–2.7) compared with never exposure. The authors adjusted for smoking, but not for other co-exposures expected in motor vehicle mechanics and repairmen exposed to leaded gasoline. Triangulation with the lung cancer findings was considered to offer reassurance about the magnitude of confounding by smoking and some other co-exposures.

Overall, the Working Group considered that the overall body of evidence supported a credible positive association between exposure to automotive gasoline and cancer of the stomach. However, because of inconsistent results in the most informative studies, the small number of exposed cases, and the crude exposure assessment

for most studies, the Working Group concluded that chance and bias could not be ruled out with reasonable confidence.

### (ii) *Cancer of other parts of the digestive system*

The Working Group also considered the evidence on associations between occupational exposure to automotive gasoline and other cancers of the digestive system, including cancer of the buccal cavity and/or pharynx ([Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Wong et al., 1999](#); [Tarvainen et al., 2008](#); [Carton et al., 2017](#); [Barul et al., 2018, 2019](#)), oesophagus ([Schnatter et al., 1993](#); [Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)), pancreas ([Norell et al., 1986](#); [Schnatter et al., 1993](#); [Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#)), liver ([Stemhagen et al., 1983](#); [Lagorio et al., 1994a](#); [Lynge et al., 1997](#); [Wong et al., 1999](#); [Sorahan, 2007](#); [Lindbohm et al., 2009](#)), and colon and/or rectum ([Siemiatycki et al., 1987a](#); [Gerhardsson de Verdier et al., 1992](#); [Arbman et al., 1993](#); [Schnatter et al., 1993](#); [Lynge et al., 1997](#); [Wong et al., 1999](#); [Weiderpass et al., 2003](#); [Patel et al., 2004](#); [Sorahan, 2007](#); [Talibov et al., 2019](#)). Overall, results were generally null, inconsistent, or based on small numbers of studies.

When considering the body of scientific evidence, the Working Group found little evidence that risks of cancers of the digestive system other than stomach cancer were positively associated with exposure to automotive gasoline.

## 2.9.9 Other solid tumours

### (a) *Female breast cancer*

The Working Group evaluated five cohort studies, one nested case–control study, and one case–control study on exposure to automotive gasoline and female breast cancer. In the United States Sister study cohort of cancer-free women with a sister diagnosed with breast cancer, no

overall association was found between self-reported ever occupational use of gasoline or other petroleum products and breast cancer incidence (HR, 0.8; 95% CI, 0.7–1.1) ([Ekenga et al., 2015](#)). There was a positive trend in the analysis restricted to the exposed-only women, with an elevated HR for breast cancer among women in the highest category of lifetime cumulative use compared with those in the lowest category (HR, 2.3; 95% CI, 1.1–4.9; 23 exposed cases). There was also a positive trend in an analysis including never exposed (*P* for trend, 0.03). In a large-scale national record-linkage study in Finland, no clear associations were found between categories of probability or level of occupational exposure to gasoline and breast cancer incidence ([Weiderpass et al., 1999](#)). There was no clear association for female gas station workers ([Lyngé et al., 1997](#)) or female filling station attendants ([Lagorio et al., 1994a](#)). In a cohort of residents with presumed community exposure to gasoline vapour from groundwater contamination in the USA, there was no association ([Patel et al., 2004](#)).

In a population-based case–control study in Canada, there was a strong positive association between ever working in the service station industry and breast cancer incidence among postmenopausal women (OR, 7.65, 90% CI, 1.29–45.3; 9 exposed cases), but not premenopausal women ([Band et al., 2000](#)). In a register-based case–control study on parental occupational exposure and breast cancer risk among female offspring in Denmark, there were no associations for either ever or cumulative maternal or paternal occupational exposure to gasoline ([Pedersen and Hansen, 2023](#)).

In view of the small body of available studies with few positive sporadic findings, as well as limitations regarding study informativeness, in particular the crude nature of the characterization of occupational exposure to automotive gasoline and the small numbers of exposed cases, the Working Group considered that the available studies did not permit a conclusion to be drawn

on the presence or absence of a causal association between exposure to automotive gasoline and female breast cancer risk.

#### (b) *Male breast cancer*

The Working Group evaluated two cohort studies, one nested case–control study, and two case–control studies on exposure to automotive gasoline and male breast cancer. There was an elevated SMR of [8.389 (95% CI, 1.016–30.285)] among male non-deck personnel in the USA, although this was based only on 2 deaths from breast cancer among marine distribution workers with minimal exposure to gasoline vapour ([Wong et al., 1993](#)). Among petroleum distribution workers in the UK, results were inconsistent between incidence- and mortality-based analyses ([Sorahan, 2007](#)).

In a multicountry case–control study in Europe, there were no clear findings, with a positive OR (OR, 1.9; 95% CI, 0.7–5.0) in the highest cumulative exposure category of gasoline exposure that was attenuated when adjusting for other solvent exposures (OR, 0.7; 95% CI, 0.2–3.0; 5 exposed cases) ([Laouali et al., 2018](#)). Although a positive significant OR of 2.2 (95% CI, 1.4–3.6) among men was reported in a register-based nested case–control study in Denmark ([Hansen, 2000](#)), the information on exposure assessment in this study was crude and based on company trade code (service station, vehicle maintenance, wholesale trade of gasoline, or car repair shops) and blue-collar worker status, and included exposure to both gasoline vapour and combustion products together (PAHs); this study was therefore considered less informative for the present evaluation. In a case–control study in France and Switzerland, there was no association between occupational exposure to gasoline and grease in the most recent job and risk of male breast cancer in cases compared with other cancer controls ([Lenfant-Pejovic et al., 1990](#)).



In view of the small body of available studies and inconsistent findings, as well as limitations regarding study informativeness related to the characterization of occupational exposure to automotive gasoline and the small numbers of exposed cases, the Working Group considered that the available studies did not permit a conclusion to be drawn on the presence or absence of a causal association between exposure to automotive gasoline and male breast cancer risk.

(c) *Cancer of the prostate*

The Working Group evaluated seven cohort studies and two case-control studies on exposure to automotive gasoline and prostate cancer. Among petroleum distribution workers in the UK, there was an SMR of [1.15] (95% CI, [0.99–1.33]; 87 deaths) for prostate cancer mortality and no association with prostate cancer incidence ([Sorahan, 2007](#)). There was no association for male marketing and distribution workers in cohorts in the USA ([Wong et al., 1999](#)) and Canada ([Schnatter et al., 1993](#)). There was no association for male gas station workers ([Lynge et al., 1997](#)), filling station attendants ([Lagorio et al., 1994a](#)), or pesticide applicators in nonfarm jobs ([Alavanja et al., 2003](#)). There was also no association in a cohort of residents with presumed community exposure to gasoline vapour from groundwater contamination in the USA ([Patel et al., 2004](#)).

In a population-based case-control study in Montreal, Canada, there were significant positive associations for prostate cancer incidence among men ever working as a service station attendant (OR, 2.30; 95% CI, 1.04–5.08; 25 exposed cases) overall and for high-grade disease (OR, 4.35; 95% CI, 1.82–10.44; 13 exposed cases), which were driven primarily by results for men who had worked for < 10 years in this occupation (OR, 4.00; 95% CI, 1.56–10.25; 11 exposed cases) ([Sauvé et al., 2016](#)). There were also positive significant associations for high-grade disease among those who had ever worked in the service station

industry (OR, 2.79; 95% CI, 1.42–5.47; 18 exposed cases). In the analysis, a total of 696 occupations and 613 industries were included. There was no association in an earlier multicase-control study in Montreal, Canada ([Siemiatycki et al., 1987a](#)).

In view of the small body of available studies with few positive findings, as well as limitations regarding the informativeness of these studies, in particular regarding the crude nature of the characterization of occupational exposure to automotive gasoline, and the small numbers of exposed cases, the Working Group considered that the available studies did not permit a conclusion to be drawn on the presence or absence of a causal association between exposure to automotive gasoline and prostate cancer risk.

(d) *Cancer of the nervous system*

The Working Group evaluated nine cohort studies and six case-control studies on exposure to automotive gasoline and risk of cancer of the brain and nervous system. Among cohort studies, [Preston-Martin \(1989\)](#) reported a PIR of [6.295] ( $P \leq 0.05$ ) in a study on occupation and industry groups at diagnosis on the basis of 2 cases of meningioma in Los Angeles County, USA. There were no associations for glioma incidence in men in large-scale national record-linkage studies in Sweden (on work in the sales work sector and the occupation of filling station attendant) ([Navas-Acién et al., 2002](#)) or for brain cancer incidence and categories of intensity of occupational exposure to gasoline among women in Finland ([Wesseling et al., 2002](#)). There were no associations for brain cancer in cohorts of male marketing and/or distribution workers in the USA ([Wong et al., 1999](#)), Canada ([Schnatter et al., 1993](#)), or the UK ([Sorahan, 2007](#)). There was no association for brain cancer among male gas station workers ([Lynge et al., 1997](#)) or mortality from CNS tumours in filling station attendants ([Lagorio et al., 1994a](#)). There was no association for brain cancer in a cohort of residents with presumed community exposure to gasoline

vapour because of groundwater contamination in the USA ([Patel et al., 2004](#)).

In the multicountry INTEROCC study, there were no associations between occupational exposure to gasoline and risk of glioma or meningioma ([McLean et al., 2014](#); [Benke et al., 2017](#)). In a multicentre hospital-based case-control study in the USA, there were no clear associations for working as a service station attendant and either glioma or meningioma ([De Roos et al., 2003](#); [Rajaraman et al., 2004](#)), although there was a positive association for acoustic neuroma (OR, 2.4; 95% CI, 1.0–6.0; 8 exposed cases) based on unconditional logistic regression models (OR, 2.5; 95% CI, 0.8–7.3; conditional logistic regression models) ([Rajaraman et al., 2004](#)). In a population-based case-control study in Iowa, USA, there was a positive association between working in the service station industry and brain glioma among men (OR, 2.4; 95% CI, 0.9–6.3; 6 exposed cases), which was stronger among men who had worked for < 10 years (OR, 4.8; 95% CI, 1.2–19.3; 3 exposed cases), but not ≥ 10 years in the industry ([Zheng et al., 2001](#)). In a hospital-based case-control study in China, there was no association between occupational exposure to petrol or diesel oil and risk of glioma or meningioma ([Hu et al., 1998, 1999](#)).

In view of the small body of available studies with few positive findings, as well as limitations regarding the informativeness of these studies, in particular regarding the crude nature of the characterization of occupational exposure to automotive gasoline and the small numbers of exposed cases, the Working Group considered that the available studies did not permit a conclusion to be drawn on the presence or absence of a causal association between exposure to automotive gasoline and cancer of the brain and nervous system.

#### (e) *Other cancer sites*

In large-scale national record-linkage studies in Finland, no clear associations were found between categories of probability or level of occupational exposure to gasoline and incidence of ovarian cancer ([Vasama-Neuvonen et al., 1999](#)) or testicular cancer ([Guo et al., 2005](#)). There were no clear findings from three case-control studies of parental occupational exposure and testicular cancer risk among offspring on the basis of a few cases with exposed parents ([Kardaun et al., 1991](#); [Olsson et al., 2018](#); [Guth et al., 2023](#)). There were also studies on cancers of the cervix, uterus, and ovary ([Lagorio et al., 1994a](#); [Lyng et al., 1997](#); [Patel et al., 2004](#)), testes ([Van den Eeden et al., 1991](#); [Wong et al., 1999](#); [Sorahan, 2007](#)); thyroid gland ([Lyng et al., 1997](#); [Wong et al., 1999](#)), and melanoma of the skin ([Schnatter et al., 1993](#); [Lagorio et al., 1994a](#); [Fritschi and Siemiatycki, 1996](#); [Lyng et al., 1997](#); [Sorahan, 2007](#)). There were no findings of increased risk and the evidence was too sparse and of insufficient informativeness to permit conclusions to be drawn on the presence or absence of causal associations between exposure to automotive gasoline and risk of cancer at any of these sites.

### 3. Cancer in Experimental Animals

Gasoline was previously evaluated by the IARC Monographs programme in 1988 (Volume 45; [IARC, 1989](#)), when the Working Group concluded that there was *limited* evidence in experimental animals for the carcinogenicity of automotive gasoline. Since the previous evaluation, there have been new studies investigating the occurrence of cancer in experimental animals in relation to exposure to gasoline. The carcinogenicity of gasoline has been assessed in long-term (2-year or lifetime) studies in mice ([Broddle et al., 1996](#)) and rats ([Maltoni et al., 1997](#); [Benson et al., 2011](#)). There have been several



initiation–promotion studies in mice ([Standeven and Goldsworthy, 1993](#); [Standeven et al., 1994a, 1995](#); [Moser et al., 1997a, b](#)), and in rats ([Short et al., 1989a](#)). In addition, two co-carcinogenicity studies in mice were available at the time of the previous evaluation but only assessed in the present monograph ([Falk and Kotin 1956](#); [Kotin et al., 1958](#)).

The results of all these studies are summarized in [Table 3.1](#).

### 3.1 Mouse

#### 3.1.1 Skin application

In a study of chronic toxicity and carcinogenicity ([Broddle et al., 1996](#)), groups of 50 male C3H/HeJ mice (age, 7–9 weeks) were sham-treated or treated with 50  $\mu$ L of neat unleaded gasoline [composition not reported] by dermal exposure twice per week for the lifetime of the animals. The test article was applied to a shaved interscapular region of the back, covering an area of  $\geq 1$  cm<sup>2</sup>. Survival of the groups treated with unleaded gasoline or sham-treated was similar throughout the study. At 24 months, survival in the group treated with unleaded gasoline was slightly higher (70%) than that observed in the sham-treated group (62%), and at 30 months, survival in both groups was low (10% for unleaded gasoline, 18% for sham-treated). [The Working Group noted that body-weight data were not reported or discussed in the publication.] Dermal irritation (erythema, alopecia, scabbing) was assessed weekly. Mice treated with unleaded gasoline exhibited very low (erythema, scabbing) or low (alopecia) signs of dermal irritation. On the basis of gross observations, skin lesions were considered to be tumours when the mass reached 1 mm in diameter. All mice underwent a limited necropsy, and the skin application site was evaluated microscopically.

In mice treated with unleaded gasoline, one animal was diagnosed as having squamous cell carcinoma of the skin, but no skin tumours were observed in the sham-treated animals. [The Working Group noted that this study was limited by the use of only one sex, one dose, and the fact that incomplete necropsy and histopathological evaluation were performed. The Working Group also noted that there was not sufficient information in the publication to verify the data described by the authors. For example, the percentage incidence in Table 4 of the publication was based on a final effective number (FEN) that was not reported and could not be calculated against the survival information in the publication. Without the FEN, the Working Group could not verify whether the tumour incidence of 1/50 animals corresponded to a percentage of 4% by using FEN. In addition, no analytical information was provided on the test article, including the percentage of benzene. Therefore, the Working Group considered that this study was uninformative for the evaluation of the carcinogenicity of gasoline in experimental animals, and it was not tabulated.]

#### 3.1.2 Inhalation (whole-body)

In a well-conducted study of chronic toxicity and carcinogenicity ([MacFarland et al., 1984](#)), groups of 100 male and 100 female B6C3F<sub>1</sub> mice (age, 6 weeks) were exposed by whole-body inhalation to unleaded automotive motor fuel (gasoline) at a mean concentration of 0, 67, 292, or 2056 ppm in filtered air for 6 hours per day, 5 days per week, for up to 103–113 weeks. [The Working Group noted that the target concentrations of the study were 0, 50, 275, and 1500 ppm. The values reported above reflect the actual exposure concentrations communicated by the authors on the basis of recalibration of the online monitoring gas chromatograph.] The unleaded gasoline used in the study was thoroughly characterized and formulated to represent

**Table 3.1 Studies of carcinogenicity in experimental animals exposed to automotive gasoline**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                       | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                              | Tumour<br>incidence                                                                                                                                                                                                                   | Significance                                        | Comments                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Mouse, B6C3F <sub>1</sub> (M)<br>6 wk<br>103–107 wk<br><a href="#">MacFarland et al. (1984)</a> | Inhalation (whole-body exposure)<br>Unleaded automotive motor fuel (gasoline), (benzene concentration, ≤ 2%)<br>Filtered air<br>0, 67, 292, 2056 ppm<br>6 h/day, 5 days/wk for up to 107 wk<br>100, 100, 100, 100<br>NR | <i>Liver</i><br>Hepatocellular adenoma or carcinoma (combined)<br>45%, 36%, 45%, 44%                                                                                                                                                  | NA                                                  | <i>Principal strengths:</i> Well-conducted study, adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, multiple doses used.<br><br><i>Principal limitations:</i> Limited reporting of results. Tumour data were combined or presented as percentages, preventing statistical analysis by the Working Group. |
| Full carcinogenicity<br>Mouse, B6C3F <sub>1</sub> (F)<br>6 wk<br>113 wk<br><a href="#">MacFarland et al. (1984)</a>     | Inhalation (whole-body exposure)<br>Unleaded automotive motor fuel (gasoline), (benzene concentration, ≤ 2%)<br>Filtered air<br>0, 67, 292, 2056 ppm<br>6 h/day, 5 days/wk for up to 113 wk<br>100, 100, 100, 100<br>NR | <i>Liver</i><br>Hepatocellular adenoma or carcinoma (combined)<br>14%, 19%, 21%, 48%<br><i>Kidney</i><br>Bilateral renal tubular adenocarcinoma<br>Total tumours, 0, 0, 0, 1<br>Papillary cystic adenoma<br>Total tumours, 0, 0, 0, 1 | NA                                                  | <i>Principal strengths:</i> Well-conducted study, adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, multiple doses used.<br><br><i>Principal limitations:</i> Limited reporting of results. Tumour data were combined or presented as percentages, preventing statistical analysis by the Working Group. |
| Full carcinogenicity<br>Rat, Sprague-Dawley (M)<br>7 wk<br>Lifetime<br><a href="#">Maltoni et al. (1997)</a>            | Oral (gavage)<br>Unleaded gasoline, (benzene concentration, 5–5.6%)<br>Olive oil<br>0, 500, 800 mg/kg bw<br>4×/wk (Monday, Tuesday, Thursday, Friday) for 104 wk<br>50, 50, 50<br>NR                                    | <i>All organs</i><br>Total malignant tumours<br>12/50, 7/50, 15/50<br><i>Nasal cavity</i><br>Total malignant tumours<br>0/50, 0/50, 3/50                                                                                              | NS<br><br>[P = 0.0355, Cochran–Armitage trend test] | <i>Principal strengths:</i> Long-term study (> 2 years), adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, two doses used.<br><br><i>Principal limitations:</i> Reporting of pathology without specific terminology (histological type).                                                                 |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                       | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                 | Tumour<br>incidence                                                                                                                                                                                                     | Significance                                                                                     | Comments                                                                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, Sprague-<br>Dawley (F)<br>7 wk<br>Lifetime<br><a href="#">Maltoni et al.<br/>(1997)</a> | Oral (gavage)<br>Unleaded gasoline, (benzene<br>concentration, 5–5.6%)<br>Olive oil<br>0, 500, 800 mg/kg bw<br>4×/wk (Monday, Tuesday, Thursday,<br>Friday) for 104 wk<br>50, 50, 50<br>NR | <i>All organs</i><br>Total malignant tumours<br>11/50, 18/50, 16/50<br><i>Mammary gland</i><br>Cancer (malignant tumour)<br>3/50, 5/50, 8/50<br><i>Uterus-vagina</i><br>Neurinosarcoma [schwannoma]<br>0/50, 2/50, 3/50 | NS<br>NS                                                                                         | <i>Principal strengths:</i> Long-term study (> 2 years), adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, two doses used.<br><br><i>Principal limitations:</i> Reporting of pathology without specific terminology (histological type). |
| Full<br>carcinogenicity<br>Rat, Sprague-<br>Dawley (M)<br>7 wk<br>Lifetime<br><a href="#">Maltoni et al.<br/>(1997)</a> | Oral (gavage)<br>Leaded gasoline, NR<br>Olive oil<br>0, 500, 800 mg/kg bw<br>4×/wk (Monday, Tuesday, Thursday,<br>Friday) for 104 wk<br>50, 50, 50<br>NR                                   | <i>All organs</i><br>Total malignant tumours<br>12/50, 17/50, 9/50<br><i>Head</i><br>All tumours<br>1/50, 2/50, 3/50                                                                                                    | NS<br>NS                                                                                         | <i>Principal strengths:</i> Long-term study (> 2 years), adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, two doses used.<br><br><i>Principal limitations:</i> Reporting of pathology without specific terminology (histological type). |
| Full<br>carcinogenicity<br>Rat, Sprague-<br>Dawley (F)<br>7 wk<br>Lifetime<br><a href="#">Maltoni et al.<br/>(1997)</a> | Oral (gavage)<br>Leaded gasoline, NR<br>Olive oil<br>0, 500, 800 mg/kg bw<br>4×/wk (Monday, Tuesday, Thursday,<br>Friday) for 104 wk<br>50, 50, 50<br>NR                                   | <i>All organs</i><br>Total malignant tumours<br>11/50, 22/50*, 17/50<br><i>Head</i><br>Osteosarcoma<br>0/50, 1/50, 2/50<br><i>Uterus-vagina</i><br>All malignant tumours<br>1/50, 10/50*, 2/50                          | [*P = 0.0163, one-tailed Fisher exact test]<br>NS<br>[*P = 0.0039, one-tailed Fisher exact test] | <i>Principal strengths:</i> Long-term study (> 2 years), adequate number of animals per group, male and female animals used, two doses used.<br><br><i>Principal limitations:</i> Reporting of pathology without specific terminology (histological type).                                                |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                            | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                | Tumour<br>incidence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Significance                                                                                                                     | Comments                                                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, F344 (CDF<br>F344 CrLBR) (M)<br>7–8 wk<br>104 wk<br><a href="#">Benson et al.<br/>(2011)</a> | Inhalation (whole-body exposure)<br>Unleaded GVC, (benzene<br>concentration, 1.9–2.1%)<br>Air<br>0, 2, 10, 20 g/m <sup>3</sup> , 6 h/day (plus<br>14 min, the time for the vapour<br>concentration to reach 90% of<br>equilibrium), 5 days/wk, 104 wk<br>50, 50, 50, 50<br>18, 22, 26, 17 | <i>Kidney</i><br>Renal tubule adenoma<br>1/50, 1/50, 4/50, 0/50<br>Renal tubule carcinoma<br>0/50, 0/50, 3/50, 0/50<br>Renal tubule adenoma or carcinoma<br>(combined)<br>1/50, 1/50, 7/50*, 0/50<br><i>Nasal cavity</i><br>Turbinate level 2: squamous cell<br>carcinoma<br>0/50, 0/50, 0/50, 1/50<br>Turbinate level 3: squamous cell<br>carcinoma<br>0/50, 0/50, 0/49, 3/50<br>Turbinate level 4: squamous cell<br>carcinoma<br>0/50, 1/50, 0/49, 3/50<br><i>Testis</i><br>Interstitial cell adenoma (Leydig cell<br>tumour)<br>48/50, 46/49, 50/50, 49/50<br>Mesothelioma<br>0/50, 0/49, 4/50, 0/50 | NS<br>NS<br>NS<br>[*P = 0.0297, one-<br>tailed Fisher exact<br>test]<br>NS<br>[P = 0.015, Cochran–<br>Armitage trend test]<br>NS | <i>Principal strengths:</i> Well-conducted long-term study,<br>adequate duration of exposure and observation, adequate<br>number of animals per group, male and female animals<br>used, multiple concentrations used.<br><br><i>Other comments:</i> Historical controls (all routes): thyroid<br>gland, follicular cell adenoma or carcinoma (combined),<br>19/1041 (1.83%). |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                       | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                | Tumour<br>incidence                                                                                                                                                                                                                                                                                                                      | Significance                                                                                                             | Comments                                                                                                                                                                                                             |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, F344 (CDF<br>F344 CrIbR) (M)<br>7–8 wk<br>104 wk<br><a href="#">Benson et al.<br/>(2011)</a><br>(cont.) |                                                                                                                                                                                                                                                                                           | <i>Thyroid gland</i><br>Follicular cell adenoma<br>0/50, 2/29, 0/27, NS<br>2/50<br>Follicular cell carcinoma<br>0/50, 0/29, 2/27, NS<br>0/50<br>Follicular cell adenoma and carcinoma<br>(combined)<br>0/50, 2/29 (7%), NS<br>2/27 (7%),<br>2/50 (4%)<br><i>Spleen</i><br>Mononuclear cell leukaemia<br>32/50, 23/34, NS<br>25/38, 32/50 |                                                                                                                          |                                                                                                                                                                                                                      |
| Full<br>carcinogenicity<br>Rat, F344 (CDF<br>F344 CrIbR) (F)<br>7–8 wk<br>104 wk<br><a href="#">Benson et al.<br/>(2011)</a>            | Inhalation (whole-body exposure)<br>Unleaded GVC, (benzene<br>concentration, 1.9–2.1%)<br>Air<br>0, 2, 10, 20 g/m <sup>3</sup> , 6 h/day (plus<br>14 min, the time for the vapour<br>concentration to reach 90% of<br>equilibrium), 5 days/wk, 104 wk<br>50, 50, 50, 50<br>29, 27, 27, 33 | <i>Spleen</i><br>Mononuclear cell leukaemia<br>13/50, 14/25*,<br>18/32**, 15/50                                                                                                                                                                                                                                                          | [* <i>P</i> = 0.0113, one-<br>tailed Fisher exact<br>test;<br>** <i>P</i> = 0.0059, one-<br>tailed Fisher exact<br>test] | <i>Principal strengths:</i> Well-conducted long-term study,<br>adequate duration of exposure and observation, adequate<br>number of animals per group, male and female animals<br>used, multiple concentration used. |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                            | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                                                                                                                                                                                          | Tumour<br>incidence                                                                                                                    | Significance                                                  | Comments                                                                                                                                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, F344 (M)<br>6 wk<br>107wk<br><a href="#">MacFarland et al. (1984)</a>                        | Inhalation (whole-body exposure)<br>Unleaded gasoline, (benzene concentration, ≤ 2%)<br>Filtered air<br>0, 67, 292, 2056 ppm<br>6 h/day, 5 days/wk for up to 107 wk<br>100, 100, 100, 100<br>NR                                                                                                                                                                                                                                                                     | <i>Kidney</i><br>Carcinoma<br>Total tumours,<br>0, 1, 2, 6<br>Carcinoma, adenoma or sarcoma (combined)<br>Total tumours,<br>0, 1, 5, 7 | NA<br><br>NA                                                  | <i>Principal strengths:</i> Well-conducted study, adequate duration of exposure and observation, male and female animals used, adequate number of animals.<br><br><i>Principal limitations:</i> Inadequate reporting of results. Tumour data were combined, limiting rat counts and preventing statistical analysis by the Working Group. |
| Full<br>carcinogenicity<br>Rat, F344 (F)<br>6 wk<br>109 wk<br><a href="#">MacFarland et al. (1984)</a>                       | Inhalation (whole-body exposure)<br>Unleaded gasoline, (benzene concentration, ≤ 2%)<br>Filtered air<br>0, 67, 292, 2056 (ppm)<br>6 h/day, 5 days/wk for up to 109 wk<br>100, 100, 100, 100<br>NR                                                                                                                                                                                                                                                                   | <i>Kidney</i><br>Carcinoma, adenoma or sarcoma (combined)<br>Total tumours,<br>0, 0, 1, 0                                              | NA                                                            | <i>Principal strengths:</i> Well-conducted study, adequate duration of exposure and observation, male and female animals used, adequate number of animals.<br><br><i>Principal limitations:</i> Inadequate reporting of results. Tumour data were combined, limiting rat counts and preventing statistical analysis by the Working Group. |
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, F344 (M)<br>5–6 wk<br>24 wk<br><a href="#">Short et al. (1989a)</a> | Inhalation (whole-body exposure)<br>Unleaded gasoline, NR<br>Filtered air<br>EHEN, EHEN + 10 ppm unleaded gasoline, EHEN + 70 ppm unleaded gasoline, EHEN + 300 ppm unleaded gasoline<br>(EHEN, 170 ppm for 2 wk in drinking-water) followed by a 4-wk holding period and then inhalation exposure to 0, 10, 70, or 300 ppm unleaded gasoline (mean concentration, 0, 10, 69, or 298 ppm) for 24 wk, and euthanized at 65–67 wk<br>30, 30, 30, 30<br>29, 29, 27, 28 | <i>Kidney</i><br>Renal cell tumours<br>0/29, 1/29, 1/27,<br>4/28                                                                       | $P < 0.05$ , [ $P = 0.0126$ ],<br>Cochran–Armitage trend test | <i>Principal strengths:</i> Adequate duration of exposure (24 wk), multiple doses.<br><br><i>Principal limitations:</i> Only males used.<br><br><i>Other comments:</i> Based on the results of the initiation–promotion studies, the possibility of a promoting effect of unleaded gasoline could not be excluded.                        |



**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                               | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                                                                                                                                                                                                                 | Tumour<br>incidence                                                 | Significance | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|---------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, F344 (M)<br>5–6 wk<br>65–67 wk<br><a href="#">Short et al. (1989a)</a> | Inhalation (whole-body exposure)<br>Unleaded gasoline, NR<br>Filtered air<br>EHEN, EHEN + 10 ppm unleaded<br>gasoline, EHEN + 70 ppm unleaded<br>gasoline, EHEN + 300 ppm<br>unleaded gasoline<br>(EHEN, 170 ppm for 2 wk in<br>drinking-water), followed by a<br>4-wk holding period and then<br>inhalation exposure to 0, 10, 70,<br>or 300 ppm unleaded gasoline<br>(mean concentration, 0, 10, 69,<br>and 298 ppm) until euthanasia at<br>65–67 wk<br>30, 30, 30, 30<br>29, 28, 27, 27 | <i>Kidney</i><br>Renal cell tumours<br>1/29, 0/28, 1/27, NS<br>2/27 |              | <i>Principal strengths:</i> Males and females used, multiple doses.<br><i>Principal limitations:</i> At 65 wk, the effects were no longer<br>observed because the incidence of renal tumours was also<br>increased in animals receiving only the initiators (intrinsic<br>limitation).<br><i>Other comments:</i> Based on the results of the initiation–<br>promotion studies, the possibility of a promoting effect of<br>unleaded gasoline could not be excluded. |
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, F344 (F)<br>5–6 wk<br>65–67 wk<br><a href="#">Short et al. (1989a)</a> | Inhalation (whole-body exposure)<br>Unleaded gasoline, NR<br>Filtered air<br>EHEN, EHEN + 10 ppm unleaded<br>gasoline, EHEN + 70 ppm unleaded<br>gasoline, EHEN + 300 ppm<br>unleaded gasoline<br>(EHEN, 170 ppm for 2 wk in<br>drinking-water), followed by a<br>4-wk holding period and then<br>inhalation exposure to 0, 10, 70,<br>or 300 ppm unleaded gasoline<br>(mean concentration, 0, 10, 69,<br>and 298 ppm) until euthanasia at<br>65–67 wk<br>31, 31, 31, 31<br>29, 29, 29, 28 | <i>Kidney</i><br>Renal cell tumours<br>1/29, 0/29, 2/29, NS<br>2/28 |              | <i>Principal strengths:</i> Males and females used, multiple doses.<br><i>Principal limitations:</i> At 65 wk, the effects were no longer<br>observed because the incidence of renal tumours was also<br>increased in animals receiving only the initiators (intrinsic<br>limitation).<br><i>Other comments:</i> Based on the results of the initiation–<br>promotion studies, the possibility of a promoting effect of<br>unleaded gasoline could not be excluded. |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Tumour<br>incidence                                                                            | Significance | Comments                                                                                                                                                                                       |
|----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Initiation–<br>promotion (tested<br>as initiator)<br>Rat, F344 (M)<br>5 to 6 wk<br>24 wk<br><a href="#">Short et al. (1989a)</a> | Inhalation (whole-body exposure)<br>Unleaded gasoline, NR<br>Filtered air<br>Control, 10 ppm unleaded<br>gasoline, 70 ppm unleaded<br>gasoline, 300 ppm unleaded<br>gasoline, EHEN, 10 ppm unleaded<br>gasoline + EHEN, 70 ppm unleaded<br>gasoline + EHEN, 300 ppm<br>unleaded gasoline + EHEN<br>Inhalation exposure to unleaded<br>gasoline at 0, 10, 70, or 300 ppm<br>(mean concentration, 0, 10, 69,<br>and 298 ppm) for 24 wk, followed<br>by 4-wk holding period, and then<br>170 ppm EHEN in drinking-water<br>for 2 wk and euthanized at 65–7 wk<br>31, 30, 30, 30, 30, 30, 30, 30<br>28, 27, 29, 26, 29, 29, 29, 27 | <i>Kidney</i><br>Renal cell tumours<br>1/28, 0/27, 0/29, NS<br>0/26, 1/29, 1/29,<br>0/29, 0/27 |              | <i>Principal strengths:</i> Multiple doses used.<br><i>Principal limitations:</i> Only males used.<br><i>Other comments:</i> Unleaded gasoline was not acting as an<br>initiator in the study. |

bw, body weight; EHEN, *N*-ethyl-*N*-hydroxyethylnitrosamine; F, female(s); F344, Fischer 344; GVC, gasoline vapour condensate; h, hour(s); M, male(s); min, minute(s); NA, not applicable; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s).

automotive gasoline sold in the USA in 1976. The concentration of benzene was adjusted to up to 2% in the formulated gasoline to align with the upper concentration of benzene in gasoline in the USA at the time. Survival was not specifically reported, although the authors stated that death rates were higher in male mice at 67 and 292 ppm than in controls, and lower in male mice at 2056 ppm than in controls. Similarly, body-weight data were not reported. The authors stated that the body weight of male mice in the group at 2056 ppm was significantly lower than that of control animals between week 66 and termination. Groups of 10 male and 10 female mice were evaluated at interim time points of 3, 6, 12, and 18 months and study termination [number not specified]. Exposure groups were evaluated when survival reached 40%, which occurred during week 107, 103, 103, and 107 for male mice in the groups at 0, 67, 292, and 2056 ppm, respectively, and during week 113 for all groups of female mice. After euthanasia, gross evaluation and necropsy were performed, with a large majority of tissues being sampled for histopathology.

In males, the incidence [given as a percentage] of hepatocellular adenoma or carcinoma (combined) was 45%, 36%, 45%, and 44% in the groups at 0, 67, 292, and 2056 ppm, respectively. In female mice at the 18-month interim, there was a dose-related increase in the incidence of hepatocellular adenoma or carcinoma (combined) 14%, 19%, 21%, and 48% of animals in the groups at 0, 67, 292, and 2056 ppm, respectively. In female mice in the group at 2056 ppm, there were two animals with renal tumours, one with bilateral renal tubular adenocarcinoma and one with a papillary cystic adenoma in the renal cortex. No renal tumours were diagnosed in the other groups of female mice. [The Working Group noted that renal neoplasms are rare in this strain.]

Several of the hepatocellular carcinomas in mice metastasized to the lungs. At study termination, lung metastases were observed in 7% of

male mice in the group at 292 ppm and 2% in the group at 2056 ppm, but not in female mice. Among moribund male mice and those that died during the study, 20% in the control group had tumours that metastasized to the lungs. In moribund female mice and those that died during the study, lung metastases were present in 6% of animals in the control group, 10% in the group at 67 ppm, and 7% in the group at 2056 ppm. [The Working Group noted that tumour incidence was reported as percentages. It was unclear how many tumour-bearing mice or how many mice in total were used for the calculation of these percentages. The Working Group also noted that statistics were not reported by [MacFarland et al. \(1984\)](#) and were not performed by the Working Group because of the limited reporting of data. Neither the number of animals with tumours nor the number of animals sampled in the period between 18 months and termination was reported. For liver tumours, only the combined incidence of hepatocellular adenoma and carcinoma was reported. Interpretation of the combined liver tumours was difficult in male mice because a meaningful increase in the incidence of hepatocellular carcinoma could be masked by reporting of the combination of benign and malignant liver tumours.]

Regarding non-neoplastic lesions, none were considered to be related to exposure to gasoline.

[The Working Group noted that this was a well-conducted study strengthened by the use of adequate durations of exposure and observation, inclusion of males and females, multiple doses, and a large number of animals per group; however, it was limited by poor reporting of results.]

### 3.1.3 Initiation–promotion studies

[The studies described below with unleaded gasoline were marked for inclusion and evaluated by the Working Group as uninformative. Specific notes are presented below each publication.]

Several studies investigated the tumour-promoting activity of unleaded gasoline ([Standeven and Goldsworthy, 1993](#); [Standeven et al., 1994a, 1995](#); [Moser et al., 1997a, b](#)). In two studies by [Standeven and Goldsworthy \(1993\)](#) and [Standeven et al. \(1994a, 1995\)](#), only the incidence of gross lesions was presented. The study by [Moser et al. \(1997a\)](#) included information about the incidence of hepatic foci; however, there was no histological description of the lesions or classification of the foci, and no presentation of the number of animals with a diagnosis of foci. Elsewhere, similar to other publications in this section, only macroscopic findings were discussed. In the study by [Moser et al. \(1997b\)](#), the total incidence of histopathological lesions was not presented. For example, the incidence of positive [for epidermal growth factor receptor (EGFR) immunoreactivity] basophilic or acidophilic foci and of adenoma and carcinoma was reported (number of EGFR-reactive adenomas/number of adenomas), but the incidence of these lesions per animal was not reported (number of animals with adenoma/number of animals). [For the above reasons, the Working Group considered that these studies were uninformative for the evaluation of the carcinogenicity of gasoline in experimental animals, and they were not tabulated.]

### 3.1.4 Administration with known carcinogens or other agents (inhalation)

In a study of chronic toxicity and carcinogenicity ([Falk and Kotin, 1956](#)), groups of 350 [male and female combined] Strain A mice (age, 10–12 weeks) were exposed by whole-body inhalation to smog or washed air for up to 52 weeks. The smog atmosphere was generated by reacting volatilized leaded gasoline and nitrogen with ozone before distribution into the exposure chamber. Over the course of the study, the concentration of ozone in the smog exposure chamber was approximately 1–3.8 ppm. [The Working

Group noted that this exposure was designed to be chemically similar and at concentrations relevant to air pollution in Los Angeles, USA. As described by the authors, the chemistry of the ultimate exposure atmosphere was complex and not well characterized. Information was not provided on the number of hours of exposure per day or number of days per week.] The animals were removed at 4-week intervals, beginning at week 16 of the study. At weeks 16, 20, and 24, 14–15 females per group were removed for interim evaluation. At weeks 28 and 32, 44–45 female mice per group were removed for evaluation. At weeks 36, 40, and 52, 75, 89, and 39 mice [males and females combined] per group, respectively, were removed for evaluation. The average body weight of animals exposed to smog was lower than that of animals exposed to washed air throughout the study duration. All mice underwent a complete necropsy, and representative sections of all visceral organs were examined histologically. The lungs were grossly examined at necropsy, using a dissecting microscope, to detect the presence of pulmonary adenomas.

The incidence of all tumours in all organs in male and female mice (combined) was significantly higher [ $P < 0.01$ , Fisher exact test] in the smog-exposed animals (181/336) than in animals exposed to washed air (control chamber) (83/338). [The Working Group noted several limitations for this study, including the poor description of the dosing regimen, lack of specific pathology diagnoses or tissue distribution of the tumours, the combination of data for males and females, and use of a single exposure concentration. Therefore, the Working Group considered that this study was uninformative for the evaluation of the carcinogenicity of gasoline in experimental animals, and it was not tabulated.]

Another study of chronic toxicity and carcinogenicity ([Kotin et al., 1958](#); some details reported in the study by [Falk and Kotin, 1956](#)) was conducted at the same time and in the same exposure chambers as that using Strain A mice

described above ([Falk and Kotin, 1956](#)). In this study, groups of 405 [male and female combined] C57BL mice (age, 10–12 weeks) were exposed by whole-body inhalation to smog [leaded gasoline] or washed air for up to 92 weeks. Survival in smog-exposed animals was significantly reduced compared with controls. This reduced survival was evident as early as week 23 of the study. The average body weight of animals in the smog chamber was lower than that of animals in the washed air chamber throughout the study duration. All mice underwent a complete necropsy, and representative sections of all visceral organs were examined histologically. The lungs were grossly examined at necropsy, using a dissecting microscope, to detect the presence of pulmonary adenomas.

The incidence of total tumours in the lung was significantly higher [ $P = 0.0373$ , Fisher exact test] in smog-exposed animals (15/405) than in animals in the control group (6/405). The incidence and severity of epithelial hyperplasia of the bronchi and bronchioles were higher in smog-exposed animals than in animals in the control group. [The Working Group noted several limitations of the design and reporting of this study. Many experimental details were not presented or were included only in the publication by [Falk and Kotin \(1956\)](#) and conflicted with details in the publication by [Kotin et al. \(1958\)](#). For example, the duration of exposure was described as 52 weeks in the paper by [Falk and Kotin \(1956\)](#), although described by [Kotin et al. \(1958\)](#) in the results text as being 92 weeks for the C57BL mice. The number of animals evaluated histologically was not clear, and therefore tumour incidence could not be generated. The reported number of animals with lung tumours in the control group was inconsistent in Tables 2, 3, and 4 in the publication. Other limitations included the lack of reporting of specific tumour pathology, the combination of data for males and females, and the use of a single exposure concentration. Therefore, the Working Group considered that

this study was uninformative for the evaluation of the carcinogenicity of gasoline in experimental animals, and it was not tabulated.]

## 3.2 Rat

### 3.2.1 Oral administration (gavage)

In a well-conducted study of chronic toxicity and carcinogenicity ([Maltoni et al., 1997](#)), groups of 50 male and 50 female Sprague-Dawley rats (age, 7 weeks) were dosed by gavage with unleaded gasoline in olive oil at daily doses of 0 (olive oil only), 500, or 800 mg/kg body weight (bw), four times per week (Monday, Tuesday, Thursday, Friday), for up to 104 weeks. After dosing for 104 weeks, the animals were kept on study until 123 weeks. The composition of the gasoline used in the study was reported by weight percentage of constituents and included 5–5.6% benzene, based on the analytical method used. The authors reported reduced survival in male and female rats dosed with unleaded gasoline at 500 and 800 mg/kg bw. [The Working Group noted that survival did not appear to have been analysed for statistical significance. Body weights were not presented in the publication.] At study termination, necropsy was conducted on all animals, and all tissues were examined microscopically.

In male rats, there was a significant positive trend [ $P = 0.0355$ , Cochran–Armitage trend test] in the incidence of total malignant tumours of the nasal cavity [considered by the Working Group to be rare tumours] – 0/50, 0/50, 3/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively. For total malignant tumours (all sites), there was a non-significant increase in incidence in the group at 800 mg/kg bw compared with controls – 12/50, 7/50, 15/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively.

In female rats, the incidence of total malignant tumours (all sites) was higher (not significantly) in the animals dosed with unleaded



gasoline than in the controls – 11/50, 18/50, 16/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively. There was a slight, non-significant, dose-related increase in the incidence of mammary tumours – 3/50, 5/50, 8/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively. The incidence of neurinosarcoma of the uterus-vagina was higher (not significantly) in the groups treated with unleaded gasoline than in the controls – 0/50, 2/50, 3/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively. [Although the incidence of neurinosarcoma of the uterus-vagina was not significantly increased, these tumours were considered rare. The Working Group considered neurinosarcomas to be malignant schwannomas.] [The Working Group noted that this was a long-term study strengthened by the use of an adequate duration of exposure and observation, an adequate number of animals per group, inclusion of males and females, and two doses; however, the study was limited by the fact that many of the pathological findings presented in the publication were not reported by histological type, but rather as a general observation (e.g. tumours of the nasal cavity, tumours of the mammary gland).]

In a concurrent, well-conducted study of chronic toxicity and carcinogenicity by [Maltoni et al. \(1997\)](#), groups of 50 male and 50 female Sprague-Dawley rats (age, 7 weeks) were dosed by gavage with leaded gasoline in olive oil at doses of 0 (olive oil only), 500, or 800 mg/kg bw, four times per week (Monday, Tuesday, Thursday, Friday), for up to 104 weeks. After dosing for 104 weeks, the animals were kept on study until 123 weeks. [The composition of the leaded gasoline was not reported.] The authors reported reduced survival in male and female rats dosed with leaded gasoline at 800 mg/kg bw and in females at 500 mg/kg bw. [The Working Group noted that survival did not appear to have been analysed for statistical significance. Body weights were not presented in the publication.] At study termination, a systematic necropsy was

conducted on all animals, and all tissues were examined microscopically.

In male rats, there was a higher incidence (17/50) of total malignant tumours in the group at 500 mg/kg bw than in the controls (12/50), although the incidence was not statistically significant. The incidence of head tumours was also higher (non-significantly) in the groups dosed with leaded gasoline than in the controls – 1/50, 2/50, and 3/50 for the groups at 0, 500, and 800 mg/kg bw, respectively.

In female rats, there was a significant increase [ $P=0.0163$ , Fisher exact test] in the incidence of total malignant tumours in the group at 500 mg/kg bw – 11/50, 22/50, 17/50 for the groups at 0 (control), 500 and 800 mg/kg bw, respectively. There was also a significant increase [ $P=0.0039$ , Fisher exact test] in the incidence of all malignant tumours of the uterus-vagina in the group at 500 mg/kg bw – 1/50, 10/50, 2/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively. The incidence of head osteosarcoma was higher, but non-significantly, in the gasoline-dosed animals than in the controls – 0/50, 1/50, and 2/50 for the groups at 0 (control), 500, and 800 mg/kg bw, respectively. [The Working Group noted that this was a long-term study strengthened by the use of an adequate duration of exposure and observation, an adequate number of animals per group, inclusion of males and females, and two doses. However, the Working Group noted that the study was limited by the fact that many of the pathological findings presented in the publication were not reported by histological type, but rather as a general observation (e.g. tumours of the nasal cavity, tumours of the mammary gland).]

### 3.2.2 Inhalation (whole-body)

In a well-conducted carcinogenicity study that complied with Good Laboratory Practice (GLP) ([Benson et al., 2011](#)), groups of 50 male and 50 female Fischer 344 (CDF F344 CrlBR) rats (age,



7–8 weeks) were exposed by whole-body inhalation to unleaded gasoline vapour condensate (GVC) at a concentration of 0, 2, 10, or 20 g/m<sup>3</sup> in air for 6 hours per day (plus 14 minutes, the time for the vapour concentration to reach 90% of equilibrium), 5 days per week, for up to 104 weeks. The GVC used in the study was well characterized and included 1.9–2.1% benzene (based on lot). There was no effect of GVC exposure on survival in males or females. At study termination, survival was 18/50, 22/50, 26/50, and 17/50 in males, and 29/50, 27/50, 27/50, and 33/50 in females, for the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively. Body weights of animals in the groups at 10 g/m<sup>3</sup> (males) and 20 g/m<sup>3</sup> (males and females) were lower than those of the controls throughout the study. In females at 10 g/m<sup>3</sup>, body weights were lower than controls after day 122. A complete necropsy was performed on all animals, and selected tissues were processed for histopathological evaluation. The tissues from the kidney and respiratory tract (lung, larynx, trachea, nasal turbinate) of male and female rats, and testes (males) were examined in all animals in all groups. All other tissues were evaluated in all animals in the control group and group at 20 g/m<sup>3</sup>, and animals that experienced early death or became moribund in all groups.

In male rats, there was a significant increase [ $P=0.0297$ , Fisher exact test] in the incidence of adenoma or carcinoma (combined) of the renal tubule at the dose of 10 g/m<sup>3</sup> – 1/50, 1/50, 7/50, and 0/50 for the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively. There was a slight but non-significant increase in the incidence of both adenoma (1/50, 1/50, 4/50, 0/50) and carcinoma (0/50, 0/50, 3/50, 0/50) of the renal tubule in the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively. However, these increases were not dose-related.

In male rats, squamous cell carcinoma of the nasal cavity at turbinate levels 2, 3, and 4 was found in animals exposed to GVC at 20 g/m<sup>3</sup>. At turbinate level 3 of the nasal cavity, there was

a significant positive trend in the incidence of squamous cell carcinoma of the nasal cavity – 0/50, 0/50, 0/49, and 3/50 in the groups at 0, 2, 10, and 20 g/m<sup>3</sup>, respectively [ $P = 0.015$ , Cochran–Armitage trend test]. At turbinate levels 2 and 4 of the nasal cavity, there was a slight but non-significant increase in the incidence of squamous cell carcinoma of the nasal cavity – 0/50, 0/50, 0/50, and 1/50 for level 2, and 0/50, 1/50, 0/49, and 3/50 for level 4, in the groups at 0, 2, 10, and 20 g/m<sup>3</sup>, respectively. The morphology of these tumours was described as having an origin in the oral mucosa and nasal passage invasion. [The Working Group noted that the incidence of squamous cell carcinoma of the nasal cavity across turbinate levels (combined) was not provided by the authors, and it did not appear that the oral cavity was evaluated histologically.] There was a non-significant increase in the incidence of follicular cell adenoma of the thyroid gland – 0/50, 2/29, 0/27, and 2/50 for the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively. There was a non-significant increase in the incidence of follicular cell carcinoma of the thyroid gland – 0/50, 0/29, 2/27, and 0/50 for the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively. There was a slight but non-significant increase in the incidence of follicular cell adenoma and carcinoma (combined) of the thyroid gland – 0/50, 2/29 (7%), 2/27 (7%), and 2/50 (4%) for the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively – with the incidence at all concentrations exceeding that for historical controls (1.83%, 19/1041; [NTP, 1999](#)). The incidence of mesothelioma of the testes and of mononuclear cell leukaemia in the spleen was not significantly increased.

In female rats, there was a significant increase in the incidence of mononuclear cell leukaemia in the spleen in the groups at 2 g/m<sup>3</sup> [ $P = 0.0113$ , Fisher exact test] and 10 g/m<sup>3</sup> [ $P = 0.0059$ , Fisher exact test] compared with the vehicle control group – 13/50, 14/25, 18/32, and 15/50 for the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively.

[The Working Group considered the adenoma or carcinoma (combined) of the renal tubule and the squamous cell carcinoma of the nasal cavity to be treatment-related. The Working Group considered that the increase in incidence of mononuclear cell leukaemia in the spleen was incidental, and that the high incidence observed in the groups at 2 and 10 g/m<sup>3</sup> was probably a result of the small number of animals evaluated in these groups. In addition, this is a very well-known background pathology of Fischer 344 (F344) rats. The Working Group noted that the incidence of all these tumours was within the range observed for historical controls.]

Regarding non-neoplastic lesions, chronic progressive nephropathy (CPN) was observed in nearly all males and females in all groups, with a slightly higher severity in rats in GVC-exposed groups than in controls. In males, average severity was 2.4 in the control group, and 2.9 in the groups at 10 and 20 g/m<sup>3</sup>. In females, average severity was 0.66 in the control group, and 0.92 and 0.86 in the groups at 10 and 20 g/m<sup>3</sup>, respectively. [The Working Group noted that other features identified in non-neoplastic lesions of the kidney, such as protein droplets or identification of  $\alpha_{2u}$ -globulin, were not reported in this publication. In addition, histopathology was not evaluated or reported at earlier time points that would allow the incidence of CPN to inform a potential mechanism.] There were statistically significant and exposure-related increases in the incidence of respiratory epithelial degeneration in the nose at turbinate level 2 (males and females) and level 3 (females). [The Working Group noted that this was a well-conducted, long-term study strengthened by the use of an adequate duration of exposure and observation, and adequate number of animals per group, inclusion of males and females, and multiple doses.]

In a well-conducted study of chronic toxicity and carcinogenicity ([MacFarland et al., 1984](#)), groups of 100 male and 100 female F344 rats (age, 6 weeks) were exposed by whole-body inhalation

to unleaded automotive motor fuel (gasoline) at mean concentrations of 0, 67, 292, and 2056 ppm in filtered air for 6 hours per day, 5 days per week, for up to 113 weeks. [The Working Group noted that the target concentrations of the study were 0, 50, 275, and 1500 ppm. The reported values aligned with the actual exposure concentrations based on recalibration of the online monitoring gas chromatograph.] The unleaded gasoline used in the study was thoroughly characterized and formulated to represent automotive gasoline used in the USA in 1976. The concentration of benzene was adjusted to up to 2% in the formulated gasoline, to align with the upper concentration of benzene in gasoline in the USA at the time. [Survival was not specifically reported.] The authors reported that male rats in the control group had a higher spontaneous death rate than those in the exposed groups after week 80. Body-weight data were not reported; the authors described rats at 2056 ppm as having significantly lower body weights than those of rats in the control group from week 13 (male) or week 26 (female) to termination. Groups of 10 males and 10 females were evaluated at interim time points of 3, 6, and 12 months, and at 18 months and study termination [number not specified]. Exposure groups were evaluated when survival reached 40%, which occurred during week 107 for all groups of male rats and week 109 for all groups of female rats. After euthanasia, gross evaluation and necropsy were performed, with a large majority of tissues being sampled for histopathology.

The incidence of renal carcinoma in male rats in the 18-month interim groups increased in a dose-related manner – 0, 1, 2, and 6 tumours in the groups at 0, 67, 292, and 2056 ppm, respectively. The total number of renal carcinomas, adenomas, or sarcomas (combined) was also increased – 0, 1, 5, and 7 tumours in the groups at 0, 67, 292, and 2056 ppm, respectively.

[The Working Group noted a discrepancy in the number of total renal tumours in male rats

reported in the text (0, 1, 6, 7) and in Table 7 (0, 1, 5, 7) of the original publication. The number of each tumour described in the text (3 adenomas, 9 carcinomas, 1 sarcoma) aligned with the number of total tumours in Table 7, and this was brought forward to [Table 3.1](#) in the present monograph.]

In female rats, one animal in the group at 292 ppm was diagnosed with a renal sarcoma. No renal tumours were diagnosed in the other groups of female rats. [Because tumour incidence was reported as total tumours for both males and females, it was unclear how many tumour-bearing mice or how many mice in total contributed to these total tumours. Therefore, no statistics could be calculated by the Working Group.]

Regarding non-neoplastic lesions, beginning at the 3-month interim time point, there were exposure-related increases in the incidence of histopathological changes in the male rat kidney. [The Working Group noted that the histopathological diagnostic terminology used in this study differed slightly from the current standard terminology for kidney lesions, particularly that relevant for  $\alpha_{2u}$ -mediated nephropathy, although the lesions were analogous to those considered for the interpretation of mechanistic effects.] The lesions observed were described as proteinaceous casts and chronic interstitial inflammation. Protein casts occurred within dilated renal tubules and were commonly located at the corticomedullary junction. Between 3 and 6 months, male rats in the groups at 2056 ppm were also observed to have mineralization with the renal pelvis. Progressive glomerulonephrosis, described as atrophied or sclerosed glomeruli, dilated renal tubules containing proteinaceous casts, tubular damage with regeneration or scarring, and the presence of foci of chronic inflammatory cells, was initially observed in a male at 2056 ppm at the 12-month interim time point. At study termination, progressive glomerulonephrosis was observed in 95–100% of males in all groups.

[The Working Group noted that this lesion was not evaluated for severity.] Mineralization was observed in a concentration-dependent manner in 5%, 63%, and 91% of male rats in the groups at 67, 292, and 2056 ppm. At study termination, there was one male rat with renal epithelial hyperplasia in the group at 292 ppm. [The Working Group noted that this study was well conducted, strengthened by the use of an adequate duration of exposure and observation, inclusion of males and females, and a large number of animals per group; however, it was limited by poor reporting of results.]

### 3.2.3 Initiation–promotion studies

An initiation–promotion study was conducted concurrently and in the same exposure chambers as described above for F344 rats ([Short et al., 1989a](#)). In this study, groups of 30 male F344 rats (age, 5–6 weeks) were initially treated with drinking-water containing the initiator *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) at 170 ppm for 2 weeks. After a 4-week holding period, the rats were subsequently exposed to unleaded gasoline (promoter; abbreviated “UG” in the paper) at a concentration of 0, 10, 70, or 300 ppm by inhalation for 24 consecutive weeks and then held until euthanasia at 65–67 weeks for histopathological analysis. The mean concentrations of unleaded gasoline in the chambers were 0, 10, 69, and 298 ppm. Spontaneous death rates were unaffected in all treated groups and did not exceed 12% in any group. No significant differences in terminal body weights were observed in any groups [statistical data were not shown]. All surviving rats were euthanized between 65 and 67 weeks (mean, 66 weeks) after the start of the experiments. All rats underwent a complete necropsy, and both kidneys were examined histologically using haematoxylin and eosin staining.

In male rats, there was a significant positive trend ( $P < 0.05$ , [ $P = 0.0126$ ], Cochran–Armitage trend test) in the incidence of renal cell tumours – 0/29 (0%), 1/29, 1/27, and 4/28 for the groups receiving EHEN, EHEN plus 10 ppm unleaded gasoline, EHEN plus 70 ppm unleaded gasoline, EHEN plus 300 ppm unleaded gasoline, respectively. All neoplasms were classified as adenomas, with no observed metastasis.

[The discussion section of the study indicated that “Statistical analysis (Cochran–Armitage trend test) of the incidence of renal cell tumours during 24 or 60 weeks of unleaded gasoline promotion demonstrated a promoting effect at 24 ( $P < 0.02$ ) but not 64 weeks ( $P < 0.01$ )”. Using the CATrend tool (<https://biostatistics.dkfz.de/CATrendv1.0/>), the analysis compared data from the initiation control (week 30, Fig. 2 of the publication) with the initiation–promotion data (Fig. 2 of the publication), and the  $P$  value was equal to 0.0126. The alternative comparison between the initiation control (Fig. 1 of the publication) with the initiation–promotion data (Fig. 2 of the publication) yielded a  $P$  value of 0.04. This suggested that the analysis probably used the initiation control at week 30 as the control group.]

Regarding preneoplastic lesions, there was a significant positive trend in the incidence of renal atypical cell foci.

In another concurrent initiation–promotion experiment (Short et al., 1989a), groups of 30 male and 30 female F344 rats were initially treated with drinking-water containing EHEN (initiator) at 170 ppm for 2 weeks. After a 4-week holding period, the rats were subsequently exposed to unleaded gasoline (promoter) at 0, 10, 70, or 300 ppm until euthanasia at 65–67 weeks for histopathological analysis.

In male rats, there was no significant increase in the incidence of renal cell tumours – 1/29, 0/28, 1/27, and 2/27 in the groups treated with EHEN, EHEN + 10 ppm unleaded gasoline, EHEN + 70 ppm unleaded gasoline, and EHEN + 300 ppm unleaded gasoline, respectively.

In female rats, there was also no significant increase in the incidence of renal cell tumours.

Regarding preneoplastic lesions, the incidence of atypical cell foci was significantly increased with a positive trend across the different groups in males. The mean scores for CPN were slightly but significantly elevated in male rats exposed to unleaded gasoline at 300 ppm in the initiation–promotion groups. However, there were no exposure-related increases in histopathological changes in the females. [CPN was characterized by tubules lined with a single epithelial layer of cells that had scant basophilic cytoplasm, surrounded by a thickened basement membrane. The affected areas exhibited an increased presence of chronic inflammatory cells and fibrocytes, and adjacent glomeruli showed segmental or diffuse sclerosis. Each kidney was assigned a four-level severity score based on the number of foci of CPN observed per slide: minimal (0–5 foci), mild (6–10 foci), moderate (11–15 foci), and severe (16 or more foci).]

[The Working Group noted that the duration of 24 weeks was adequate. However, at 65 weeks, the effects were no longer observed because the incidence of renal tumours was also increased in animals receiving only the initiators. This was an intrinsic limitation of the study design.]

In a third concurrent study that was part of the initiation–promotion protocol (Short et al., 1989a), groups of 30 male F344 rats were initially exposed to unleaded gasoline at 0, 10, 70, or 300 ppm for 24 weeks, followed by a 4-week holding period, and then treated with drinking-water containing EHEN (promoter) at 170 ppm for 2 weeks and euthanized at 65–67 weeks for histopathological analysis.

There were no differences across all groups in the incidence of renal cell tumours – 1/28, 0/27, 0/29, 0/26, 1/29, 1/29, 0/29, and 0/27 for the control group and groups receiving 10 ppm unleaded gasoline, 70 ppm unleaded gasoline, 300 ppm unleaded gasoline, EHEN, 10 ppm unleaded gasoline plus EHEN, 70 ppm unleaded



gasoline plus EHEN, and 300 ppm unleaded gasoline plus EHEN, respectively.

Regarding preneoplastic lesions, there were no exposure-related increases in histopathological changes in the rat kidneys.

[Overall, the Working Group noted that, on the basis of the results of the initiation–promotion studies, the possibility that unleaded gasoline has a promoting effect could not be excluded. The Working Group also noted that in the study by [Short et al. \(1989a\)](#), unleaded gasoline was not acting as an initiator.]

### 3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of unleaded gasoline has been assessed in one GLP study in male and female F344 (CDF F344 CrIBR) rats treated by inhalation (whole-body) ([Benson et al., 2011](#)). The carcinogenicity of gasoline was also evaluated in well-conducted studies that did not comply with GLP. Specifically, unleaded gasoline was tested by oral administration (gavage) in male and female Sprague-Dawley rats ([Maltoni et al., 1997](#)); by skin application in male C3H/HeJ mice ([Broddle et al., 1996](#)); by inhalation (whole-body) in two concurrent studies in male and female B6C3F<sub>1</sub> mice and in male and female F344 rats ([MacFarland et al., 1984](#)). The carcinogenicity of leaded gasoline was tested by oral administration (gavage) in male and female Sprague-Dawley rats ([Maltoni et al., 1997](#)). In addition, several initiation–promotion studies using administration by inhalation were conducted in male B6C3F<sub>1</sub> mice ([Standeven et al., 1995](#)) and female B6C3F<sub>1</sub> mice ([Standeven and Goldsworthy, 1993](#); [Standeven et al., 1994a](#); [Moser et al., 1997a, b](#)); and in male and female F344 rats (24 and 65–67 weeks) ([Short et al., 1989a](#)). Moreover, two co-carcinogenicity studies using administration by inhalation (whole-body) were available in male and female

(combined) Strain A mice ([Falk and Kotin, 1956](#); [Kotin et al., 1958](#)).

In a well-conducted study that complied with GLP in male and female F344 (CDF F344 CrIBR) rats treated by inhalation (whole-body) ([Benson et al., 2011](#)), the incidence of renal tubule adenoma or carcinoma (combined) in males was significantly higher than in the controls. There was also a significant positive trend in the incidence of squamous cell carcinoma of the nasal cavity (turbinate level 3) in males. In females, the incidence of mononuclear cell leukaemia in the spleen was significantly higher than in the controls. [The Working Group considered the adenoma or carcinoma (combined) of renal tubule and squamous cell carcinoma of the nasal cavity to be treatment-related; however, the Working Group considered the increase in mononuclear cell leukaemia in the spleen to be incidental, because the observed incidence was inside the range for historical control data and this is a very well-known background pathology of F344 rats. The statistical significance was probably a result of the small number of animals evaluated in these groups.]

In a well-conducted study in male and female Sprague-Dawley rats treated with unleaded gasoline by oral administration (gavage) ([Maltoni et al., 1997](#)), there was a significant positive trend in the incidence of total malignant tumours of the nasal cavity in males. [Malignant tumours of the nasal cavity were considered to be rare neoplasms.]

In the well-conducted study in male and female B6C3F<sub>1</sub> mice treated with unleaded gasoline by inhalation (whole-body) ([MacFarland et al., 1984](#)), there was an increase in the incidence of hepatocellular adenoma or carcinoma (combined) in females. [The Working Group noted that, for liver tumours, only the combined incidence of hepatocellular adenoma and carcinoma was reported. Interpretation of the combined liver tumours was difficult in male mice because a meaningful increase in the

incidence of hepatocellular carcinoma could be masked by reporting of the combination of benign and malignant liver tumours.]

In the well-conducted study in male and female F344 rats treated with unleaded gasoline by inhalation (whole-body) ([MacFarland et al., 1984](#)), there was an increase in the number of renal carcinomas and the total number of renal carcinomas, adenomas, or sarcomas (combined) in males. [Because tumour incidence was reported as total tumours, it was unclear how many tumour-bearing rats or how many rats in total contributed to these total tumours. Therefore, no statistics could be calculated by the Working Group.]

In the well-conducted study in male and female Sprague-Dawley rats treated with leaded gasoline by oral administration (gavage) ([Maltoni et al., 1997](#)), there was a significant increase in the incidence of total malignant tumours and of all malignant tumours of the uterus-vagina in females.

In the initiation–promotion study in male F344 rats treated with unleaded gasoline by inhalation (24 weeks) ([Short et al., 1989a](#)), there was a significant positive trend in the incidence of renal cell tumours.

No significant increases in the incidence of neoplasms were observed in male and female F344 rats treated by inhalation (whole-body) (65–67 weeks) ([Short et al., 1989a](#)). [The Working Group noted that the duration of 24 weeks was adequate. However, at 65 weeks, the effects were no longer observed because the incidence of renal tumours also increased in animals receiving only the initiators. This was an intrinsic limitation of the study design.]

Studies in male C3H/HeJ mice treated by skin application ([Broddle et al., 1996](#)), and in male and female B6C3F<sub>1</sub> mice ([Standeven and Goldsworthy, 1993](#); [Standeven et al., 1994a](#); [1995](#); [Moser et al., 1997a, b](#)), male and female Strain A mice ([Falk and Kotin, 1956](#)), and male and female C57BL mice ([Kotin et al., 1958](#)) treated

by inhalation, were considered to be uninformative for the evaluation of the carcinogenicity of gasoline in experimental animals.

## 4. Mechanistic Evidence

### 4.1 Absorption, distribution, metabolism, and excretion

#### 4.1.1 Exposed humans

No specific data on the absorption, distribution, metabolism, and excretion (ADME) of automotive gasoline were available to the Working Group. The majority of its components (BTEX; alkanes, including branched alkanes such as trimethylpentane (TMP); cycloalkanes; alkenes; and cycloalkenes) are small non-polar molecules that are volatile and easily pass across barriers, such as cell membranes, in the lungs and elsewhere. Numerous studies in exposed humans have assessed the ADME of single gasoline components, including BTEX, and several alkanes and alcohols (not described here), which are easily absorbed and are widely distributed in tissues. Studies in workers exposed to gasoline, such as service station attendants, have shown that they excrete larger quantities of metabolites of major gasoline components, such as methylhippuric acid, SPMA, hippuric acid, and mandelic acid, than non-exposed workers (see Section 1.1, and Table S1.9).

[The Working Group noted that most gasoline components are absorbed via inhalation and through the skin, redistributed to various organs, and to a significant degree eliminated via exhalation and urinary excretion after exposure. This generalization might not apply to some of the many gasoline additives, including anti-knock agents, antioxidants, detergents, and dyes, or to two-stroke oil, which may be more polar and far less volatile. However, the metabolism and subsequent excretion of metabolites may



vary substantially, depending on the components present in the gasoline. In addition, the composition of inhaled gasoline vapour differs from that of liquid gasoline, owing to differences in the evaporation rates of the components (see Section 1.1.4). In addition, the percentage of each component of the gasoline that is absorbed by inhalation may differ, because of differences in blood solubility (or more correctly, blood/air partitioning). This also applies to dermal absorption, owing to differences in skin permeability.]

#### 4.1.2 Experimental systems

##### (a) *Non-human mammalian systems in vivo*

[Travis et al. \(1992\)](#) studied the metabolic pathway and interactions between benzene and gasoline vapour in male F344 rats exposed in a closed-chamber gas uptake exposure system. Exposure to gasoline vapour was associated with a reduction in the metabolism of benzene compared with the metabolism of benzene alone. The toxicokinetics of other gasoline components were not investigated.

[Benson et al. \(2001, 2003\)](#) compared the toxicokinetics of MTBE in rats that were exposed to radiolabelled  $^{14}\text{C}$ -MTBE only with those of rats exposed to gasoline containing MTBE. Male F344 rats were exposed nasally once for 4 hours to 4, 40, or 400 ppm  $^{14}\text{C}$ -MTBE, or to 20 or 200 ppm of the light fraction of unleaded gasoline containing 4 and 40 ppm  $^{14}\text{C}$ -MTBE, respectively. The effects of repeated inhalation of gasoline vapour on the fate of the inhaled MTBE were studied by repeated (4 hours per day, 7 days) exposure to gasoline at 20 or 200 ppm, followed on day 8 by exposure to gasoline containing  $^{14}\text{C}$ -MTBE. The co-exposure to gasoline, both acute and repeated, reduced the concentrations of MTBE equivalents in all the tissues studied (lung, liver, kidney, heart, brain, and testes), and increased the elimination rates of MTBE and its metabolites. The toxicokinetics of the other components of gasoline were not studied.

##### (b) *Physiologically based pharmacokinetic modelling*

[Dennison et al. \(2003, 2004a, b\)](#) developed a four-compartment physiologically based pharmacokinetic (PBPK) model and used a lumping approach to describe the toxicokinetics of gasoline in rats. The model tracked five target components (benzene, toluene, ethylbenzene, *o*-xylene, and *n*-hexane) and a lumped chemical group that represented all the non-target components. Competitive metabolic inhibition was assumed to occur between all the target components and the lumped chemical group. The model was fitted to the time courses of the changes in the concentrations of chemicals in chamber air during the closed-chamber exposure of male F344 rats to the target chemicals or gasoline for 6 hours, with starting concentrations of between approximately 200 and 2000 ppm. The kinetic parameters of the target chemicals were refined by studies performed with either the single chemical alone or with all five chemicals together. A six-chemical interaction model for the lumped chemical group was used to estimate the lumped chemical partitioning and metabolic parameters for a winter blend of gasoline containing MTBE and a summer blend not containing any oxygenate additive. The computer simulations were highly consistent with the experimental data for the single chemicals and the five-chemical mixture, as well as those for the winter and summer blends of gasoline. The modelling indicated that the metabolism of the individual components was inhibited by up to 27% during the closed-chamber experiments using gasoline. As for benzene, the predicted inhibition of metabolism was 17% at the highest simulated level of gasoline exposure, of 400 ppm. Because the various gasoline components differ in their volatility, the gasoline PBPK model was subsequently extended to better reflect the composition of the gasoline vapour that would be inhaled after the release of

gasoline into the environment ([Dennison et al., 2004a](#)).

[Jasper et al. \(2016\)](#) used a seven-compartment PBPK model to characterize the toxicokinetics of inhaled gasoline vapour in rats. Ten target chemicals (*n*-pentane, 2-methylpentane, toluene, *n*-hexane, cyclohexane, benzene, *m*-xylene, ethylbenzene, *p*-xylene, and *o*-xylene) were tracked separately, and 109 additional chemicals were lumped into clusters, which were iteratively increased in size from 0 to 99 chemicals. The model was fitted to experimental data derived from the nasal exposure of female Long–Evans rats to 1000 ppm gasoline for 6 hours. The data obtained were the chemical concentrations in the blood and tissues during exposure, measured repeatedly during the first hour post-exposure and after 4 and 6 hours. The simulations were improved by incorporating competitive inhibition. [The Working Group noted that the authors stated that blood and tissues were analysed, but the tissues were not listed, and no concentrations were presented, other than for blood.]

### Synopsis

[Overall, the Working Group noted that there was a lack of studies regarding the ADME of gasoline vapour. However, on the basis of the properties of the major gasoline components, experimental studies of single components, and bio-monitoring data from gasoline-exposed workers (reviewed in Section 1), the Working Group considered that most gasoline components can be absorbed via inhalation and through the skin, redistributed to various organs and, to a significant degree, eliminated via exhalation.

A few *in vivo* studies have shown that gasoline exposure reduces the metabolism of benzene and increases that of MTBE. PBPK modelling based on limited experimental data has indicated that the observed decreases in the metabolism of individual components could be explained by competitive inhibition.]

## 4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens (KCs) ([Smith et al., 2016](#)) encompassed by the agent automotive gasoline. Evidence was available for the KCs “is electrophilic or can be metabolically activated to an electrophile”, “is genotoxic”, “alters DNA repair and causes genomic instability”, “induces oxidative stress”, “induces epigenetic alterations”, “induces chronic inflammation”, “is immunosuppressive”, “modulates receptor-mediated effects”, and “alters cell proliferation, cell death, or nutrient supply”. No data were available for the KC “causes immortalization”. The studies in exposed humans used to support the mechanistic evidence were evaluated for the quality of the study design, exposure assessment, and assay accuracy and precision, and were found to have used suitable methods for epidemiological studies (see also Section 1.6.2). The evaluated studies in humans also accounted for important confounding and modifying variables. The determination of the Working Group was also sustained by mechanistic evidence from experimental systems.

### 4.2.1 *Is electrophilic or can be metabolically activated to an electrophile*

See [Table 4.1](#).

[The Working Group noted that the literature indicated that the metabolites of components of automotive gasoline, such as benzene, are potent electrophiles capable of forming DNA adducts.]

In two studies identified by the Working Group ([Naidoo et al., 2016](#); [Ahmadkhaniha et al., 2021](#)), DNA adducts were found to be associated with gasoline exhaust components, specifically those of benzo[*a*]pyrene and 1,3-butadiene. [The Working Group noted that these are key constituents of the gasoline exhaust, rather than gasoline vapour, and are known for their carcinogenic

**Table 4.1 End-points relevant to “is electrophilic” or “can be metabolically activated to an electrophile” in humans exposed to automotive gasoline**

| End-point                      | Biological sample | Location Setting, study design                                                    | Duration of exposure<br>No. of study participants                                                                       | Response <sup>a</sup><br>(significance) | Covariates controlled                                                                                                     | Comments                                                                                                                                                                                                                           | Reference                                  |
|--------------------------------|-------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| DNA adducts (BPDE)             | Peripheral blood  | Durban, South Africa<br>Cross-sectional<br>Service station attendants             | Workers exposed to fuel for 5.8 yr<br><i>n</i> = 101 exposed<br><i>n</i> = 50 controls                                  | + ( <i>P</i> < 0.0001)                  | The exposed and non-exposed participants in the study sample varied substantially with respect to key demographic factors | <i>Limitations:</i> No data about the environmental levels of benzene. The adducts were not related to gasoline vapour, but instead to gasoline exhaust.                                                                           | <a href="#">Naidoo et al. (2016)</a>       |
| DNA strand breaks (% tail DNA) |                   |                                                                                   |                                                                                                                         | + ( <i>P</i> < 0.01)                    |                                                                                                                           | <i>Other results:</i> Correlation between the BPDE–DNA adducts and % tail DNA. Cigarette smokers had higher % tail DNA and higher BPDE–DNA adduct concentrations than did non-smokers. Other parameters not related to end-points. |                                            |
| MHBVal–haemoglobin adduct      | Peripheral blood  | Tehran, Islamic Republic of Iran<br>Cross-sectional<br>Service station attendants | 13.82 µg/m <sup>3</sup> airborne levels of 1,3-butadiene for 60 days<br><i>n</i> = 25 exposed<br><i>n</i> = 25 controls | + ( <i>P</i> < 0.05)                    | Age, smoking habits (all non-smokers), sex (all men)                                                                      | <i>Limitation:</i> No data about exposure time. The adducts were not related to gasoline vapour, but instead to gasoline exhaust.                                                                                                  | <a href="#">Ahmadkhaniha et al. (2021)</a> |

BPDE, benzo[*a*]pyrene diol epoxide; MHBVal, 1- and 2-hydroxy-3-butenyl valine; yr, year(s).<sup>a</sup> +, positive.

potential and adverse effects on health ([Sjöström et al., 2019](#)). Therefore, the Working Group considered that these findings were not informative for the evaluation of automotive gasoline.]

#### 4.2.2 *Is genotoxic*

##### (a) *Humans*

##### (i) *Exposed humans*

See [Table 4.2](#).

[The Working Group noted that several studies evaluated multiple genotoxicity endpoints in exposed workers, mostly service station attendants, and performed correlation analysis of the duration of exposure (mostly, work shift hours and years of working), measured markers of exposure, and adjusted for covariates.]

[Rekhadevi et al. \(2010\)](#) reported significant increases in micronucleus frequency and DNA strand breaks in the peripheral blood lymphocytes of 200 service station attendants in India, compared with 200 non-exposed control participants. There were positive correlations between these genotoxic markers and factors such as the overall duration of exposure and the daily length of the exposure. Additionally, specific correlations were identified: the frequency of micronuclei positively correlated with benzene concentration in the air and xylene concentration in the blood; and the DNA strand breaks correlated with BTX concentrations in the air, and with xylene concentration in the blood. [Rekhadevi et al. \(2011\)](#), in the same population, observed a significant rise in the frequencies of micronuclei in exfoliated buccal cells and of chromosomal aberrations in peripheral blood lymphocytes in exposed individuals. [The Working Group noted that the strength of these studies were the significant sample size and the study design, which controlled for sex, age, smoking, and alcohol consumption. In addition, the authors examined the influence of factors such as the years of exposure and hours of

exposure per day, on DNA damage (micronuclei and DNA strand breaks), using a combination of exposure assessment markers (BTX in blood, BTX in air, and BTX in the breathing zone).]

[Keretetse et al. \(2008\)](#) identified increased levels of DNA strand breaks in 20 service station attendants from Potchefstroom, South Africa. The authors reported a significant positive relationship between the duration of exposure and the extent of basal DNA damage. Individuals who were exposed for >10 years showed the highest level of basal DNA damage, whereas those exposed for <1 year had minimal DNA damage, indicating a cumulative effect of long-term occupational exposure. [The Working Group noted that the study had a small sample size, but that factors such as sex, age, and smoking were controlled for.]

[Xiong et al. \(2016\)](#) documented increases in DNA strand breaks in peripheral blood lymphocytes and micronuclei frequency in exfoliated buccal cells from 200 service station attendants in Nanning, China, along with higher concentrations of oxidative stress markers in blood, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), than in non-exposed study participants (see also Section 4.2.5). They found that the levels of BTEX in refuelling areas were significantly higher than those in the office areas of the same service stations. In the refuelling areas, the average air levels of benzene, toluene, ethylbenzene, *m*-xylene, *p*-xylene, and *o*-xylene were 60.03, 317.76, 40.91, 113.85, 37.18, and 57.51 µg/m<sup>3</sup>, respectively. After adjustment for confounding factors, the frequency of micronuclei was found to be affected by the number of years of exposure. [The Working Group noted that one strength of the study was the comprehensive adjustment for potential confounding factors.]

**Table 4.2 End-points relevant to genotoxicity in humans exposed to automotive gasoline**

| End-point                         | Biological sample                    | Location Setting, study design                                               | Duration of exposure<br>No. of study participants                                                                                                                                             | Response <sup>a</sup><br>(significance)                                                                                                                                                                                          | Covariates controlled                  | Comments                                                                                                                                                                                                                                                                                                      | Reference                               |
|-----------------------------------|--------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| MN formation<br>DNA strand breaks | Peripheral blood<br>Peripheral blood | Hyderabad, India<br>Cross-sectional<br>Service station attendants            | Workers exposed to fuel for ~5 h per day, workers dispensed ~665 L of fuel per day for ≥ 5 yr (10.66 yr ± 3.58)<br><i>n</i> = 200 exposed<br><i>n</i> = 200 controls                          | + ( <i>P</i> < 0.05)<br><br>+ ( <i>P</i> < 0.05)<br>Positive correlations: MN with benzene in the air and with xylene in the blood; DNA strand breaks with benzene, toluene, and xylene in the air, and with xylene in the blood | Sex, age, smoking, alcohol consumption | Combination of markers of exposure: ↑ BTX in ambient air; ↑ BTX in breathing-zone air; ↑ benzene, xylene in blood.<br>Benzene in ambient air: 1322 µg/m <sup>3</sup> (0.414 ppm <sup>b</sup> ).<br>Age, years of exposure, hours per day of exposure, and smoking habits influenced MN and DNA strand breaks. | <a href="#">Rekhadevi et al. (2010)</a> |
| Chromosomal aberrations           | Peripheral blood lymphocytes         | Hyderabad, India<br>Cross-sectional<br>Service station attendants            | Workers exposed to fuel for ~8 h per day; workers dispensed ~665 L of fuel per day, for ≥ 5 yr (10.66 yr ± 3.58)<br><i>n</i> = 200 exposed<br><i>n</i> = 200 controls                         | + ( <i>P</i> < 0.05)                                                                                                                                                                                                             | Sex, age, smoking, alcohol consumption | Exposure measured using benzene and benzene metabolites (phenol, <i>t,t</i> -MA, SPMA) in urine, not corrected for creatinine concentration.                                                                                                                                                                  | <a href="#">Rekhadevi et al. (2011)</a> |
| MN formation                      | Exfoliated buccal cells              |                                                                              |                                                                                                                                                                                               | + ( <i>P</i> < 0.05)                                                                                                                                                                                                             |                                        |                                                                                                                                                                                                                                                                                                               |                                         |
| DNA strand breaks                 | Peripheral blood                     | Potchefstroom, South Africa<br>Cross-sectional<br>Service station attendants | Workers exposed to fuel for ≤ 1 yr to > 10 yr.<br>Recruited from a station with an average of 1258.38 ± 288.43 L fuel being sold per month<br><i>n</i> = 20 exposed<br><i>n</i> = 20 controls | + ( <i>P</i> < 0.05)<br>Individuals exposed for > 10 yr showed the highest level of basal DNA damage.<br>Other results: Antioxidative stress status ↓ Calculated repair capacity                                                 | Sex, age, smoking                      | Small sample size.<br>Personal exposure to benzene: 0.65 mg/m <sup>3</sup> (0.203 ppm <sup>b</sup> ).                                                                                                                                                                                                         | <a href="#">Keretetse et al. (2008)</a> |

**Table 4.2 (continued)**

| End-point         | Biological sample                  | Location Setting, study design                                                          | Duration of exposure<br>No. of study participants                                                                             | Response <sup>a</sup><br>(significance)                                                        | Covariates controlled                                                                                                                                                                            | Comments                                                                                                                                                                                                                                        | Reference                                         |
|-------------------|------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| DNA strand breaks | Peripheral blood lymphocytes       | Nanning, China<br>Cross-sectional<br>Service station attendants                         | Workers exposed to fuel for an average of 8.7 yr<br><i>n</i> = 200 exposed<br><i>n</i> = 52 controls                          | + ( <i>P</i> < 0.01)                                                                           | Age, sex, smoking, and alcohol consumption                                                                                                                                                       | Air monitoring: 60.03 µg/m <sup>3</sup> (0.019 ppm <sup>b</sup> ) benzene in the workplace).                                                                                                                                                    | <a href="#">Xiong et al. (2016)</a>               |
| MN formation      | Exfoliated buccal cells            |                                                                                         |                                                                                                                               | + ( <i>P</i> < 0.01)                                                                           |                                                                                                                                                                                                  |                                                                                                                                                                                                                                                 |                                                   |
| DNA strand breaks | Peripheral blood mononuclear cells | Campeche, Chiapas, and Tabasco, Mexico<br>Cross-sectional<br>Service station attendants | Workers exposed during work shifts of 8, 12, or 24 h per day for 4.5–5.6 yr<br><i>n</i> = 82 exposed<br><i>n</i> = 8 controls | + ( <i>P</i> < 0.05)<br>Impact of the length of the working day on DNA damage                  | Impacts of alcohol and tobacco consumption and physical exercise on DNA damage parameters were assessed. No statistically significant differences in DNA damage based on these lifestyle factors | Small sample size for the control group. No markers of exposure were evaluated.                                                                                                                                                                 | <a href="#">Martínez-Salinas et al. (2024)</a>    |
| MN formation      | Exfoliated buccal cells            | Los Mochis, Mexico<br>Cross-sectional<br>Service station attendants                     | Workers exposed to fuel for 1–29 yr<br><i>n</i> = 60 exposed<br><i>n</i> = 60 controls                                        | + ( <i>P</i> < 0.001)                                                                          | Age, smoking, alcohol consumption                                                                                                                                                                | Markers of exposure were not assessed.                                                                                                                                                                                                          | <a href="#">Martínez-Valenzuela et al. (2017)</a> |
| DNA strand breaks | Peripheral blood                   | Bangkok, Thailand<br>Cross-sectional<br>Service station attendants                      | 8-h work shift per day<br><i>n</i> = 29 exposed<br><i>n</i> = 27 controls                                                     | + ( <i>P</i> < 0.001)<br>Other results:<br>↓ DNA repair capacity (cytogenetic challenge assay) | Sex, age, smoking                                                                                                                                                                                | Small sample size; no data about years of service. A combination of markers of exposure were evaluated:<br>↑ benzene blood level;<br>↑ urinary <i>t,t</i> -MA.<br>Air monitoring: 64.78 ppb (0.065 ppm <sup>b</sup> ) benzene in the workplace. | <a href="#">Navasumrit et al. (2005)</a>          |



**Table 4.2 (continued)**

| End-point         | Biological sample            | Location Setting, study design                                      | Duration of exposure<br>No. of study participants                                                                                                                            | Response <sup>a</sup><br>(significance)                                                                                                              | Covariates controlled                                                                                                                                                      | Comments                                                                                                                                                                                                                                                           | Reference                                |
|-------------------|------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| DNA strand breaks | Peripheral blood             | Bangkok, Thailand<br>Cross-sectional<br>Service station attendants  | <i>n</i> = 31 exposed<br><i>n</i> = 35 controls                                                                                                                              | + ( <i>P</i> < 0.001)<br>Other results:<br>↓ DNA repair capacity by the cytogenetic challenge assay<br>Biomarkers of exposure evaluated:<br>↑ 8-OHdG | Sex, age, smoking                                                                                                                                                          | Small sample size; no data about years of service or working hours.<br>Air monitoring:<br>207.94 µg/m <sup>3</sup> (0.065 ppm <sup>b</sup> ) benzene in the workplace.<br>High levels of benzene in blood.                                                         | <a href="#">Ruchirawat et al. (2010)</a> |
| DNA strand breaks | Peripheral blood lymphocytes | Rio Grande, Brazil<br>Cross-sectional<br>Service station attendants | Workers on 8-h work shifts, exposed to fuel on average for 9.7 ± 1.1 yr (range, 0.5–32 yr)<br><i>n</i> = 43 service station attendants<br><i>n</i> = 28 non-exposed controls | + ( <i>P</i> < 0.001)                                                                                                                                | Sex                                                                                                                                                                        | Comet assay scoring carried out by manual visual classification.<br>Combination of markers of exposure evaluated: ↑ urinary <i>t,t</i> -MA of exposed group.<br>Personal benzene exposure: Exposed, 76.20 µg/m <sup>3</sup><br>controls, 42.01 µg/m <sup>3</sup> . | <a href="#">Moro et al. (2013)</a>       |
| MN formation      | Exfoliated buccal cells      |                                                                     |                                                                                                                                                                              | + ( <i>P</i> < 0.001)                                                                                                                                |                                                                                                                                                                            |                                                                                                                                                                                                                                                                    |                                          |
| DNA strand breaks | Peripheral blood lymphocytes | Lucknow, India<br>Cross-sectional<br>Service station attendants     | Workers exposed to fuel for ≥ 6 mo; minimum, 7.3 ± 7.1 yr (range, 0.5–35 yr)<br><i>n</i> = 100 exposed<br><i>n</i> = 100 controls                                            | + ( <i>P</i> < 0.01)                                                                                                                                 | Statistical analysis (ANOVA) showed that lifestyle factors, smoking, tobacco chewing, and alcohol consumption had little effect on the DNA damage detected in either group | An in vitro study in human lymphocytes showed that benzene and its metabolites caused significant levels of DNA damage at concentrations of ≥ 10 µM.<br>Air monitoring: benzene in the workplace, 100–250 ppb.                                                     | <a href="#">Pandey et al. (2008)</a>     |
| MN formation      | Peripheral blood lymphocytes |                                                                     | In a subset of the population studied, <i>n</i> = 39 exposed (blood benzene, 7.94 ppb)<br><i>n</i> = 18 controls (blood benzene, 2.82 ppb)                                   | + ( <i>P</i> < 0.001)                                                                                                                                | Age, sex, smoking, and alcohol consumption                                                                                                                                 |                                                                                                                                                                                                                                                                    |                                          |

**Table 4.2 (continued)**

| End-point               | Biological sample            | Location Setting, study design                                                            | Duration of exposure<br>No. of study participants                                                                                                                                                                                                                                                                 | Response <sup>a</sup><br>(significance)                                                                                                         | Covariates controlled                   | Comments                                                                                                                                                                                                                                                                        | Reference                              |
|-------------------------|------------------------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| DNA strand breaks       | Peripheral blood             | Rio de Janeiro, Brazil<br>Cross-sectional<br>Service station attendants                   | Workers on 8-h work shifts, exposed to fuel for 1–10 yr, > 10–20 yr, or > 20 yr<br><i>n</i> = 154 exposed<br><i>n</i> = 100 controls                                                                                                                                                                              | + ( <i>P</i> < 0.05)                                                                                                                            | Alcohol consumption                     | Variation in the number of individuals sampled for each assay.<br>Comet assay analyses only considered four classes of DNA damage, and therefore the extent of DNA damage may have been underestimated.<br>Biological monitoring: urinary <i>t,t</i> -MA, 0.10 mg/g creatinine. | <a href="#">Poça et al. (2021)</a>     |
| MN formation            | Peripheral blood             |                                                                                           | 126 exposed<br>110 controls                                                                                                                                                                                                                                                                                       | + ( <i>P</i> < 0.001)                                                                                                                           |                                         |                                                                                                                                                                                                                                                                                 |                                        |
| DNA strand breaks       | Peripheral blood lymphocytes | Italy<br>Cross-sectional<br>Service station attendants, gasoline pump maintenance workers | Workers on 8-h work shifts, exposed to fuel for 14.2 ± 14.1 yr (attendants) or 10.7 ± 10.4 yr (pump workers)<br><i>n</i> = 28 service station attendants<br><i>n</i> = 33 petrochemical industry operators<br><i>n</i> = 21 gasoline pump maintenance worker<br><i>n</i> = 51 non-exposed workers (control group) | + ( <i>P</i> < 0.05)<br>Other results: positive correlations between the benzene air level and SPMA and genotoxicity (comet test) were reported | Age, sex, duration of exposure, smoking | Small sample size.<br>Biomarkers of exposure evaluated:<br>Biological monitoring: <i>t,t</i> -MA (↑); SPMA (↑).<br>Air monitoring: 40 µg/m <sup>3</sup> (0.013 ppm <sup>b</sup> ) benzene in the workplace.                                                                     | <a href="#">Fracasso et al. (2010)</a> |
| Chromosomal aberrations | Peripheral blood lymphocytes | Service station attendants                                                                | <i>n</i> = 19 exposed<br><i>n</i> = 16 controls                                                                                                                                                                                                                                                                   | –                                                                                                                                               |                                         |                                                                                                                                                                                                                                                                                 |                                        |

**Table 4.2 (continued)**

| End-point         | Biological sample            | Location Setting, study design                                        | Duration of exposure<br>No. of study participants                                                                          | Response <sup>a</sup><br>(significance)                                                                | Covariates controlled                                              | Comments                                                                                                                                                                                                                                                                          | Reference                              |
|-------------------|------------------------------|-----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| MN formation      | Peripheral blood lymphocytes | Shebin El-Kom, Egypt<br>Cross-sectional<br>Service station attendants | Workers on 9.22 ± 1.6-h work shifts, exposed to fuel for 8.63 ± 9.22 yr<br><i>n</i> = 62 exposed<br><i>n</i> = 62 controls | + ( <i>P</i> = 0.001)                                                                                  | Age, sex, smoking, alcohol consumption                             | Air monitoring:<br>Exposed: benzene, 3.69 ppm; toluene, 120.59 ppm; ethylbenzene, 133.70 ppm; xylene, 114.35 ppm.<br>Controls: benzene, 0.05 ppm; toluene, 0.11 ppm; ethylbenzene, 0.02 ppm; xylene, 0.04 ppm.                                                                    | <a href="#">Salem et al. (2018)</a>    |
| MN formation      | Exfoliated buccal cells      | Bangalore, India<br>Cross-sectional<br>Service station attendants     | Workers on 13.2 ± 6.4-h work shifts, exposed to fuel for 3.2 ± 2.0 yr<br><i>n</i> = 26 exposed<br><i>n</i> = 27 controls   | + ( <i>P</i> < 0.01)<br>Other results:<br>↑ relative mtDNA copy number ( <i>P</i> = 0.001)<br>↑ 8-OHdG | Age, BMI, sex, educational level, smoking, and alcohol consumption | Biomarkers of exposure:<br>VOCs, < 290 ± 50 µg/m <sup>3</sup> ; PM <sub>10</sub> , 318 ± 134 µg/m <sup>3</sup> , and CO, 1050 ± 260 µg/m <sup>3</sup> ;<br>Biological monitoring (urinary phenol):<br>Exposed, 20.5 ± 9.4 mg/g creatinine<br>Controls, 8.4 ± 5.7 mg/g creatinine. | <a href="#">Gaikwad et al. (2020)</a>  |
| MN formation      | Exfoliated buccal cells      | Mersin, Türkiye<br>Cross-sectional<br>Service station attendants      | Workers on 8-h work shifts, exposed to fuel for 4.44–6.46 yr<br><i>n</i> = 50 exposed<br><i>n</i> = 50 controls            | + ( <i>P</i> < 0.01)                                                                                   | Age, sex, smoking                                                  | Biological monitoring (urinary phenol):<br>Exposed, 19.85 mg/g creatinine<br>Controls, 7.25 mg/g creatinine.                                                                                                                                                                      | <a href="#">Celik et al. (2003)</a>    |
| DNA strand breaks | Peripheral blood lymphocytes | Rome, Italy<br>Cross-sectional<br>Service station attendants          | Workers on 8-h work shifts<br><i>n</i> = 12 exposed<br><i>n</i> = 12 controls                                              | + ( <i>P</i> = 0.028)                                                                                  | Age, smoking                                                       | Small sample size; years of working not reported.<br>Air monitoring: benzene, 0.3 µg/m <sup>3</sup> .                                                                                                                                                                             | <a href="#">Andreoli et al. (1997)</a> |

**Table 4.2 (continued)**

| End-point    | Biological sample            | Location Setting, study design                                         | Duration of exposure<br>No. of study participants                                                                                  | Response <sup>a</sup><br>(significance)                                                                                                       | Covariates controlled                                          | Comments                                                                                              | Reference                              |
|--------------|------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|----------------------------------------|
| MN formation | Peripheral blood lymphocytes | Bari, Italy<br>Cross-sectional<br>Service station attendants           | Workers on 8-h work shifts, exposed to fuel for 15 ± 13 yr (range, 1–42 yr)<br><i>n</i> = 21 exposed<br><i>n</i> = 19 controls     | + ( <i>P</i> < 0.01)<br>Other results: smoking habits had no effect on the frequency of MN in either the controls or the exposed participants | Age, sex, smoking                                              | Small sample size. Air monitoring: benzene, 229 µg/m <sup>3</sup> ; toluene, 1097 µg/m <sup>3</sup> . | <a href="#">Bukvic et al. (1998)</a>   |
| MN formation | Peripheral blood lymphocytes | Sweden,<br>Cross-sectional<br>Gasoline pump mechanics                  | <i>n</i> = 15 exposed<br><i>n</i> = 15 controls                                                                                    | + ( <i>P</i> < 0.016)                                                                                                                         | Age, sex, smoking                                              | Small sample size, years of working not reported. Air monitoring: benzene, 1 mg/m <sup>3</sup> .      | <a href="#">Högstedt et al. (1991)</a> |
| MN formation | Exfoliated buccal cells      | Paraná region, Brazil<br>Cross-sectional<br>Service station attendants | Workers on 7.39 ± 0.86-h work shifts, exposed to fuel for ≥ 5 yr<br><i>n</i> = 40 exposed<br><i>n</i> = 40 controls                | + ( <i>P</i> < 0.05)                                                                                                                          | Age, sex, smoking, alcohol consumption                         | Markers of exposure were not assessed.                                                                | <a href="#">Filho et al. (2018)</a>    |
| MN formation | Exfoliated buccal cells      | Pelotas, Brazil<br>Cross-sectional<br>Service station attendants       | Workers exposed to fuel for 4.6 ± 5.6 yr (range, 1–20 yr)<br><i>n</i> = 30 exposed<br><i>n</i> = 30 controls                       | + ( <i>P</i> < 0.001)                                                                                                                         | Age, sex, smoking, alcohol consumption, duration of employment | Markers of exposure were not assessed.                                                                | <a href="#">Benites et al. (2006)</a>  |
| MN formation | Buccal exfoliated cells      | Porto Alegre, Brazil<br>Cross-sectional<br>Service station attendants  | Workers working 40 h per week, exposed to fuel for 3–6 mo, 6 mo–5 yr, or > 5 yr<br><i>n</i> = 27 exposed<br><i>n</i> = 27 controls | + ( <i>P</i> < 0.001)                                                                                                                         | Age, smoking, alcohol consumption                              | Air monitoring: toluene, 0.80 ppm; xylene, 0.16 ppm.                                                  | <a href="#">da Rosa et al. (2013)</a>  |
| MN formation | Exfoliated buccal cells      | Santos city, Brazil<br>Cross-sectional<br>Service station attendants   | Workers working 40 h per week, exposed to fuel for ≥ 5 yr<br><i>n</i> = 23 exposed<br><i>n</i> = 23 controls                       | + ( <i>P</i> < 0.05)                                                                                                                          | Age, sex, smoking                                              | Small sample size. Markers of exposure were not assessed.                                             | <a href="#">Martins et al. (2009)</a>  |

**Table 4.2 (continued)**

| End-point                 | Biological sample            | Location Setting, study design                                                    | Duration of exposure<br>No. of study participants                                                                                                                 | Response <sup>a</sup><br>(significance) | Covariates controlled                       | Comments                                                                        | Reference                                |
|---------------------------|------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|---------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------|
| MN formation              | Exfoliated buccal cells      | Tehran, Islamic Republic of Iran<br>Cross-sectional<br>Service station attendants | Workers exposed to fuel for $5.85 \pm 1.07$ yr<br>$n = 110$ exposed<br>$n = 100$ controls                                                                         | $+$ ( $P = 0.0001$ )                    | Age, sex, smoking, alcohol consumption      | Markers of exposure were not assessed; working hours per day were not reported. | <a href="#">Shahsavari et al. (2022)</a> |
| MN formation              | Exfoliated buccal cells      | Puducherry, India<br>Cross-sectional<br>Service station attendants                | Workers exposed to fuel for $19.36 \pm 8.41$ yr<br>$n = 50$ exposed<br>$n = 50$ controls                                                                          | $+$ ( $P < 0.001$ )                     | Age, smoking                                | Markers of exposure were not assessed; working hours per day were not reported. | <a href="#">Arul et al. (2017)</a>       |
| MN formation              | Exfoliated buccal cells      | Gujrat, Pakistan<br>Cross-sectional<br>Service station attendants                 | Workers exposed to fuel for $\leq 10$ yr, $> 10$ yr (11–20 yr, 21–30 yr and 31–40 yr)<br>$n = 100$ exposed<br>$n = 100$ controls                                  | $+$ ( $P < 0.001$ )                     | Age, sex, smoking                           | Markers of exposure were not assessed; working hours per day were not reported. | <a href="#">Butt et al. (2017)</a>       |
| DNA strand breaks         | Peripheral blood lymphocytes | Ankara and Amasya, Türkiye<br>Cross-sectional<br>Service station attendants       | Workers exposed to fuel for $\geq 1$ yr (mean $\pm$ SD, $13.4 \pm 9.8$ ), split into two groups, $< 7$ yr or $\geq 7$ yr<br>$n = 53$ exposed<br>$n = 61$ controls | $+$ ( $P < 0.001$ )                     | Age, sex, smoking, alcohol consumption, BMI | Markers of exposure were not assessed.                                          | <a href="#">Elkama et al. (2024)</a>     |
| Chromosomal aberrations   |                              |                                                                                   |                                                                                                                                                                   | $+$ ( $P < 0.002$ )                     |                                             |                                                                                 |                                          |
| MN formation              |                              |                                                                                   | $n = 47$ exposed<br>$n = 40$ controls                                                                                                                             | $+$ ( $P < 0.001$ )                     |                                             |                                                                                 |                                          |
| Sister-chromatid exchange |                              |                                                                                   |                                                                                                                                                                   | $+$ ( $P < 0.001$ )                     |                                             |                                                                                 |                                          |

**Table 4.2 (continued)**

| End-point                        | Biological sample            | Location Setting, study design                                                        | Duration of exposure<br>No. of study participants                                                                                       | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                      | Covariates controlled | Comments                                                                                                                                                       | Reference                                          |
|----------------------------------|------------------------------|---------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| MN formation                     | Peripheral blood lymphocytes | Ahmedabad, India<br>Cross-sectional<br>Service station attendants                     | <i>n</i> = 70 exposed<br><i>n</i> = 70 controls                                                                                         | + ( <i>P</i> < 0.001)                                                                                                                                                                                                                                                                        |                       |                                                                                                                                                                | <a href="#">Shaikh et al. (2022)</a>               |
| Sister-chromatid exchange        | Peripheral blood lymphocytes |                                                                                       |                                                                                                                                         | + ( <i>P</i> < 0.001)                                                                                                                                                                                                                                                                        |                       |                                                                                                                                                                |                                                    |
| Chromosomal aberrations          | Peripheral blood lymphocytes | Rio de Janeiro and Sao Paulo, Brazil<br>Cross-sectional<br>Service station attendants | Workers exposed to fuel for 6 mo to ~22 yr<br><i>n</i> = 30 exposed<br><i>n</i> = 24 controls                                           | + ( <i>P</i> < 0.05) only for deletions                                                                                                                                                                                                                                                      | Age, smoking          | Markers of exposure were not assessed; there were some discrepancies between the information in the text and tables on years of working.<br>Small sample size. | <a href="#">Santos-Mello and Cavalcante (1992)</a> |
| MN formation                     | Exfoliated buccal cells      | Rio Grande, Brazil<br>Cross-sectional<br>Service station attendants                   | Workers on 8 h per day work shift, exposed to fuel for 11.03 ± 1.1 yr<br><i>n</i> = 43 exposed<br><i>n</i> = 22 controls (taxi drivers) | (–)<br>Other results:<br>↑ 8-OHdG<br>+ Correlation between 8-OHdG and MN and DNA strand breaks, assessed by combining the gas station attendants and taxi drivers.<br>The MN test in buccal cells only assessed MN and no other parameters (negative, but an increase of 3.75× was observed) | Age, sex, smoking     | Biological monitoring: urinary <i>t,t</i> -MA, 440 µg/g creatinine.                                                                                            | <a href="#">Göethel et al. (2014)</a>              |
| DNA strand breaks (visual score) | Peripheral blood             |                                                                                       |                                                                                                                                         | + ( <i>P</i> < 0.001)                                                                                                                                                                                                                                                                        |                       |                                                                                                                                                                |                                                    |



**Table 4.2 (continued)**

| End-point         | Biological sample            | Location Setting, study design                                        | Duration of exposure<br>No. of study participants                                                                                         | Response <sup>a</sup><br>(significance)                                                                                                                                          | Covariates controlled                                                                                              | Comments                                                                                                                                                                                                                                                               | Reference                            |
|-------------------|------------------------------|-----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| DNA strand breaks | Peripheral blood             | Durban, South Africa<br>Cross-sectional<br>Service station attendants | Workers exposed to fuel for a mean of 5.8 yr<br><i>n</i> = 101 exposed<br><i>n</i> = 50 controls                                          | + ( <i>P</i> < 0.01)                                                                                                                                                             | The exposed and non-exposed participants in the study varied substantially with respect to key demographic factors | Markers of exposure were not assessed.                                                                                                                                                                                                                                 | <a href="#">Naidoo et al., 2016</a>  |
| MN formation      | Peripheral blood lymphocytes | Rome, Italy<br>Cross-sectional<br>Service station attendants          | Workers on 8 h per day work shifts, exposed to fuel for 22.4 ± 2.3 yr (range, 3–42 yr)<br><i>n</i> = 12 exposed<br><i>n</i> = 12 controls | –                                                                                                                                                                                | Age, sex, smoking                                                                                                  | Small sample size. Hyperploidy rates were measured on chromosomes 7, 11, 18 and X; no differences were found. Air monitoring: benzene, 0.32 mg/m <sup>3</sup> . Biological monitoring: urinary benzene, 517.6 µg/g creatinine; <i>t,t</i> -MA, 824.09 µg/g creatinine. | <a href="#">Carere et al. (1995)</a> |
| MN formation      | Peripheral blood             | Rome, Italy<br>Cross-sectional<br>Service station attendants          | Workers on 8 h per day work shifts, exposed to fuel for 22.4 ± 2.3 yr (range, 3–42 yr)<br><i>n</i> = 12 exposed<br><i>n</i> = 12 controls | (–)<br>Hyperploidy and polyploidy, using FISH (centromere)<br>Combination of biomarkers of benzene exposure: (↑) in air samples, (↑) urinary <i>t,t</i> -MA, and urinary benzene | Age, sex, smoking                                                                                                  | <i>Limitations:</i> Small sample size, but samples matched according to age and smoking habits. High frequency of MN in both the control and exposed groups. Air monitoring: 0.32 mg/m <sup>3</sup> (0.1 ppm <sup>b</sup> ) benzene in the workplace.                  | <a href="#">Carere et al. (1998)</a> |

**Table 4.2 (continued)**

| End-point                                                    | Biological sample            | Location Setting, study design                                                          | Duration of exposure<br>No. of study participants                                                                                                                 | Response <sup>a</sup><br>(significance)                                                                                                                                                        | Covariates controlled                      | Comments                                                                                                                                                                                                                                                          | Reference                              |
|--------------------------------------------------------------|------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| MN formation (specific FISH marker of 1cen and 1q12 lesions) | Peripheral blood             | Rome, Italy<br>Cross-sectional<br>Service station attendants                            | Workers on 8 h per day work shifts, exposed to fuel for 22.4 ± 2.3 yr (range 3–42 yr)<br><i>n</i> = 12 exposed<br><i>n</i> = 12 controls                          | (+) ( <i>P</i> < 0.001)                                                                                                                                                                        | Age, sex, smoking                          | <i>Limitations:</i> Small sample size, but samples matched according to age and smoking habits. Same population as that sampled for the previous study ( <a href="#">Carere et al., 1998</a> ).<br>Air monitoring: 0.1 ppm <sup>b</sup> benzene in the workplace. | <a href="#">Marcon et al. (2002)</a>   |
| Chromosomal aberrations                                      | Peripheral blood lymphocytes | Southern region, Brazil<br>Cross-sectional<br>Service station attendants                | Workers on 8 h per day work shifts, exposed to fuel for 3.5 yr (median)<br><i>n</i> = 19 exposed<br><i>n</i> = 6 local controls<br><i>n</i> = 38 general controls | –                                                                                                                                                                                              | Age                                        | Small sample size.<br>BTX not detected in blood.                                                                                                                                                                                                                  | <a href="#">Trevisan et al. (2014)</a> |
| DNA strand breaks                                            | Peripheral blood lymphocytes | Santa Maria, Rio Grande do Sul, Brazil<br>Cross-sectional<br>Service station attendants | Workers mostly on 6–8 h per day work shifts, exposed to fuel for 1 to > 15 yr<br><i>n</i> = 17 exposed<br><i>n</i> = 16 controls                                  | –<br>Other results:<br>↑ 8-OHdG<br>Specific inflammatory parameters correlated with DNA strand breaks and 8-OHdG level.<br>Inflammatory parameters: ↑ IL-1, IL-6, TNFα, IFNγ, and IL-10 levels | Age, sex, smoking, and alcohol consumption | <i>Limitations:</i> Small sample size; no data about environmental levels of benzene, confounders not considered in the analysis.                                                                                                                                 | <a href="#">Brum et al. (2021)</a>     |

**Table 4.2 (continued)**

| End-point                 | Biological sample            | Location Setting, study design                                    | Duration of exposure<br>No. of study participants                                                                                                   | Response <sup>a</sup><br>(significance) | Covariates controlled             | Comments                                                                                                                                                                                                                                                                          | Reference                              |
|---------------------------|------------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| MN formation              | Peripheral blood lymphocytes | Barcelona, Spain<br>Cross-sectional<br>Service station attendants | Workers mostly on 8 h per day work shifts, exposed to fuel for 1 to > 38 yr<br>(13.24 ± 1.32 yr)<br><i>n</i> = 50 exposed<br><i>n</i> = 43 controls | –                                       | Age, smoking, alcohol consumption | Air monitoring: workplace BTX, phenol, and HA levels below the LOD.<br>Biological monitoring: MHA in urine, below the LOD; benzene, 0.91 mg/m <sup>3</sup> ; toluene, 1.58 mg/m <sup>3</sup> ; xylene, 0.89 mg/m <sup>3</sup> ; urinary phenol, 5.06; HA, 433.08 mg/g creatinine. | <a href="#">Pitarque et al. (1996)</a> |
| Chromosomal aberrations   | Peripheral blood lymphocytes | Mersin, Türkiye<br>Cross-sectional<br>Service station attendants  | Workers mostly on an 8-h per day work shift, exposed to fuel for 7 to > 14 yr<br><i>n</i> = 30 exposed<br><i>n</i> = 30 controls                    | –<br><br>+ ( <i>P</i> < 0.05)           | Smoking                           | Biological monitoring (urinary phenol): 19.85 mg/g creatinine.                                                                                                                                                                                                                    | <a href="#">Celi and Akbaş (2005)</a>  |
| Sister-chromatid exchange |                              |                                                                   |                                                                                                                                                     |                                         |                                   |                                                                                                                                                                                                                                                                                   |                                        |

ANOVA, analysis of variance; BMI, body mass index; BTX, benzene, toluene, xylene; CO, carbon monoxide; FISH, fluorescence in situ hybridization; h, hour(s); HA, hippuric acid; IFN $\gamma$ , interferon gamma; IL, interleukin; mo, month(s); LOD, limit of detection; MHA, methylhippuric acid; MN, micronucleus; mtDNA, mitochondrial DNA; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PM<sub>10</sub>, particulate matter of  $\leq 10 \mu\text{m}$  in diameter; ppb, parts per billion; ppm, parts per million; SD, standard deviation; SPMA, S-phenylmercapturic acid; TNF $\alpha$ , tumour necrosis factor alpha; *t,t*-MA, *trans,trans*-muconic acid; VOC, volatile organic compound; yr, year(s).

<sup>a</sup> +, positive; –, negative; (+) or (–), positive or negative findings in a study of limited quality; ↑, increase; ↓, decrease.

<sup>b</sup> The conversion of benzene concentrations from mg/m<sup>3</sup> to ppm was performed using the formula  $\text{ppm} = [(\text{mg}/\text{m}^3) \times 24.45]/78.11$ , where 24.45 is the molar volume of an ideal gas under standard temperature and pressure conditions and 78.11 is the molar mass of benzene. This approach ensured consistent transformations, on the basis that 1 ppm of benzene is approximately equivalent to 3.19 mg/m<sup>3</sup>.

[Martínez-Salinas et al. \(2024\)](#) and [Martínez-Valenzuela et al. \(2017\)](#) observed significant increases in DNA strand breaks in peripheral blood lymphocytes and micronuclei in buccal exfoliated cells in service station workers in the states of Campeche, Chiapas, and Tabasco, and in the city of Los Mochis, in Mexico. [Martínez-Salinas et al. \(2024\)](#) showed that longer work shifts (12 and 24 hours) were associated with greater DNA damage than were either 8-hour shifts or no exposure. [Martínez-Valenzuela et al. \(2017\)](#) grouped the participants into three groups, with 1–2, 3–10, or 11–29 years of work in the relevant environment. As a result of gasoline exposure, the micronucleus frequency and other nuclear abnormalities in buccal epithelial cells were higher at the beginning of exposure to gasoline vapour in these three groups than in non-exposed individuals, but these frequencies tended to decrease over subsequent years, albeit that there were no statistically significant differences among the mean values. [The Working Group noted that a strength of the two studies was the adjustment for smoking and alcohol consumption, and a limitation was the lack of assessment of markers of exposure.]

In other studies in which genotoxicity end-points were evaluated, there was no analysis of the duration of the exposure to gasoline vapour, but markers of exposure were assessed and adjusted for covariates. [Navasumrit et al. \(2005\)](#) and [Ruchirawat et al. \(2010\)](#) observed increases in DNA strand breaks in the peripheral blood of service station attendants from the Bangkok area of Thailand (approximately 30 in each study) compared with a control group. A number of markers of benzene exposure were measured, and higher blood benzene levels and urinary *t,t*-MA concentrations were identified. The level of benzene in the workplace in both studies was 0.065 ppm. [Navasumrit et al. \(2005\)](#) reported that the individual blood benzene exposure levels of the service station attendants were relatively high, averaging 121.67 ppb, whereas

the mean level of the control study participants was only 4.77 ppb. In both studies, a reduction in DNA repair capacity was observed in exposed individuals, which exacerbated the effects of DNA damage over time (see also Section 4.2.3). [The Working Group noted that a strength of these studies was that variables such as sex, age, and smoking habits were controlled for.]

[Moro et al. \(2013\)](#) reported that 43 service station attendants from Brazil showed significant increases in DNA strand breaks in peripheral blood lymphocytes and increases in the frequency of micronuclei in exfoliated buccal cells, which positively correlated with the personal air concentration of benzene and the urinary *t,t*-MA concentration. [The Working Group noted that the levels of personal benzene exposure and urinary *t,t*-MA levels were both below the ACGIH limits (1600 µg per m<sup>3</sup>) established in the year of assessment.]

Similarly, [Pandey et al. \(2008\)](#) observed significant increases in DNA strand breaks and the frequency of micronuclei in the peripheral blood lymphocytes of 100 service station attendants in India. [The Working Group noted that this study showed associations of both low and high blood benzene levels with the increase of micronucleus frequency in peripheral blood lymphocytes.]

[Poça et al. \(2021\)](#) observed significant increases in the frequency of micronuclei and DNA strand breaks in the peripheral blood lymphocytes of 126 and 154 Brazilian service station attendants, respectively. The levels of urinary *t,t*-MA were higher in service station attendants than in control office workers with no known exposure to gasoline. In addition, immunophenotyping results revealed increases in the percentages of natural killer (NK) cells and memory T-helper lymphocytes, and reductions in the percentages of helper lymphocytes and naive T-helper lymphocytes (see Section 4.2.7). [The Working Group noted that a strength of the study was that alcohol consumption and smoking habits were controlled for. The authors

also measured the same parameters in convenience store workers from the same service station as an additional control group, which helped them account for gasoline exhaust exposure. The Working Group noted that only four classes of DNA damage were reported for the comet assay, which could have led to an underestimation of the extent of the DNA damage.]

[Fracasso et al. \(2010\)](#) observed a significant increase in DNA strand breaks in peripheral blood lymphocytes from 28 service station attendants from Italy, compared with a non-exposed control group. Markers of exposure, including personal benzene exposure and urinary metabolite excretion of *t,t*-MA and SPMA, were also elevated in the service station attendants. There were positive correlations between DNA damage and the concentrations of glutathione (GSH) and SPMA in urine samples from the exposed workers. No significant increase in chromosomal aberrations was observed in a smaller subset of 19 exposed individuals versus 16 control individuals. [The Working Group noted that although this study had a small sample size, a link between markers of benzene exposure and DNA strand breaks was identified.]

[Salem et al. \(2018\)](#) showed a significant increase in the frequency of micronuclei in peripheral blood lymphocytes from service station attendants ( $n = 62$ ) from Egypt compared with non-exposed controls ( $n = 62$ ). [The Working Group noted that in this study, levels of BTEX were measured in the ambient air of service stations and in control areas.]

[Gaikwad et al. \(2020\)](#) found a higher micronucleus frequency in buccal cells and higher levels of urinary 8-OHdG (see Section 4.2.5) in service station attendants ( $n=26$ ) from India than in non-exposed study participants ( $n=27$ ). [The Working Group noted that the findings were adjusted for age, BMI, sex, education level, and smoking and alcohol consumption habits. In addition, the authors measured urinary phenol and  $PM_{10}$  concentrations and total VOCs. The

TWA  $PM_{10}$  value was  $318 \pm 134 \mu\text{g}/\text{m}^3$ , and it was below the LOD for VOCs.]

In Türkiye, [Celik et al. \(2003\)](#) observed an increase in micronucleus frequency in exfoliated buccal cells from 50 service station attendants. [The Working Group noted that urinary phenol levels were significantly higher in service station attendants (19.85 mg/g/L creatinine) than in the control study participants (7.25 mg/g/L creatinine).]

[Andreoli et al. \(1997\)](#) observed a statistically significant difference in DNA strand breaks in the peripheral blood lymphocytes of 12 service station attendants from Italy compared with 12 non-exposed control individuals. [The Working Group noted that the benzene exposure of service station attendants was  $0.3 \text{ mg}/\text{m}^3$ , on an 8-hour TWA basis.]

Micronucleus frequency was also increased in the peripheral blood of service station attendants from Italy who had been exposed to fuel for  $15 \pm 13$  years ([Bukvic et al., 1998](#)). [The Working Group noted that the authors controlled for age, sex, and smoking as covariates, and reported air monitoring data for benzene ( $229 \mu\text{g}/\text{m}^3$ ) and toluene ( $1097 \mu\text{g}/\text{m}^3$ ).]

[Högstedt et al. \(1991\)](#) also noted an increase in the frequency of micronuclei in the peripheral blood of gasoline pump workers from Sweden compared with construction workers. The gasoline pump mechanics were exposed to  $1 \text{ mg}/\text{m}^3$  of benzene as a TWA over 8 hours, with a peak of  $20 \text{ mg}/\text{m}^3$  over 10 minutes of indoor work. [The Working Group noted that the mechanics were exposed to gasoline when they worked at service stations, where they dismantled the pumps, transported them by car to a workshop, or repaired them. The Working Group further noted that these studies had small sample sizes but evaluated relevant markers of exposure.]

In other studies, genotoxicity end-points were evaluated and covariates were adjusted for. For instance, consistent significant increases in the frequency of micronuclei in exfoliated buccal

cells were observed in several studies conducted in Brazil ([Benites et al., 2006](#); [Martins et al., 2009](#); [da Rosa et al., 2013](#); [Filho et al., 2018](#)), one in Iran ([Shahsavari et al., 2022](#)), one in India ([Arul et al., 2017](#)), and one in Pakistan ([Butt et al., 2017](#)). [The Working Group noted that some of these studies had a small sample size, and most did not involve the measurement of markers of exposure; however, the findings of the studies were consistent.]

In addition, the frequency of micronuclei and alterations in other genotoxicity end-points were measured in peripheral blood lymphocytes in other studies. [Elkama et al. \(2024\)](#) reported increases in chromosomal aberrations, the frequency of micronuclei, and DNA strand breaks in the peripheral blood lymphocytes of service station attendants from Türkiye. [The Working Group noted that this was a well-conducted study that involved adjustment of the results for several covariates, but markers of exposure were not evaluated.]

[Shaikh et al. \(2022\)](#) reported higher frequencies of micronuclei and increased sister-chromatid exchange (SCE) in the peripheral blood lymphocytes of 70 service station attendants from India, compared with a control group. [Santos-Mello and Cavalcante \(1992\)](#) documented a significant increase in chromosomal aberrations (specifically attributed to deletion mutations) in service station attendants from Brazil. [The Working Group noted that markers of exposure were not evaluated in this study, but that age, sex, and smoking habits were controlled for.]

[Göethel et al. \(2014\)](#) reported a non-significant increase in the frequency of micronuclei in exfoliated buccal cells from 43 service station attendants in Brazil. The increase in micronuclei was concomitant with an increase in oxidative stress, as measured using 8-OHdG levels, and in DNA strand breaks, measured by the comet assay (see also Section 4.2.5), compared with a control group of taxi drivers. Notably, the levels

of *t,t*-MA in the exposed group were significantly elevated. [The Working Group noted that there was a discrepancy between micronucleus formation and oxidative stress, suggesting that oxidative damage to DNA may occur without this being immediately translated into observable chromosomal damage: prolonged exposure may be required for this to manifest. In addition, the micronucleus frequency was 3.75 times higher, albeit not significantly, in the exposed group compared with the control group. No correlation analysis was performed for the relation between genotoxicity end-points and urinary *t,t*-MA or whole-blood carboxyhaemoglobin concentrations.]

[Naidoo et al. \(2016\)](#) also observed an increase in DNA strand breaks in peripheral blood cells sampled from 101 service station attendants in Durban, South Africa. [The Working Group noted, however, that the study had limitations in the absence of assessment of markers of exposure and a lack of control for covariates.]

[Carere et al. \(1995, 1998\)](#), and [Marcon et al. \(2002\)](#) evaluated the same group of service station attendants from Italy but obtained different results. Whereas Marcon et al. detected specific chromosomal aberrations using fluorescence in situ hybridization markers (1cen and 1q12 lesions), Carere et al. found no significant increases in the frequency of micronuclei or in DNA strand breaks in peripheral blood lymphocytes. [The Working Group noted that the study by Marcon et al. showed that the average benzene exposure of the service station attendants was  $0.1 \pm 0.01$  ppm. The studies were limited by the small sample sizes of the exposed and control groups, which were matched according to age and smoking habits.]

A few studies have shown no genotoxicity associated with gasoline exposure. [Trevisan et al. \(2014\)](#) did not observe increases in chromosomal aberrations or SCE in the peripheral blood lymphocytes of 19 service station attendants in Brazil. [The Working Group noted that



the blood concentrations of BTX were measured, but these substances were not detected in either the exposed or control groups.] [Brum et al. \(2021\)](#) did not report a significant increase in DNA strand breaks in the peripheral blood lymphocytes of 17 service station attendants in Brazil. Notably, the authors observed increases in end-points relevant to oxidative stress and inflammatory processes, such as 8-OHdG, interleukins 6 and 10 (IL-6, IL-10), tumour necrosis factor alpha (TNF $\alpha$ ), and interferon gamma (IFN $\gamma$ ) (see also Sections 4.2.5, and 4.2.6). [The Working Group noted several limitations of the study, such as the small sample size, the lack of exposure data, and the absence of consideration of potential confounders.] [Pitarque et al. \(1996\)](#) did not report any increase in the frequency of micronuclei in peripheral lymphocytes from service station attendants in Spain. [Celi and Akbaş \(2005\)](#) reported no increase in chromosomal aberrations, but observed a significant increase in the level of SCE, in the peripheral blood lymphocytes of service station attendants in Türkiye. [The Working Group noted that the analysis was based on a small sample size.]

[The Working Group reviewed a few other studies, and considered them to be less informative, because of limitations in the study design and data interpretation.] For example, [Roma-Torres et al. \(2006\)](#) reported significant increases in chromosomal aberrations, micronucleus frequency, and DNA damage (assessed via the comet assay) in workers at a petroleum refinery in Portugal compared with the control group. [The Working Group noted that the interpretation of the study findings was limited by the fact that the workers evaluated in the study were exposed to both gasoline and other derivatives of petroleum.]

[Khisroon et al. \(2015\)](#) evaluated genotoxicity in a group of 98 workers in Pakistan (68 service station attendants and 30 automobile workshop workers). The authors observed a statistically significant increase in DNA strand breaks in the

exposed group compared with the control group ( $n = 92$ ). [The Working Group noted that the automobile workshop workers could have been exposed to other carcinogenic agents besides automotive gasoline, unlike the service station attendants. In addition, it was noted that the automobile workshop mechanics had a significantly higher mean total comet score than the service station attendants.] [Gattás et al. \(2001\)](#) evaluated the frequency of micronuclei in exfoliated buccal cells of service station attendants in Brazil during three different time periods. [The Working Group noted that the study mainly focused on the evaluation of ethanol versus methanol as an automotive fuel, rather than on the analysis of automotive gasoline.] [Edwards and Priestly \(1993\)](#) in Australia and [Tunsaringkarn et al. \(2011\)](#) in Thailand reported significantly higher levels of SCE in service station attendants than in control participants. [The Working Group noted that these studies had small sample sizes and inadequate adjustment for known confounders.]

#### (ii) *Human cells in vitro*

See [Table 4.3](#).

[The Working Group identified three studies in which the genotoxic effects of various gasoline components and related chemicals were assessed in human cells in vitro. These studies may provide valuable mechanistic insights into how exposure to these substances could induce DNA damage, including single-strand breaks, double-strand breaks, and gene mutations.]

[Chen et al. \(2008\)](#) assessed the genotoxic effects of individual gasoline components, namely MTBE and BTEX, on human lymphocytes using the alkaline and neutral comet assays. They found that all the tested chemicals induced DNA damage, including DNA single- and double-strand breaks. Specifically, MTBE, benzene, and xylene caused DNA double-strand breaks at a concentration of 200  $\mu$ M. To investigate the role of free radicals in DNA damage, spin traps such as 5,5-dimethyl-pyrroline-N-oxide

**Table 4.3 End-points relevant to genotoxicity in human cells in vitro exposed to automotive gasoline**

| End-point                                         | Tissue, cells                                                   | Results <sup>a</sup>               |                                 | Concentration<br>(LEC or HIC)                   | Comments                                                                                                                                                                                                                                                                                                   | Reference                            |
|---------------------------------------------------|-----------------------------------------------------------------|------------------------------------|---------------------------------|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
|                                                   |                                                                 | Without<br>metabolic<br>activation | With<br>metabolic<br>activation |                                                 |                                                                                                                                                                                                                                                                                                            |                                      |
| DNA strand breaks (alkaline, comet assay)         | Lymphocytes (primary)                                           | +                                  | NT                              | MTBE, 50 µM                                     | A visual score was used to assess strand breaks.<br><i>Limitation:</i> Comet assay with only 10 minutes of unwinding, without metabolic activation. The maximum concentration was determined on the basis of the cytotoxicity and solubility of the substances.                                            | <a href="#">Chen et al. (2008)</a>   |
|                                                   |                                                                 | +                                  |                                 | Benzene, 50 µM                                  |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | Ethylbenzene, 100 µM                            |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | Toluene, 50 µM                                  |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | <i>m</i> -Xylene, 100 µM                        |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | <i>o</i> -Xylene, 100 µM                        |                                                                                                                                                                                                                                                                                                            |                                      |
| DNA strand breaks (neutral comet assay)           |                                                                 | +                                  | NT                              | <i>p</i> -Xylene, 50 µM                         | A visual score was used to assess strand breaks.                                                                                                                                                                                                                                                           |                                      |
|                                                   |                                                                 | +                                  |                                 | MTBE, 50 µM                                     |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | Benzene, 50 µM                                  |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | –                                  |                                 | Ethylbenzene, 200 µM                            |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | –                                  |                                 | Toluene, 200 µM                                 |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | <i>m</i> -Xylene, 50 µM                         |                                                                                                                                                                                                                                                                                                            |                                      |
| DNA strand breaks (modified comet assay with Fpg) |                                                                 | +                                  | NT                              | <i>o</i> -Xylene, 50 µM                         | A visual score was used to assess strand breaks.<br>Also relevant to Section 4.2.5.                                                                                                                                                                                                                        |                                      |
|                                                   |                                                                 | +                                  |                                 | <i>p</i> -Xylene, 50 µM                         |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | MTBE, 200 µM                                    |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | Benzene, 200 µM                                 |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | –                                  |                                 | Ethylbenzene, 200 µM                            |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | –                                  |                                 | Toluene, 200 µM                                 |                                                                                                                                                                                                                                                                                                            |                                      |
| DNA strand breaks (% tail DNA, analysis by CASP)  | Adenocarcinoma alveolar basal epithelial cells (A549 cell line) | +                                  | NT                              | <i>m</i> -Xylene, 200 µM                        | Cell cultures were exposed to gasoline vapour from their apical side for 24 h (a new method for static air/liquid interface studies: static exposure system for VOCs using glass chambers).<br>The maximum concentration was determined on the basis of the cytotoxicity and solubility of the substances. | <a href="#">Sayyed et al. (2022)</a> |
|                                                   |                                                                 | +                                  |                                 | <i>o</i> -Xylene, 200 µM                        |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | <i>p</i> -Xylene, 200 µM                        |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 | Unleaded commercial gasoline 95 octane, 229 ppm |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 |                                                 |                                                                                                                                                                                                                                                                                                            |                                      |
|                                                   |                                                                 | +                                  |                                 |                                                 |                                                                                                                                                                                                                                                                                                            |                                      |

**Table 4.3 (continued)**

| End-point                                  | Tissue, cells                   | Results <sup>a</sup>               |                                 | Concentration<br>(LEC or HIC)                          | Comments                                                                                                                                                                                                                                                  | Reference                                    |
|--------------------------------------------|---------------------------------|------------------------------------|---------------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
|                                            |                                 | Without<br>metabolic<br>activation | With<br>metabolic<br>activation |                                                        |                                                                                                                                                                                                                                                           |                                              |
| Gene mutation<br>(TK <sup>+/-</sup> locus) | Lymphoblasts (TK6<br>cell line) | (-)                                | (-)                             | Unleaded gasoline and<br>2,2,4-trimethylpentane 5% v/v | <i>Limitations:</i> Single concentrations<br>tested. It was difficult to determine the<br>exact concentrations used, owing to the<br>lack of specific information regarding<br>the initial concentration of the test<br>gasoline in the saturated medium. | <a href="#">Richardson<br/>et al. (1986)</a> |
| Sister-chromatid<br>exchange               |                                 | (-)                                | (-)                             |                                                        |                                                                                                                                                                                                                                                           |                                              |

CASP, comet analysis software package; Fpg, formamidopyrimidine-DNA glycosylase; HIC, highest ineffective concentration; LEC, lowest effective concentration; MTBE, methyl-*tert*-butyl ether; NT, not tested; ppm, parts per million; TK, thymidine kinase; VOCs, volatile organic compounds; v/v, volume per volume.

<sup>a</sup> +, positive; -, negative; (+) or (-), positive or negative findings in a study of limited quality

and *N-tert*-butyl- $\alpha$ -phenylnitrone were used. These traps reduced the level of DNA damage in lymphocytes exposed to BTEX at 200  $\mu$ M, suggesting that free radicals contribute to the DNA-damaging effects of these chemicals. Additionally, post-treatment with formamidopyrimidine-DNA glycosylase, which recognizes oxidized DNA purines, revealed that increased DNA damage was associated with benzene and xylene, indicating oxidative damage (see also Section 4.2.5). After exposure to MTBE and BTEX at concentrations between 50–200  $\mu$ M, cell viability was > 95%, suggesting that the observed DNA damage was not the result of cell death. Owing to the limited solubility of the chemicals, 200  $\mu$ M was the highest concentration tested. [The Working Group noted that the limitations of the study included the short unwinding time of only 10 minutes in the comet assay and the absence of the use of metabolic activation in the assays. However, these limitations did not limit the reliability of the findings. The study was considered informative, because single components of gasoline were compared in the same experiment.]

[Sayyed et al. \(2022\)](#) investigated the toxicity of gasoline vapours (VOCs) using a static exposure system equipped with glass chambers. Human alveolar basal epithelial A549 adenocarcinoma cells were exposed to gasoline vapour via their apical side for 24 hours. To determine the half-maximal inhibitory concentration ( $IC_{50}$ ) of gasoline, A549 cells were exposed to a range of liquid crude gasoline volumes equivalent to a range of evaporated gasoline concentrations (from 0 to 735 ppm). The authors reported that gasoline-derived VOCs inhibited cell viability with an  $IC_{50}$  of 485 ppm immediately after exposure and 516 ppm after 24 hours. DNA strand breaks were observed, as measured by tail DNA percentage in the comet assay, as well as a disruption of cell membrane integrity, indicated by lactate dehydrogenase leakage, and caspase-independent apoptosis. The maximum concentration

tested was determined on the basis of the cytotoxicity and solubility of the tested compounds. Notably, the study was not performed in the presence of a metabolic activation system (S9), because the A549 cells selected express metabolic enzymes involved in the bioactivation of gasoline-derived VOCs. [The Working Group noted that the high sensitivity of the in vitro exposure system, combined with agreement with the findings of other studies, confirmed the reliability of this system for assessment of the toxicity of inhaled VOC mixtures.]

[Richardson et al. \(1986\)](#) assessed the genotoxic effects (gene mutations at the thymidine kinase (*TK*) locus and SCE) of unleaded gasoline and 2,2,4-trimethylpentane in the human lymphoblast TK6 cell line in vitro. Neither unleaded gasoline nor 2,2,4-trimethylpentane, both tested at a concentration of 5% v/v, induced mutations at the *TK* locus or caused SCE, either in the presence or absence of a metabolizing system (rat liver homogenate). [The Working Group noted that the study was of limited informativeness because of the testing of single concentrations, insufficient information on the initial concentration of gasoline in the saturated medium, and difficulty assessing the exact concentrations used. In fact, the maximum concentration for testing was determined only on the basis of the solubility and cytotoxicity of the two tested substances.]

#### (b) *Experimental systems*

##### (i) *Non-human mammalian systems in vivo*

See [Table 4.4](#).

[Schreiner et al. \(2014\)](#) assessed the genotoxic effects of vapour condensates of baseline gasoline (BGVCs) and gasoline mixed with oxygenates, including MTBE, ETBE, TAME, DIPE, TBA, and ethanol. Sprague-Dawley rats exposed to 0, 2000, 10 000, or 20 000 mg/m<sup>3</sup> of each condensate, for 6 hours per day, 5 days per week, over 4 weeks, were tested for micronucleus formation

**Table 4.4 End-points relevant to genotoxicity in experimental systems in vivo exposed to automotive gasoline**

| End-point                                 | Species, strain (sex)                     | Tissue, cells | Results <sup>a</sup>                                                                          | Route, duration, dosing regimen                                                                                                                                                        | Concentration (LEC or HIC) or dose (LED or HID) | Comments                                                             | Reference                               |
|-------------------------------------------|-------------------------------------------|---------------|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|----------------------------------------------------------------------|-----------------------------------------|
| MN formation                              | Rats, Sprague-Dawley (M and F)            | Bone marrow   | –                                                                                             | Both sexes were exposed for 6 h/day on 5 days per week for 4 wk. Inhalation of BGVC (2000 mg/m <sup>3</sup> ), or gasoline with an oxygenate (MTBE, ETBE, TAME, DIPE, TBA, or ethanol) | 2000 mg/m <sup>3</sup> BGVC                     | Only one dose of ETBE yielded positive results in females.           | <a href="#">Schreiner et al. (2014)</a> |
| Sister-chromatid exchange                 |                                           | Blood         | + BGVC alone<br>+ gasoline with MTBE<br>+ gasoline with TAME<br>(no difference between sexes) |                                                                                                                                                                                        |                                                 |                                                                      |                                         |
| Chromosomal aberrations                   | Rats, Sprague-Dawley CD (M and F)         | Bone marrow   | –                                                                                             | Unleaded gasoline (containing 2% benzene). 6–48 h after intraperitoneal injections of 0.03–0.3 mL/rat or after five daily intraperitoneal injections of 0.013–0.13 mL/rat per day      | 0.13 mL/rat per day (5 days)                    |                                                                      | <a href="#">Conaway et al. (1984)</a>   |
| Unscheduled and replicative DNA synthesis | Rats, F344/CrlBR (M)                      | Hepatocytes   | –                                                                                             | Unleaded gasoline (containing 2% benzene; PS-6) (gavage, 100–5000 mg/kg; measured 2, 12, 24, and 48 h after intragastric administration)                                               | 5000 mg/kg                                      | <i>Limitation:</i> Small number of animals treated. Relevant to KC3. | <a href="#">Loury et al. (1986)</a>     |
| Unscheduled and replicative DNA synthesis | Mice, B6C3F <sub>1</sub> /CrlBR (M and F) | Hepatocytes   | –                                                                                             | Unleaded gasoline (containing 2% benzene; PS-6) (gavage, 2000 mg/kg)                                                                                                                   | 5000 mg/kg                                      | <i>Limitation:</i> Number of animals treated. Relevant to KC3.       | <a href="#">Loury et al. (1986)</a>     |
| Unscheduled and replicative DNA synthesis | Rats, F344/CrlBR (M and F)                | Kidney cells  | –                                                                                             | Unleaded gasoline (containing 2% benzene; PS-6): inhalation (2000 ppm) or gavage (5000 mg/kg)                                                                                          | 5000 mg/kg                                      | <i>Limitation:</i> Small number of animals treated. Relevant to KC3. | <a href="#">Loury et al. (1987a)</a>    |

BGVC, baseline gasoline vapour condensate; DIPE, diisopropyl ether; ETBE, ethyl *tert*-butyl ether; F, female(s); F344, Fischer 344; h, hour(s); HIC, highest ineffective concentration; HID, highest ineffective dose; KC, key characteristic of carcinogens; KC3, key characteristic of carcinogens 3, alters DNA repair or causes genomic instability; LEC, lowest effective concentration; LED, lowest effective dose; M, male(s); MTBE, methyl *tert*-butyl ether; ppm, parts per million; TAME, *tert*-amyl methyl ether; TBA, *tert*-butyl alcohol.

<sup>a</sup> +, positive; –, negative.

in the blood and bone marrow, and SCE in the blood. Specifically, the average cell generation time, cell cycle delay, and mitotic index were assessed. No significant increase in the frequency of micronuclei was observed for any of the tested substances, and only one dose was positive in female rats after exposure to ETBE. However, there was a significant increase in SCE in rats exposed to BGVC or to mixtures of gasoline and MTBE or gasoline and TAME. The inclusion of oxygenates in gasoline did not increase the effects of baseline gasoline. [The Working Group considered the study to be of relevance, because all the gasoline components were assessed simultaneously, providing a comprehensive assessment of their potential genotoxic effects.]

[Conaway et al. \(1984\)](#) assessed the level of chromosomal aberrations in the bone marrow of Sprague-Dawley CD rats exposed via intraperitoneal injection to unleaded gasoline containing 2% benzene at doses of 0.03–0.3 mL per rat 6–48 hours before the analysis, or at doses of 0.013–0.13 mL per rat daily for 5 days. No increase in chromosomal aberrations was observed in the bone marrow. [The Working Group noted, as a limitation of the study, the lack of information on whether the doses were selected through a previous dose range-finding study.] [Conaway et al. \(1984\)](#) reported no induction of gene mutations at the *Tk* locus in L5178Y *Tk*<sup>+/-</sup> cells in a mouse lymphoma assay. The cells were treated with unleaded gasoline containing 2% benzene at concentrations of 0–1.0 µL/mL (see [Table 4.4](#)).

### (iii) Other species

See [Table 4.5](#).

In a study conducted by [Krug et al. \(2024\)](#), the mutagenic potential of photo-oxidation products from gasoline vapour was evaluated using *Salmonella typhimurium* (strain TA100). The authors observed an increase in the frequency of point mutations after exposure to gasoline vapour at 2.4 parts per million by carbon

(ppmC), in the absence of metabolic activation. The research focused on reacted gasoline vapour, which originated from an initial mixture with a carbon content of 10.8 ppmC. Upon ultraviolet-B irradiation (300–400 nm) in a steady-state atmosphere chamber in the presence of nitrogen oxides, the gasoline vapour mixture primarily generated formaldehyde and acetaldehyde, along with peroxyacetyl nitrate (88 ppmC) and peroxypropionyl nitrate (23 ppmC), suggesting that the photo-oxidation of gasoline yields several compounds with mutagenic potential. Notably, the most mutagenic products identified were toluene, ethylbenzene, *o*-, *m*-, and *p*-xylene, and 1,2,4- and 1,3,5-trimethylbenzene. Additionally, a time-response relationship was identified, indicating that the mutagenic activity varied according to the duration of exposure to the photo-oxidation products. [The Working Group noted that the study was limited by a lack of testing that incorporated metabolic activation and the use of only one *Salmonella* strain.]

[Conaway et al. \(1984\)](#) investigated the potential mutagenicity of unleaded gasoline containing 2% benzene in various *S. typhimurium* strains (TA100, TA1535, TA1537, TA1538, and TA98). They used both the plate incorporation and suspension tests, and concentrations ranging from 0.001 to 30 µL/mL. Unleaded gasoline did not induce frameshift or point mutations, either in the presence or absence of metabolic activation.

[Nylander et al. \(1978\)](#) investigated the mutagenic effects of commercial gasoline (purchased from a service station in the USA and containing 0.04% lead) and two of its components, benzene and 1,2-dichloroethane, in *Drosophila melanogaster*. The chemicals were administered to larvae through the food. The frequency of somatic mutations affecting eye pigmentation was assessed in a genetically unstable sex-linked test system, driven by a transposable genetic element. Gasoline and 1,2-dichloroethane significantly increased the frequency of somatic mutations, whereas benzene did not have any mutagenic



**Table 4.5 End-points relevant to genotoxicity in non-human mammalian experimental systems in vitro and other species exposed to automotive gasoline**

| End-point                                 | Species, strain (sex), or cells                          | Results <sup>a</sup>         |                           | Experimental design                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Concentration (LEC or HIC)               | Comments                                                                                 | Reference                             |
|-------------------------------------------|----------------------------------------------------------|------------------------------|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------------------------------------------------------|---------------------------------------|
|                                           |                                                          | Without metabolic activation | With metabolic activation |                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                          |                                                                                          |                                       |
| Non-human mammalian systems               |                                                          |                              |                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                          |                                                                                          |                                       |
| Unscheduled and replicative DNA synthesis | Rats, F344/CrlBR (M), primary hepatocytes                | –                            | NR                        | Unleaded gasoline (containing 2% benzene; PS-6) (0.05 or 0.10% v/v)                                                                                                                                                                                                                                                                                                                                                                                                        | 0.10% v/v                                | Limitation: Small number of animals treated.<br>Other results: Cytotoxicity above 0.20%. | <a href="#">Loury et al. (1986)</a>   |
| Unscheduled and replicative DNA synthesis | Mice, B6C3F <sub>1</sub> /CrlBR (M), primary hepatocytes | –                            | NR                        | Unleaded gasoline (containing 2% benzene; PS-6) (0.01% v/v)                                                                                                                                                                                                                                                                                                                                                                                                                | 0.01% v/v                                | Limitation: Small number of animals treated.<br>Other results: Cytotoxicity above 0.03%. | <a href="#">Loury et al. (1986)</a>   |
| Unscheduled and replicative DNA synthesis | Rats, F344/CrlBR (M), primary kidney cells               | –                            | NR                        | Unleaded gasoline (containing 2% benzene; PS-6) (0.005–0.010% v/v)                                                                                                                                                                                                                                                                                                                                                                                                         | 0.01% v/v                                | Limitation: Small number of animals treated.<br>Other results: Cytotoxicity above 0.05%. | <a href="#">Loury et al. (1987a)</a>  |
| Gene mutation (Tk <sup>+</sup> /– locus)  | Mouse L5178Y TK <sup>+</sup> /– cells                    | –                            | –                         | Unleaded gasoline (containing 2% benzene), tested at concentrations of 0–1.0 µL/mL                                                                                                                                                                                                                                                                                                                                                                                         | 1.0 µL/mL                                |                                                                                          | <a href="#">Conaway et al. (1984)</a> |
| Other species                             |                                                          |                              |                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                          |                                                                                          |                                       |
| Gene mutation                             | Salmonella typhimurium (TA100)                           | +                            | NR                        | Photo-oxidation products of gasoline vapours. Reacted gasoline vapour: initial mixture had a carbon level of 10.8 ppmC. The irradiated gasoline mixture formed mainly formaldehyde and acetaldehyde (atmospheric concentrations of peroxyacetyl nitrate 88 ppmC and peroxypropionyl nitrate 23 ppmC; initiated with precursor VOCs). Photo-oxidation of gasoline (most mutagenic products): toluene; ethylbenzene; o-, m-, and p-xylene; 1,2,4- and 1,3,5-trimethylbenzene | 2.4 ppmC (VOC; lights on; time relation) | Limitation: Lack of assay with metaboization and assay with only one strain.             | <a href="#">Krug et al. (2024)</a>    |

**Table 4.5 (continued)**

| End-point                             | Species, strain (sex), or cells                                        | Results <sup>a</sup>         |                           | Experimental design                                                                                                                        | Concentration (LEC or HIC) | Comments                                                                                                                                                                                                                                                                                                                                                             | Reference                                            |
|---------------------------------------|------------------------------------------------------------------------|------------------------------|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
|                                       |                                                                        | Without metabolic activation | With metabolic activation |                                                                                                                                            |                            |                                                                                                                                                                                                                                                                                                                                                                      |                                                      |
| Frameshift mutation and gene mutation | <i>Salmonella typhimurium</i> (TA1535, TA1537, TA1538, TA98, or TA100) | –                            | –                         | Unleaded gasoline (containing 2% benzene); either the plate incorporation (0.001–5 µL/plate) or suspension method (3.75–30 µL/mL) was used | 30 µL/mL                   |                                                                                                                                                                                                                                                                                                                                                                      | <a href="#">Conaway et al. (1984)</a>                |
| Somatic mutation (eye pigmentation)   | <i>Drosophila melanogaster</i>                                         | (+)                          | NA                        |                                                                                                                                            | 1% (with 0.04% lead)       | <i>Limitations:</i> The origin of the organisms used was unclear, no toxicity test was conducted to select the doses, the positive result presented for the mutagenic effects involved little difference from the control, and the total duration of exposure of the organisms was not provided.<br><i>Other results:</i> Benzene (–); 1,2-dichloroethane (+; 0.1%). | <a href="#">Nylander et al. (1978)</a>               |
| DNA strand breaks                     | <i>Corbicula fluminea</i> (mollusc)                                    | +                            | NA                        | Test of the WSFG                                                                                                                           | 5% WSFG, 6 h               | <i>Limitations:</i> Only four classes were considered in the comet assay, visual score.                                                                                                                                                                                                                                                                              | <a href="#">Fedato et al. (2010)</a>                 |
| MN formation                          |                                                                        | +                            |                           |                                                                                                                                            | 5% WSFG, 96 h              |                                                                                                                                                                                                                                                                                                                                                                      |                                                      |
| Chromosomal aberrations               | <i>Allium cepa</i> (plant)                                             | –                            | NA                        | Test of the WSFG                                                                                                                           | 50% WSFG, 48 h             | <i>Limitation:</i> The chemical composition of the WSFG sample was not provided.                                                                                                                                                                                                                                                                                     | <a href="#">Oliveira-Martins and Grisolia (2009)</a> |
| MN formation                          | <i>Oreochromis niloticus</i> (fish)                                    | –                            | NA                        |                                                                                                                                            | 50% WSFG, 96 h             |                                                                                                                                                                                                                                                                                                                                                                      |                                                      |
| MN formation                          | <i>Astyanax altiparanae</i> (fish)                                     | –                            | NA                        | Test of the WSFG                                                                                                                           | 1.5% WSFG, 96 h            |                                                                                                                                                                                                                                                                                                                                                                      | <a href="#">Galvan et al. (2016)</a>                 |

**Table 4.5 (continued)**

| End-point         | Species, strain (sex), or cells                    | Results <sup>a</sup>         |                           | Experimental design | Concentration (LEC or HIC) | Comments                                                                                                                                             | Reference                             |
|-------------------|----------------------------------------------------|------------------------------|---------------------------|---------------------|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
|                   |                                                    | Without metabolic activation | With metabolic activation |                     |                            |                                                                                                                                                      |                                       |
| DNA strand breaks | <i>Danio rerio</i> (ZFL fish hepatocyte cell line) | +                            | NR                        | Test of the WSFG    | 5% WSFG, 1 h               | <i>Limitation:</i> The chemical composition of the WSFG sample was not provided. Only four classes were considered in the comet assay, visual score. | <a href="#">Lachner et al. (2015)</a> |

F344, Fischer 344; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male(s); NA, not applicable; NR, not reported; ppmC, parts per million per carbon; TK, thymidine kinase; VOC, volatile organic compound; v/v, volume per volume; WSFG, water-soluble fraction of gasoline.

<sup>a</sup> +, positive; –, negative; (+) or (–), positive or negative findings in a study of limited quality.

activity under the test conditions. [The Working Group noted that the study had some limitations, including a lack of clarity on the origin of the organisms tested, the absence of toxicity testing to determine the most appropriate doses, and a lack of information on the total duration of exposure.]

[Fedato et al. \(2010\)](#) investigated the toxicity of water-soluble fractions of gasoline (WSFGs) obtained from regular unleaded gasoline without additives, collected from a service station in Brazil, in the bivalve mollusc *Corbicula fluminea*. Quantitative analysis of the undiluted WSFGs revealed high levels (a total concentration of 24 800 µg/L) of BTEX, of which ethylbenzene, xylene, and benzene were the most abundant components. Of the 12 PAHs analysed, naphthalene was the most abundant, followed by anthracene and phenanthrene. The total concentration of PAHs was 2203.7 µg/L, and all other PAHs analysed were present at concentrations below the detection limit (0.05 µg/L). *C. fluminea* was exposed to 5% diluted WSFG or clean water (negative control) in static acute toxicity tests over 6, 24, or 96 hours, and the haemolymph and gill cells were then analysed for DNA damage, using the comet and micronucleus assays. DNA strand breaks were detected in the haemocytes and gill cells at all time points, whereas an increase in the frequency of micronuclei was observed only after 96 hours. Results after a 6-hour recovery period, after WSFG exposure, showed a reduction in damaged nucleosides, and hence repair of DNA damage, compared with control levels. [The Working Group noted that the study had a limitation in relation to the comet assay: the authors considered only four classes of visual scoring. Findings based on only four classes of damage restrict the analysis and can hinder comparison between studies, because visual assessment of the comet assay is recommended to include five classes of damage (classes 0 to 4).]

[Galvan et al. \(2016\)](#) observed no increase in the frequency of micronuclei in the freshwater fish *Astyanax altiparanae* after 96 hours of exposure to an acute, sublethal dose of WSFG (0.5% v/v) in a semi-static system. Chemical analysis of the WSFG revealed a total BTEX concentration of 78 921.40 µg/L and a total PAH concentration of 147.74 µg/L. The WSFG used in the study was prepared by mixing one part homogenized unleaded gasoline, which contained ethanol (21.6% v/v) and benzene (0.6% v/v), with nine parts distilled water. The recovery process was also assessed after contaminant depuration. The recovery treatments included gradual depuration, consisting of 7 days in WSFG, followed by 8 days in clean water; total depuration in clean water for 15 days; and total depuration for 30 days.

[Oliveira-Martins and Grisolia \(2009\)](#) investigated the genotoxicity of wastewater from eight service stations in Brasília, Brazil, in tilapia fish (*Oreochromis niloticus*). Although no micronuclei were detected in the erythrocytes of *O. niloticus*, there were increases in nuclear abnormalities and fish mortality, indicating toxicity. The fish were kept in aquaria containing 30 L of continuously aerated wastewater for 96 hours. In the same study, the authors did not observe chromosomal aberrations or chromosomal altered segregation in *Allium cepa* root cells exposed to 10% or 50% diluted wastewater for 48 hours, with continuous shaking. Despite significant inhibition of proliferation of *A. cepa* root cells, genotoxic effects were only observed in two samples. [The Working Group noted that wastewater from processed gasoline station effluent can include toxic compounds but might not exhibit genotoxic potential. The Working Group also noted that the chemical composition of the service station effluent samples was not provided.]

[Lachner et al. \(2015\)](#) assessed the genotoxic potential of dilutions of WSFG in a *Danio rerio* hepatocyte cell line (ZFL) using the comet assay. The authors observed a dose-related increase in DNA damage, as measured by frequency of

nucleoids and the total number of damaged nucleoids, after 1, 3, or 6 hours exposure to 5% or 10% dilutions of the gasoline fractions. Concentration-dependent cytotoxicity and lower cell viability with longer durations of exposure were observed, without any alteration in oxidative stress, assessed by quantifying reactive oxygen species (ROS) and the total antioxidant capacity for peroxy radicals. [The Working Group noted that the comet assay analysis did not follow standard protocol recommendations, limiting the informativeness of the study.]

### Synopsis

[Overall, the Working Group noted that, in many studies, exposure to automotive gasoline is associated with an increase in DNA strand breaks and micronucleus formation in peripheral blood lymphocytes and in exfoliated buccal cells of occupationally exposed populations (mainly service station attendants). The findings of these studies were consistent across populations and countries, including Brazil, China, India, Mexico, and Thailand. Some of these studies characterized exposure through environmental workplace measurements, such as airborne benzene or BTEX, and/or biomarkers of internal exposure, such as urinary *t,t*-MA, hippuric acid, and phenol, even though it is recognized that such biomarkers may not be optimal means of detecting low levels of exposure. In addition, several studies examined multiple end-points, including micronuclei, DNA strand breaks, chromosomal aberrations, and SCE. Confounders such as sex, age, smoking, and alcohol consumption were controlled for in most of these studies, to facilitate the establishment of clear links between exposure and genotoxicity. There was also some evidence of genotoxicity in experimental systems, including human cells and rodents *in vivo*, which also provided information on single components of gasoline. However, the Working Group noted that the findings of the studies varied, probably because of differences in

design, the end-points measured, and the formulation of the agent.]

### 4.2.3 Alters DNA repair or causes genomic instability

See [Table 4.6](#).

#### (a) Exposed humans

Four studies were identified that described the impact of exposure to automotive gasoline and its components on DNA repair in occupational settings.

In a study by [Navasumrit et al. \(2005\)](#), a group of 29 service station attendants from Bangkok, Thailand, who were exposed to high levels of benzene were identified. Benzene exposure was associated with a significant increase in DNA damage, as measured by the olive tail moment, compared with the control group (see Section 4.2.2). The exposure was associated with high urinary *t,t*-MA (217.30 ng/mL urine) and blood benzene (641.84 parts per trillion, ppt) concentrations and individual benzene air exposure (121.67 ppm, measured using an organic vapour monitor near the breathing zone), indicating substantial benzene absorption. DNA repair capacity was assessed using a cytogenetic challenge assay, in which blood lymphocytes from the workers were cultured and exposed to ionizing radiation (100 cGy, using a <sup>137</sup>Cs source at a dose of 5 Gy/minute) to induce DNA damage. After a 50-hour culture period, the cells were harvested, and metaphase spreads were prepared. Analysis of 50 metaphase cells per individual revealed a significant increase in dicentric chromosomes (1.4×) and chromosome deletions (2.4×) in service station attendants compared with controls, indicating a reduced ability to repair DNA damage. [The Working Group noted that covariates such as sex, age, and smoking were controlled for in this study.]

**Table 4.6 End-points relevant to alteration of DNA repair or to genomic instability in humans exposed to automotive gasoline**

| End-point (assay)                                                                     | Biological sample | Location, study design                                                  | Exposure level<br>No. of study participants                                                                                                          | Response <sup>a</sup><br>(significance) | Covariates controlled | Comments                                                                                                                                                                                                                                                     | Reference                                |
|---------------------------------------------------------------------------------------|-------------------|-------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| DNA repair capacity (by the cytogenetic challenge assay after irradiation)            | Peripheral blood  | Bangkok, Thailand<br>Cross-sectional<br>Service station attendants      | 64.78 ppb (0.065 ppm) benzene in the workplace<br><i>n</i> = 29 exposed<br><i>n</i> = 27 non-exposed controls                                        | + ( <i>P</i> < 0.001)                   | Sex, age, smoking     | Dicentric chromosomes (↑ 1.4×) and chromosome deletions (↑ 2.4×) after challenge assay. Exposure to radiation during cell culture procedures.                                                                                                                | <a href="#">Navasumrit et al. (2005)</a> |
| DNA repair capacity (by the cytogenetic challenge assay after irradiation)            | Peripheral blood  | Bangkok area, Thailand<br>Cross-sectional<br>Service station attendants | 207.94 µg/m <sup>3</sup> (0.065 ppm <sup>b</sup> ) benzene air level in the workplace<br><i>n</i> = 31 exposed<br><i>n</i> = 34 non-exposed controls | + ( <i>P</i> < 0.001)                   | Sex, age, smoking     | Dicentric chromosomes (↑ 1.6×) and chromosome deletions (↑ 2.4×) after challenge assay. Exposure to radiation during cell culture procedures. Small sample size; no data about the years of service or working hours.                                        | <a href="#">Ruchirawat et al. (2010)</a> |
| DNA repair capacity (by the cytogenetic challenge assay after irradiation)            | Peripheral blood  | Bangkok area, Thailand<br>Cross-sectional<br>Service station attendants | 112.41 ppb individual benzene exposure of the service station attendants<br><i>n</i> = 31 exposed<br><i>n</i> = 34 controls                          | + ( <i>P</i> < 0.001)                   | Sex, smoking          | Dicentric chromosomes (↑ 1.6×) and chromosome deletions (↑ 2.4×) after challenge assay. Exposure to radiation during cell culture procedures. No clear influence of polymorphisms in the <i>CYP2E1</i> , <i>NQO1</i> , <i>GSTT1</i> , or <i>XRCC1</i> genes. | <a href="#">Chanvaivit et al. (2007)</a> |
| DNA repair capacity (cytogenetic challenge assay with H <sub>2</sub> O <sub>2</sub> ) | Peripheral blood  | Durban, South Africa<br>Cross-sectional<br>Service station attendants   | Personal exposure to benzene, 0.65 mg/m <sup>3</sup> (0.203 ppm <sup>b</sup> )<br><i>n</i> = 20 exposed<br><i>n</i> = 20 controls                    | (–)                                     | Sex, age, smoking     | Unclear results. Exposure to H <sub>2</sub> O <sub>2</sub> for 10 minutes ex vivo (without cell culture).                                                                                                                                                    | <a href="#">Keretetse et al. (2008)</a>  |

H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; ppb, parts per billion; ppm, parts per million.

<sup>a</sup> +, positive; –, negative; (+) or (–), positive or negative findings in a study of limited quality.

<sup>b</sup> The conversion of benzene concentrations from mg/m<sup>3</sup> to ppm was performed using the formula ppm = [(mg/m<sup>3</sup>) × 24.45]/78.11, where 24.45 is the molar volume of an ideal gas under standard temperature and pressure conditions, and 78.11 is the molar mass of benzene. This approach ensured consistent transformations, on the basis that 1 ppm of benzene is approximately equivalent to 3.19 mg/m<sup>3</sup>.



Similarly, [Ruchirawat et al. \(2010\)](#) identified 31 service station attendants from the Bangkok area of Thailand who were exposed to elevated levels of benzene. Benzene exposure was associated with higher individual benzene levels (average of 360.84  $\mu\text{g}/\text{m}^3$  for the exposed group and 4.46  $\mu\text{g}/\text{m}^3$  for the control group), high formation of 8-OHdG ( $29 \times 8\text{-OHdG per } 10^5$  2'-deoxyguanosine), and an increase in the olive tail moment (1.7 $\times$ ) compared with the control group (see Sections 4.2.2 and 4.2.5). To assess DNA repair capacity, the researchers used a cytogenetic challenge assay (also employed by [Navasumrit et al., 2005](#)). The authors observed significant increases in dicentric chromosomes (1.6 $\times$ ) and chromosome deletions (2.4 $\times$ ) in the service station attendants compared with the controls, indicating a reduced ability to repair DNA damage. [The Working Group noted that the accumulation of chromosomal abnormalities suggested that DNA repair mechanisms were impaired in the service station attendants, increasing their vulnerability to genotoxic insults and potential long-term health consequences. Additionally, the individual benzene exposure of service station attendants significantly correlated with their DNA repair capacity ( $r=0.426$ ,  $P<0.001$  for dicentric chromosomes;  $r=0.673$ ,  $P<0.001$  for the number of deletions per metaphase). The Working Group also noted that the authors controlled for similar variables to those in the study by Navasumrit et al.]

In the study by [Chanvaivit et al. \(2007\)](#), the effect of exposure to benzene was assessed in 31 service station attendants from Bangkok, Thailand, compared with 34 control participants. Occupational exposure to benzene was monitored through the assessment of exposure biomarkers in urine and blood samples. DNA repair capacity was assessed as a marker of an early effect, in a similar way to that described by [Navasumrit et al. \(2005\)](#). In addition, the authors investigated the impact of polymorphisms in metabolic genes (*CYP2E1*), detoxification genes

(*NQO1*, encoding NAD(P)H quinone dehydrogenase 1, and *GSTT1*, encoding glutathione S-transferase theta 1), and DNA repair gene *XRCC1*, encoding X-ray repair cross complementing 1 (at codon 399) on biomarker levels, but did not identify any clear relation with exposure. DNA repair capacity was assessed by means of a cytogenetic challenge assay using radiation, and this showed a significantly higher frequency of dicentric chromosomes per metaphase cell and an increased frequency of deletions in the service station attendants versus the controls, implying a lesser ability to repair DNA. [The Working Group noted that the findings were consistent with those reported by [Ruchirawat et al. \(2010\)](#), suggesting that similar populations were assessed, and comparable outcomes were reported. These results from the same group showed that the mean individual benzene exposure of the service station attendants ( $112.41 \pm 13.92$  ppb) was significantly higher than that of controls ( $1.39 \pm 0.17$  ppb,  $P < 0.001$ ). The blood benzene concentrations were significantly elevated in the service station attendants (483.46 ppt), and post-shift urine samples contained higher levels of *t,t*-MA (0.20 mg/g creatinine). The Working Group also noted that polymorphisms in the studied genes did not influence the results; however, the small sample size could have limited the accuracy of such an assessment.]

[Keretetse et al. \(2008\)](#) observed elevated levels of DNA strand breaks (see Section 4.2.2) in 20 service station attendants from Durban, South Africa, who were exposed to benzene. The highest level of damage to DNA was observed in workers who had >10 years of exposure. A significant relationship was established between the volume of gasoline sold during a shift (1258.38 L), and the average concentrations of benzene (0.65  $\text{mg}/\text{m}^3$ ), toluene (0.73  $\text{mg}/\text{m}^3$ ), and all the VOCs measured (46.57  $\text{mg}/\text{m}^3$ ; excluding xylene, 0.31  $\text{mg}/\text{m}^3$ ). DNA repair was assessed using the comet assay, and the DNA repair

capacity was calculated using the formula: repair capacity =  $[1 - (\% \text{ tail DNA at 60 minutes} / \% \text{ tail DNA after H}_2\text{O}_2 \text{ exposure})]$  (600  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  for 10 minutes at 37 °C). The 60-minute time point was selected because, in some exposed individuals, comets could not be automatically scored at the 90-minute time point, indicating near-complete or complete DNA recovery. [The Working Group noted that the duration of exposure influenced both the level of DNA damage and the calculated repair capacity. Despite their high levels of DNA damage, the exposed group exhibited slightly more efficient DNA repair capacity than the non-exposed group. This suggests that DNA repair started in response to oxidative damage in the exposed group, but that this was insufficient to fully counteract the damage caused by the exposure to the agent of interest. Additionally, DNA repair capacity was notably delayed in smokers, regardless of whether or not they had been exposed to gasoline-derived VOCs.] The study also revealed that DNA repair capacity diminished with longer exposure to these compounds. Workers with >10 years of exposure exhibited higher basal DNA damage and lower DNA repair capacity than those with shorter exposure periods. Specifically, the repair capacities were 0.29 for those exposed for >10 years and 0.46 for those exposed for <1 year, indicating that prolonged exposure impairs DNA repair mechanisms. [The Working Group noted that covariates such as sex, age, and smoking were controlled for. However, there were also limitations, including a lack of clarity and robustness in the results.]

#### (b) *Experimental systems*

See [Table 4.4](#).

[Loury et al. \(1986, 1987a\)](#) assessed unscheduled DNA synthesis (UDS) and replicative DNA synthesis (RDS) in F344/CrlBR rats and B6C3F<sub>1</sub>/CrlBR mice exposed to unleaded gasoline containing 2% benzene (PS-6). Limited UDS and RDS activities were observed in the

hepatocytes of F344 rats exposed to gasoline by gavage at doses ranging between 100 and 5000 mg/kg (three animals per dose group) for 2, 12, 24, or 48 hours. Weak UDS activity was also observed in the hepatocytes of male and female B6C3F<sub>1</sub> mice exposed to 2000 mg/kg of unleaded gasoline via gavage. [The Working Group noted that the small number of animals treated in these studies represented a limitation, and would potentially affect the statistical power of the analyses and the reliability of the findings. In addition, the Working Group recognized the low sensitivity of the UDS assay for the detection of some instances of DNA damage, with the potential for false negative results.]

[Loury et al. \(1986, 1987a\)](#) also assessed UDS and RDS in primary cultures of hepatocytes and kidney cells treated in vitro with unleaded gasoline containing 2% benzene (PS-6). In cultures of hepatocytes from male F344 rats, weak UDS activity was observed at concentrations of 0.05% or 0.10% (v/v), with cytotoxicity occurring at concentrations >0.20%. Similarly, cultures of hepatocytes from male B6C3F<sub>1</sub> mice exhibited weak UDS activity at 0.01% (v/v), with signs of cytotoxicity at concentrations exceeding 0.03%. No UDS activity was detected in cultures of kidney cells from male F344 rats that were exposed to concentrations of unleaded gasoline of between 0.005% and 0.010% (v/v). Cytotoxicity was also observed at concentrations exceeding 0.05% ([Loury et al., 1986, 1987a](#)). [The Working Group noted that the small number of animals used to generate the cultures of primary cells may have affected the robustness of the results, limiting the informativeness of the studies.]

#### *Synopsis*

[The Working Group noted that some studies indicated a reduced DNA repair capacity in service station attendants. Exposure to benzene and VOCs has been reported to play a role in this reduction. In addition, a high level of benzene exposure was associated with high concentrations

of 8-OHdG, a biomarker of oxidative damage to DNA (see Section 4.2.5). The Working Group noted some limitations in the number of studies available and the study design.]

#### 4.2.4 Induces epigenetic alterations

See [Table 4.7](#).

##### *Exposed humans*

[The Working Group considered the relevance of end-points associated with epigenetic alterations, as described by [Wu and Morris \(2001\)](#), and knowledge regarding the epigenetic alterations induced by environmental exposures ([Baccarelli and Bollati, 2009](#); [Bollati and Baccarelli, 2010](#); [Alegría-Torres et al., 2011](#); [Collotta et al., 2013](#)) and those that occur in various cancer types ([Herceg and Vaissière, 2011](#); [Dawson and Kouzarides, 2012](#); [Herceg et al., 2018](#); [Ilango et al., 2020](#), [Hanahan, 2022](#)).]

Bollati and colleagues assessed the DNA methylation profile of *LINE-1* (encoding long interspersed nuclear elements) and *Alu* retroelements, the methylation of *MAGE-1* (a gene that encodes a tumour-specific antigen and is commonly hypomethylated in malignancies; [De Smet et al., 1996](#)) and of *p15* (a tumour suppressor gene that is commonly hypermethylated in AML; [Juhl-Christensen et al., 2012](#)), and the phenomenon of loss of imprinting in *H19 locus* in benzene-exposed individuals and controls in Milan, Italy ([Bollati et al., 2007](#)). The benzene-exposed group consisted of 78 service station attendants and 77 urban traffic officers, and the control group comprised 58 office workers. A progressive increase in benzene concentrations was observed in office workers, urban traffic officers, and service station attendants, the latter group showing the highest levels. *LINE-1* hypomethylation and *p15* hypermethylation were observed in the benzene-exposed groups, relative to the control groups. When the methylation levels of the different elements and genes

were correlated with airborne benzene levels, an inverse correlation was observed for *LINE-1* and *Alu*, whereas *p15* methylation levels were directly correlated. The differences identified remained significant after adjustment for confounding variables.

[The Working Group noted that the methylation profile of retroelements such as *LINE* and *SINE* (short interspersed nuclear element), represented by *Alu*, has been used as a proxy for the global methylation level, owing to their high representation within the human genome ([Roy-Engel, 2012](#)). In addition, owing to their transposition potential, these elements are usually highly methylated, which helps maintain genomic stability ([Bhat et al., 2022](#)). Therefore, differences in the methylation levels of these elements after exposures are likely to contribute to genomic instability. The Working Group noted that the strengths of this study included the use of quantitative methodology to determine the DNA methylation level and the adjustment for several confounding factors; in particular, age, smoking, alcohol consumption, the genotype of metabolizing enzymes, and blood cell count.]

In the same cohort in Milan, Italy (78 service station attendants, 77 police officers, and 33 workers in a modern petrochemical plant), [Carugno et al. \(2012\)](#) reported that DNA methylation of the same targets correlated with mitochondrial DNA (mtDNA) copy number. High mtDNA copy number correlated with lower *LINE-1* and higher *p15* methylation levels. [The Working Group noted that variations in mtDNA have been associated with various types of cancers ([Kopinski et al., 2021](#)); therefore, the results suggest an association between exposure to automotive gasoline and DNA methylation alterations with cancer development.

**Table 4.7 End-points relevant to epigenetic alterations in humans exposed to automotive gasoline**

| End-points                                                                                                            | Biological sample | Location, setting, study design                                                    | Exposure level<br>No. of study participants                                                                                                                                                                                                                                                                                                                                                 | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                             | Covariates controlled                                                                                                                                                                                                                                             | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Reference                             |
|-----------------------------------------------------------------------------------------------------------------------|-------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| <i>LINE-1</i> methylation<br><i>Alu</i> methylation<br><i>MAGE-1</i> methylation<br><i>p15</i> methylation            | Peripheral blood  | Milan, Italy<br>Cross-sectional<br>Service station attendants                      | Median personal benzene exposure:<br>Service station attendants, 61 µg/m <sup>3</sup><br>Traffic police officers, 22.0 µg/m <sup>3</sup><br>Non-exposed control group, 6.0 µg/m <sup>3</sup><br><i>n</i> = 78 service station attendants<br><i>n</i> = 77 traffic police officers<br><i>n</i> = 58 non-exposed controls                                                                     | ↓ <i>LINE-1</i> methylation<br>↓ <i>Alu</i> methylation<br>↓ <i>MAGE-1</i> methylation<br>↑ <i>p15</i> methylation in benzene-exposed groups<br>(Airborne benzene level negatively correlated with <i>LINE-1</i> and <i>Alu</i> methylation, and positively correlated with <i>p15</i> methylation) | Age, sex, smoking status (never, ex, or current), urine cotinine, cigarettes/day, pack-years, years since smoking cessation, alcohol consumption, metabolic polymorphisms ( <i>NQO1</i> and <i>CYP2E1</i> ), and percentage lymphocytes in the differential count | <i>Strengths:</i> The use of quantitative methodology (pyrosequencing) to determine the DNA methylation levels, adjustment for several confounding factors, and the exposure measurement (airborne benzene).                                                                                                                                                                                                                                                                                                                                                                                                                     | <a href="#">Bollati et al. (2007)</a> |
| <i>LINE-1</i> methylation<br><i>Alu</i> methylation<br><i>MAGE-1</i> methylation<br><i>p15</i> methylation<br>mtDNAcn | Peripheral blood  | Genoa, Milan, and Cagliari, Italy<br>Cross-sectional<br>Service station attendants | Median personal benzene exposure:<br>Service station attendants, 60.9 µg/m <sup>3</sup><br>Police officers, 21.8 µg/m <sup>3</sup><br>Controls, 6.3 µg/m <sup>3</sup> ;<br>Exposed participants:<br><i>n</i> = 153 bus drivers<br><i>n</i> = 78 gas station attendants<br><i>n</i> = 77 police officers<br><i>n</i> = 33 workers in a modern petrochemical plant<br><i>n</i> = 178 controls | ↓ <i>LINE-1</i> methylation, negatively correlated with mtDNAcn<br>No change in <i>Alu</i> methylation<br>No change in <i>MAGE-1</i> methylation<br>↑ <i>p15</i> methylation, positively correlated with mtDNAcn<br>↑ mtDNAcn                                                                       | Age, sex, smoking, and number of cigarettes per day                                                                                                                                                                                                               | <i>Strengths:</i> The use of quantitative methodologies to determine DNA methylation levels (pyrosequencing) and mtDNAcn (qPCR), adjustment for confounding factors, the exposure measurement (airborne benzene), correlation between epigenetic alterations and a genetic alteration.<br><br><i>Limitation:</i> Blood cell count was not accounted for.<br><br><i>Controls:</i> Occupationally active people from the same area as the exposed participants, residents of towns located 2–5 km from the petrochemical plant (close referents), and residents from an area further (≥ 20 km) from the plant (distant referents). | <a href="#">Carugno et al. (2012)</a> |

**Table 4.7 (continued)**

| End-points                                                                                                                                                                                                                                            | Biological sample | Location, setting, study design                                     | Exposure level<br>No. of study participants                                                                                                                                                                                                   | Response <sup>a</sup><br>(significance)                                                                                  | Covariates controlled                                                                             | Comments                                                                                                                                                                                                                                                                                             | Reference                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| DNA methylation of <i>LINE-1</i> subfamilies <i>L1HS</i> , <i>L1PA5</i> , <i>L1PA2</i> , and <i>L1Ta</i> <i>Alu</i> subfamilies <i>AluSx</i> , <i>AluYb8</i> , and <i>AluYd6</i> <i>HERV</i> subfamilies <i>MLT1D</i> , <i>ERV1</i> , and <i>ERV9</i> | Peripheral blood  | Milan, Italy<br>Cross-sectional study<br>Service station attendants | <i>n</i> = 20 service station attendants with high mean airborne benzene exposure ( $78.6 \pm 42.5 \mu\text{g}/\text{m}^3$ )<br><i>n</i> = 20 office workers with low mean airborne benzene exposure ( $7.0 \pm 5.5 \mu\text{g}/\text{m}^3$ ) | ↓ <i>L1PA2</i> methylation in the exposed group (methylation level inversely correlated with the airborne benzene level) | Age and smoking                                                                                   | <i>Strengths</i> : The use of quantitative methodology (pyrosequencing) to determine DNA methylation levels, adjustment for confounding factors and an exposure measurement (airborne benzene).<br><br><i>Limitation</i> : Blood cell count was not accounted for.                                   | <a href="#">Byun et al. (2013a)</a> |
| mtDNA methylation in three regions: tRNA phenylalanine ( <i>MT-TF</i> ), 12S rRNA ( <i>MT-RNR1</i> ) gene and “D-loop” control region                                                                                                                 | Peripheral blood  | Milan, Italy<br>Cross-sectional study<br>Service station attendants | <i>n</i> = 20 service station attendants with high mean airborne benzene exposure ( $78.6 \pm 42.5 \mu\text{g}/\text{m}^3$ )<br><i>n</i> = 20 office workers with low mean airborne benzene exposure ( $7.0 \pm 5.5 \mu\text{g}/\text{m}^3$ ) | No change                                                                                                                | Age and smoking status. All the participants were male. Blood leukocyte subtype was accounted for | <i>Strengths</i> : The use of quantitative methodology (pyrosequencing) to determine DNA methylation levels, adjustment for confounding factors, use of an exposure measurement (airborne benzene).<br><br><i>Limitations</i> : Biological effects of mtDNA methylation are still poorly understood. | <a href="#">Byun et al. (2013b)</a> |

**Table 4.7 (continued)**

| End-points                                                                                                         | Biological sample | Location, setting, study design                                     | Exposure level<br>No. of study participants                                                                                                                                                                                 | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                                                                 | Covariates controlled                                                                                                                                | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Reference                               |
|--------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| <i>LINE-1</i> DNA methylation and RNA expression<br><i>Alu</i> DNA methylation and RNA expression<br>H3K9me3 level | Peripheral blood  | Milan, Italy<br>Cross-sectional study<br>Service station attendants | <i>n</i> = 89 service station workers (all men) with median benzene exposure of 59 µg/m <sup>3</sup><br><i>n</i> = 90 non-occupationally exposed participants (all men) with median benzene exposure of 4 µg/m <sup>3</sup> | ↓ <i>L1-Pa5</i> ( <i>LINE-1</i> subfamily) methylation in the exposed group<br>Direct correlation between the expression of <i>Alu-Y</i> and <i>Alu-J</i> and urinary benzene level<br>Inverse correlation between the methylation levels of <i>Alu-YD6</i> and <i>L1-Ta</i> and urine MTBE level                                       | Age, sex, BMI, smoking, medications, use of products containing solvents during the previous 24 h, means of transport, and time taken to get to work | <i>Strengths:</i> The use of quantitative methodologies to determine DNA methylation (pyrosequencing) and expression levels (PCR), adjustment for several confounding factors, exposure measurements (airborne benzene, and urinary benzene, MTBE, <i>t,t</i> -MA, and SPMA), analysed both retroelements methylation and expression.<br><i>Limitations:</i> Details of the H3K9me3 analysis were not provided and the blood cell count was not accounted for. | <a href="#">Rota et al. (2018)</a>      |
| Global DNA methylation<br><i>LINE-1</i> DNA methylation<br><i>Alu</i> DNA methylation                              | Peripheral blood  | Milan, Italy<br>Cross-sectional study<br>Service station attendants | <i>n</i> = 78 service station attendants with median benzene exposure of 61 µg/m <sup>3</sup><br><i>n</i> = 58 office and hospital workers (controls) with median benzene exposure of 6 µg/m <sup>3</sup>                   | ↓ Global DNA methylation<br>↓ <i>LINE-1</i> methylation in the exposed group<br>↓ <i>Alu</i> methylation in the exposed group (airborne benzene level inversely correlated with the global, <i>LINE-1</i> , and <i>Alu</i> methylation levels; <i>t,t</i> -MA level inversely correlated with <i>LINE-1</i> and <i>Alu</i> methylation) | Age and smoking                                                                                                                                      | <i>Strengths:</i> The use of quantitative methodology (pyrosequencing) to determine DNA methylation levels, adjustment for confounding factors, exposure measurements (airborne benzene; urinary benzene, <i>t,t</i> -MA, and SPMA).<br><i>Limitation:</i> Blood cell count was not accounted for.                                                                                                                                                             | <a href="#">Fustinoni et al. (2012)</a> |



**Table 4.7 (continued)**

| End-points                                                                                                            | Biological sample | Location, setting, study design                                                 | Exposure level<br>No. of study participants                                                                                                                                                                                                         | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                      | Covariates controlled           | Comments                                                                                                                                                                                                                                                                                                                                                                                 | Reference                                   |
|-----------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| miR-221 expression                                                                                                    | Peripheral blood  | Southern China<br>Cross-sectional study<br>Service station attendants           | $n = 97$ Service station attendants (59 men and 38 women) with mean airborne benzene of $73.0 \pm 18.8 \text{ mg/m}^3$<br>$n = 103$ residents of the same cities (61 men and 42 women) with mean airborne benzene of $7.47 \pm 1.15 \text{ mg/m}^3$ | (↑) miR-221 expression in the exposed group (in multivariate logistic regression, miR-221 expression level was associated with the plasma benzene concentration, type of job, exposure age, and protective measures)                         | Alcohol consumption and smoking | <i>Strengths:</i> The use of quantitative methodology to determine expression levels (qPCR), adjustment for confounding factors, and the use of exposure measurements (airborne and plasma benzene).<br><i>Limitations:</i> Details of the expression analysis were not provided; blood cell count not accounted for.                                                                    | <a href="#">Hu et al. (2016)</a>            |
| <i>IL6</i> promoter DNA methylation<br><i>iNOS</i> promoter DNA methylation<br><i>CYP2E1</i> promoter DNA methylation | Peripheral blood  | Léon, Guanajuato, Mexico<br>Cross-sectional study<br>Service station attendants | $n = 36$ service station attendants with mean benzene exposure $0.19 \text{ mg/m}^3$<br>$n = 66$ administrative personnel from a public university with mean benzene exposure, $0.001 \text{ mg/m}^3$                                               | ↑ <i>IL6</i> methylation level in the exposed group<br><i>iNOS</i> methylation level correlated with the cumulative duration of exposure and the urinary SPMA level<br><i>CYP2E1</i> methylation inversely correlated with air benzene level | Age, sex, and smoking           | <i>Strengths:</i> The use of quantitative methodology to determine DNA methylation levels (pyrosequencing), adjustment for confounding factors, exposure measurements (BTEX, <i>n</i> -hexane, MEK, methyl acetate, and acetone were measured in the breathing zone).<br><i>Limitations:</i> Blood cell count was not accounted for; exposure was not measured for all the participants. | <a href="#">Jiménez-Garza et al. (2017)</a> |

**Table 4.7 (continued)**

| End-points                                                                                                                                          | Biological sample | Location, setting, study design                                                 | Exposure level<br>No. of study participants                                                                                                                                                                      | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                                      | Covariates controlled           | Comments                                                                                                                                                                                                                                                                                                                             | Reference                                   |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Promoter DNA methylation:<br><i>SOD1</i><br><i>TOP2A</i><br><i>TNFA</i><br><i>COX2</i><br><i>GSTP1</i><br><i>HMOX1</i><br><i>iNOS</i>               | Peripheral blood  | Léon, Guanajuato, Mexico<br>Cross-sectional study<br>Service station attendants | <i>n</i> = 36 service station attendants with mean benzene exposure 0.19 mg/m <sup>3</sup><br><i>n</i> = 66 administrative personnel from a public university with mean benzene exposure 0.001 mg/m <sup>3</sup> | <i>SOD1</i> methylation level inversely correlated with benzene exposure<br><i>TOP2A</i> methylation level directly correlated with toluene and ethylbenzene and inversely correlated with MEK<br><i>TNFA</i> methylation level directly correlated with the acetone level<br>No changes for other promoters | Age, sex, and smoking           | <i>Strengths:</i> The use of quantitative methodology to determine DNA methylation levels (pyrosequencing), adjustment for confounding factors, exposure measurements (up to 8 VOCs were measured in the breathing zone).<br><i>Limitations:</i> Blood cell count not accounted for; exposure not measured for all the participants. | <a href="#">Jiménez-Garza et al. (2018)</a> |
| <i>p14</i> <sup>ARF</sup> promoter DNA methylation<br><i>p16</i> <sup>INK4A</sup> promoter DNA methylation<br><i>GSTP1</i> promoter DNA methylation | Peripheral blood  | Rio de Janeiro, Brazil<br>Cross-sectional study<br>Service station attendants   | <i>n</i> = 59 service station attendants (21 women and 38 men)<br><i>n</i> = 68 age-matched volunteers (30 women and 38 men) with no previous history of occupational exposure to BTEX                           | (↑) <i>p14</i> <sup>ARF</sup> methylation<br>(↑) <i>p16</i> <sup>INK4A</sup> methylation<br>No change in <i>GSTP1</i> (no association observed between phenotype and methylation status)                                                                                                                     | Smoking and alcohol consumption | <i>Strengths:</i> Adjustment for confounding factors.<br><i>Limitations:</i> The use of a semiquantitative methodology to determine DNA methylation level (MSP); lack of exposure measurements; blood cell count not accounted for.                                                                                                  | <a href="#">Silvestre et al. (2020)</a>     |

**Table 4.7 (continued)**

| End-points                                            | Biological sample | Location, setting, study design | Exposure level<br>No. of study participants                                                          | Response <sup>a</sup><br>(significance)                                                      | Covariates controlled | Comments                                                                                                                                                                                                                                                                                                                                                     | Reference                           |
|-------------------------------------------------------|-------------------|---------------------------------|------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| ~800 promoters of cancer associated genes methylation | Peripheral blood  | NR                              | 6 benzene-exposed workers (2 men, 4 women)<br><i>n</i> = 4 non-exposed participants (2 men, 2 women) | (↓) <i>RUNX3</i> methylation<br>(↑) <i>MSH3</i> methylation<br>(↑) <i>SEMA3C</i> methylation | NR                    | <i>Strengths:</i> The use of high-throughput methodology to assess DNA methylation (microarray).<br><br><i>Limitations:</i> Methodology was poorly described (groups were not properly defined; exposure measurements and statistical parameters were not provided; confounding factors were not considered) and a small number of individuals was analysed. | <a href="#">Zhang et al. (2010)</a> |

BMI, body mass index; h, hour(s); IL, interleukin; miR, microRNA; MEK, methyl ethyl ketone; MSP, methylation-specific PCR; MTBE, methyl *tert*-butyl ether; mtDNA, mitochondrial DNA; mtDNAcn, mitochondrial DNA copy number; NR, not reported; PCR, polymerase chain reaction; rRNA, ribosomal ribonucleic acid; qPCR, quantitative polymerase chain reaction; SPMA, *S*-phenylmercapturic acid; *t,t*-MA, *trans,trans*-muconic acid; tRNA, transfer RNA; VOC, volatile organic compound.

<sup>a</sup> ↑, increase; ↓, decrease; (↑), (↓), increase or decrease in a study of limited quality; or no change.

Additionally, the Working Group considered that the use of quantitative methodologies to determine the DNA methylation level (such as pyrosequencing) and the mtDNA copy number (such as quantitative real-time polymerase chain reaction), the adjustment for confounding factors, and the measurement of airborne benzene exposure strengthen the informativeness of the study. The analysis was conducted according to region for all the study participants, service station attendants, and police officers. One study limitation was noted: the lack of adjustment for cell type composition.]

In a study conducted by Byun and colleagues, the methylation levels of *LINE-1* (*L1HS*, *L1PA5*, *L1PA2*, and *L1Ta*), *Alu* (*AluSx*, *AluYb8*, and *AluYd6*), and human endogenous retroviruses (*HERV* – *MLT1D*, *ERV1*, and *ERV9*) were analysed in 20 service station attendants and 20 office workers in Milan, Italy (Byun et al., 2013a). *L1PA2* was significantly hypomethylated in service stations attendants, compared with controls, and its methylation inversely correlated with the airborne benzene level. This correlation was also significant after adjustment for age and smoking status. [The Working Group noted that strengths of the study included the use of a quantitative assessment of specific subfamilies of retroelements, rather than just a global DNA methylation assessment, and the adjustment for relevant confounding factors.]

In addition to altered methylation of genomic DNA, Byun et al. (2013b) also identified alterations in mtDNA sampled from the same service station attendants in Milan, Italy. When they analysed three regions (tRNA phenylalanine, 12S rRNA gene and “D-loop” control region), the authors did not observe differences between the service station attendants and office workers, who had a low level of exposure. [The Working Group noted that the interpretation of the results was difficult, because the biological effects of mtDNA methylation are still poorly understood.]

Service station attendants ( $n=89$ , all men) and non-occupationally exposed controls ( $n=90$ , all men) in the Milan area, Italy, were recruited for a study conducted by Rota et al. (2018). They measured biomarkers of exposure, including airborne benzene and MTBE, and urinary benzene, *t,t*-MA, and SPMA concentrations. Excepting the urinary concentration of *t,t*-MA, the concentrations of these biomarkers were higher in the exposed group than in the control group. *LINE-1* and *Alu* methylation, as well as RNA expression of the same elements, indicative of their demethylation and consequent activation, were measured in peripheral blood samples. In addition, the authors measured the levels of the histone H3K9me3. Only L1-Pa5 was hypomethylated in the service station attendants versus the control group. However, the expression of *AluY* and *AluJ* directly correlated with the urinary benzene concentration, and methylation levels of *AluYD6* and *L1-Ta* inversely correlated with urinary MTBE concentrations in the service station attendants.

[The Working Group noted that details of this analysis were not provided, limiting the interpretability of the findings. Nevertheless, the Working Group noted that the assessment of both DNA methylation and the expression levels of retroelements was a strength of the study, adding evidence regarding the activation of these elements. However, the associations did not involve the same repetitive elements, or the same urinary biomarkers, suggesting that the alterations identified might be linked to specific chemical components of the automotive gasoline. The Working Group could not exclude the possibility that the observed effects might have been affected by other exposures, behaviours, and specific genetic features, such as associated gene variants involved in detoxification.]

Global DNA methylation was measured in 78 service station attendants and 58 controls in Milan, Italy, by assessing 5-methylcytosine levels by GC-MS analysis and assessing the DNA

methylation levels of *Alu* and *LINE-1* repetitive elements ([Fustinoni et al., 2012](#)). Both the global and *LINE-1* methylation levels were lower in the exposed group. In addition, the airborne benzene level inversely correlated with the global, *LINE-1*, and *Alu* methylation levels; whereas the *t,t*-MA concentration inversely correlated with *LINE-1* and *Alu* methylation. The results remained significant after adjustment for age and sex. Smoking was shown to have a significant impact on benzene-related biomarkers, but not on methylation. [The Working Group noted that the quantitative assessment of exposure biomarkers and DNA methylation and the adjustment for confounding factors were strengths of the study.]

[The Working Group noted that microRNAs (miRNAs) are small non-coding RNAs involved in the post-transcriptional regulation of gene expression. These molecules recognize their target messenger RNAs (mRNAs) by base complementarity, which leads to their degradation or a cessation of protein translation. A single miRNA can have many different targets, which are cell type-dependent ([Saliminejad et al., 2019](#)).]

[Hu et al. \(2016\)](#) analysed the expression of a single miR, miR-221, previously described as a driver of leukaemogenesis (the development of leukaemia), in a cohort in southern China ([Zhang et al., 2020, 2021](#); [Li et al., 2024](#)). Exposure to automotive gasoline was estimated by measuring benzene concentrations both in the air of a breathing zone (1.4 m), and in the plasma of service station attendants (the exposed group) and residents in the same cities (the control group). These groups did not differ in the prevalence of the potential confounding factors alcohol consumption and smoking habits. Higher miR-221 expression was shown in the lymphocytes of service station attendants than in those of the control group. In addition, the miRNA expression level correlated with the concentration of benzene in plasma. Expression of miR-221 was associated with benzene concentration, type of job, age of exposure, and protective measures

in multivariate logistic regression. [The Working Group noted that the study lacked some details on the technical and analytical procedures regarding miRNA expression. In addition, the authors used different expression units in the various analyses.]

The methylation of the *IL6*, *iNOS*, and *CYP2E1* promoters was assessed in workers in a plastic shoe factory and in service station workers, both groups occupationally exposed to solvents, and was compared with methylation levels in administrative personnel (control group) in León, Guanajuato, Mexico ([Jiménez-Garza et al., 2017](#)). [The Working Group noted that IL-6 is one of the most important pro-inflammatory cytokines in the tumour microenvironment and is upregulated in almost all tumour types ([Kumari et al., 2016](#)). Nitric oxide is a free radical that can modify all biological molecules and is a key inflammatory mediator ([Janakiram and Rao, 2012](#)). *CYP2E1* encodes an enzyme that is involved in the metabolism of several carcinogens, such as ethanol, nitrosamines, and benzene; polymorphisms in this gene have been frequently associated with cancer risk; and its expression is modulated by the level of exposure to carcinogens ([Schoedel and Tyndale, 2003](#); [Dougherty et al., 2008](#); [Yin et al., 2018](#)).] The authors observed higher levels of *IL6* promoter methylation in 36 service station workers than in 66 controls, and this difference remained significant after adjustment for confounding factors. In addition, the *iNOS* promoter methylation level positively correlated with the cumulative duration of exposure and the urinary concentration of SPMA. A significant inverse correlation was observed between *CYP2E1* promoter methylation and the airborne benzene level. [The Working Group noted that the study contained relevant data on the epigenetic modulation of inflammation-associated genes.]

[Jiménez-Garza et al. \(2018\)](#) reported no differences in the promoter DNA methylation levels of seven genes (*COX2*, *iNOS*, *SOD1*,

*HMOX1*, *GSTP1*, and *TNFA*, which encode proteins involved in oxidative stress and inflammatory pathways; [Jiménez-Garza et al. \(2018\)](#); and *TOP2A*, which is involved in DNA breaking and rejoining during DNA replication; [Nitiss, 2009](#)) between service station attendants from Léon, Guanajuato, Mexico (the same individuals studied by [Jiménez-Garza et al., 2017](#)) and the control group ([Jiménez-Garza et al., 2018](#)). However, the *SOD1* promoter methylation level was inversely correlated with the individual benzene exposure level; the *TOP2A* promoter methylation level was directly correlated with toluene and ethylbenzene exposure, and was inversely correlated with methyl ethyl ketone exposure; and the *TNFA* promoter methylation level directly was correlated with acetone exposure. [The Working Group noted that the level of exposure was not measured in all the study participants.]

[Silvestre et al. \(2020\)](#) observed a higher frequency of *p14* and *p16* hypermethylation in peripheral blood samples collected from service station attendants in Rio de Janeiro, Brazil, than in controls ([Silvestre et al., 2020](#)). [The Working Group noted that these two tumour suppressor genes can be affected by different molecular mechanisms, including by DNA methylation, during carcinogenesis ([Zhao et al., 2016](#)).] The differences between methylation levels remained significant when exposed and non-exposed individuals were placed into subgroups according to their smoking habits and alcohol consumption. Female service attendants who had experienced miscarriage showed hypermethylation of at least two of the three genes analysed (*p14*, *p16*, and *GSTP1*). In addition, the frequency of *p16* hypermethylation was significantly higher in service station attendants who had chromosomal abnormalities. [The Working Group noted that DNA methylation was assessed using a semiquantitative method.]

## Synopsis

[The Working Group noted that epigenetic alterations might reflect a difference in the proportions of specific cell types, because changes in methylation are associated with cell differentiation ([Jaenisch and Bird, 2003](#)). In all the studies, whole blood or lymphocytes were used to assess the variations associated with exposures, which could have biased the epigenetic analyses. In addition, all the studies presented here had a cross-sectional design, which limited the causal inferences that could be made. The main challenge was to determine whether exposures other than to automotive gasoline might have been involved in the observed effects. Even when well-known confounding factors were adjusted for, it was not possible to determine which variables might be associated with epigenetic alterations. In particular, the geographical location (rural or urban) of the participants should have been described for all the studies. The available studies investigated potential associations between automotive gasoline exposure and the gene-specific and global DNA methylation levels in service station attendants. Most of these studies included an exposure assessment that consisted of the measurement of the airborne benzene level, comparison of the epigenetic alteration end-points in the exposed and non-exposed groups, and correlation analysis of the individual benzene level (airborne, urinary, or plasma). The techniques used to assess epigenetic alterations were consistent with the technology available at the time of the study, and the results were appropriately interpreted. The analyses focused on genes that are normally associated with the metabolism of chemical components of automotive gasoline, which would be expected to be affected by the exposure. However, alterations in genes associated with cancer, inflammation, and oxidative stress, as well as repetitive elements (which were used as proxies for global DNA methylation, but are also indicative of genomic



instability) were also investigated. *LINE-1* hypomethylation was the most frequent finding, being observed in various studies of the same populations, and this was shown to be independent of confounding factors such as smoking and age. The Working Group noted that such alterations in the methylation profile of *LINE-1* are consistently observed in cancer ([Deininger and Batzer, 1999](#); [Xiao-Jie et al., 2016](#); [Burns, 2017](#)), and have been suggested to be an early alteration during carcinogenesis ([Cho et al., 2009](#); [Kitkumthorn and Mutirangura, 2011](#); [Dumitrescu, 2012](#); [Murata et al., 2013](#)).]

#### 4.2.5 Induces oxidative stress

[The Working Group noted that the impact of oxidative stress induced by automotive gasoline has been a significant area of research. This section summarizes the evidence for oxidative stress induced by exposure to gasoline in studies in exposed human and in experimental systems.]

##### (a) Humans

##### Exposed humans

See [Table 4.8](#).

[Several studies have explored the impact of automotive gasoline exposure on oxidative stress markers in humans, especially in service station attendants and individuals with related jobs. The Working Group noted that in most of the studies, comparisons were made with groups with no history of occupational exposure to automotive gasoline or heavy metals, service station cashiers, drivers in different urban areas, fibreglass or plastic workers, or painters. In addition, service station attendants from different regional areas and/or with different durations of employment were compared.]

Elevated levels of 8-OHdG, an end-point related to oxidative damage to DNA, were found in service station attendants, and also in one study in car mechanics and refinery workers ([Lagorio et al., 1994b](#); [Göethel et al., 2014](#); [Kamal](#)

[et al., 2016](#); [Fenga et al., 2017](#); [Carrieri et al., 2019](#); [Tranfo et al., 2019](#); [Gaikwad et al., 2020](#)). [The Working Group noted that the urinary 8-OHdG concentration can be considered to be a reliable marker of cumulative oxidative stress in such populations of workers, relevant to the duration of the exposure or the years of employment. In contrast, the blood 8-OHdG concentration provides an index of the immediate occurrence of oxidative damage to DNA, and this parameter was less commonly measured in these studies ([Ruchirawat et al., 2010](#); [Göethel et al., 2014](#); [Xiong et al., 2016](#); [Brum et al., 2021](#)).] In addition, urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) concentration was associated with the occupation, as well as the working hours, of service station attendants ([Beerappa et al., 2013](#)). These results were in line with several of the studies reported in Sections 4.2.2 and 4.2.3, in which greater DNA damage, decreased DNA repair activity, micronucleus formation, increased mtDNA copy number, and cytogenetic damage were identified in service station attendants ([Rekhadevi et al., 2010](#); [Ruchirawat et al., 2010](#); [Carugno et al., 2012](#); [Moro et al., 2019](#); [Gaikwad et al., 2020](#); [Brum et al., 2021](#)). [The Working Group noted that oxidative damage to DNA was probably caused by ROS produced during benzene metabolism, and was probably an additional effect of co-exposure to toluene and/or xylene.]

In addition, other end-points related to oxidative stress in the blood were investigated in individuals exposed to gasoline. The blood concentrations of malondialdehyde (MDA) and 4-hydroxynonenal, markers of lipid peroxidation (LPO), were significantly higher in service station attendants than in non-exposed individuals ([Xiong et al., 2016](#); [Xia et al., 2017](#); [Alsés and Alzeer, 2021](#); [Toson et al., 2022](#)).

**Table 4.8 End-points relevant to oxidative stress in humans exposed to automotive gasoline**

| End-point, assay                                             | Biological sample | Location, setting, study design                                   | Exposure duration or level<br>No. of study participants                                                                                                                                                        | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                                                                            | Covariates controlled                                             | Comments                                                                                                                                                                                                                                               | Reference                              |
|--------------------------------------------------------------|-------------------|-------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| 8-OHdG, by HPLC                                              | Urine             | Italy<br>Observational<br>Service station attendants              | Benzene,<br>0.45 ± 0.94 mg/m <sup>3</sup> ;<br>toluene,<br>0.68 ± 1.67 mg/m <sup>3</sup> ;<br>xylene,<br>0.36 ± 1.09 mg/m <sup>3</sup><br><i>n</i> = 65 service station attendants                             | + Correlation between 8-OHdG and benzene exposure<br>Dose–response relationship for 8-OHdG (per unit increase in benzene exposure)                                                                                                                                                                                                                 | Age, length of employment, smoking, exposure to diagnostic X-rays | Benzene, toluene, and xylene exposure measured over 1 yr using repeated personal samplers.                                                                                                                                                             | <a href="#">Lagorio et al. (1994b)</a> |
| 8-OHdG, by commercial kit                                    | Urine, blood      | Brazil<br>Cross-sectional<br>Service station attendants           | Years of working<br>11.03 ± 1.1 for service station attendants<br>17.23 ± 1.7 for taxi drivers<br><i>n</i> = 52 service station attendants<br><i>n</i> = 34 taxi drivers<br><i>n</i> = 22 non-exposed controls | ↑ 8-OHdG in service station attendants and taxi drivers ( <i>P</i> < 0.05)<br>+ Correlation between benzene exposure and 8-OHdG<br>+ Correlation between 8-OHdG and increase in MN and DSBs (assessed in a combination of the service station attendants and taxi drivers; see also <a href="#">Table 4.2</a> and <a href="#">Section 4.2.2</a> ). | Age, sex, smoking                                                 | Markers of exposure assessed:<br><i>t,t</i> -MA, 440 µg/g creatinine for service station attendants and 118 µg/g creatinine for non-exposed controls.                                                                                                  | <a href="#">Goethel et al. (2014)</a>  |
| 8-OHdG<br>SOD,<br>by ELISA and Marklund method, respectively | Blood and urine   | Pakistan<br>Cross-sectional<br>Car mechanics and refinery workers | Duration of employment for both groups, 12 ± 5.8 yr<br><i>n</i> = 25 car mechanics<br><i>n</i> = 30 refinery workers<br><i>n</i> = 34 age-matched non-exposed controls                                         | ↑ 8-OHdG ( <i>P</i> < 0.01)<br>No change in SOD                                                                                                                                                                                                                                                                                                    | Age                                                               | Markers of exposure were assessed:<br>PAH metabolites: monohydroxy-PAHs, 1.02–4.1 µmol/mol creatinine for mechanics and 0.78–3.88 µmol/mol creatinine for refinery workers.<br><br><i>Limitation:</i> Multiple work environments with other exposures. | <a href="#">Kamal et al. (2016)</a>    |

**Table 4.8 (continued)**

| End-point, assay                       | Biological sample | Location, setting, study design                                       | Exposure duration or level<br>No. of study participants                                                                                                   | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                     | Covariates controlled                                              | Comments                                                                                                                                                                                                                                                                                                                                   | Reference                              |
|----------------------------------------|-------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| 8-OHdG, by ELISA                       | Urine             | India<br>Cross-sectional<br>Service station attendants                | Average duration of employment, $3.2 \pm 2$ yr, $13.2 \pm 6.4$ h per day<br>$n = 26$ service station attendants<br>$n = 27$ non-exposed controls          | $\uparrow$ 8-OHdG ( $P = 0.01$ )                                                                                                                                                                                                            | Age, BMI, sex, educational level, smoking, and alcohol consumption | Markers of exposure were assessed: Urinary phenol, $20.5 \pm 9.4$ mg/g creatinine, vs non-exposed, $8.4 \pm 5.7$ mg/g creatinine<br>Multiple end-points of DNA damage evaluated.                                                                                                                                                           | <a href="#">Gaikwad et al. (2020)</a>  |
| 8-OHdG, by ELISA                       | Urine             | Sicily, Italy<br>Cross-sectional<br>Service station attendants        | Years of occupational exposure to fuel, $13.74 \pm 5.47$<br>$n = 80$ service station attendants<br>$n = 63$ office workers (non-exposed control group)    | $\uparrow$ 8-OHdG ( $P < 0.05$ )<br>Significant correlations between 8-OHdG and $t,t$ -MA concentration, job seniority, and age                                                                                                             | Sex, smoking, alcohol consumption, BMI                             | Markers of exposure were assessed: $t,t$ -MA, $0.83 \pm 0.54$ $\mu\text{g/mL}$ urine                                                                                                                                                                                                                                                       | <a href="#">Fenga et al. (2017)</a>    |
| 8-oxoGua<br>8-oxodG<br>8-oxoGuo, by MS | Urine             | Saudi Arabia and Italy<br>Observational<br>Service station attendants | $n = 29$ in Saudi Arabia (10–12-h shifts; samples collected before and after shifts)<br>$n = 102$ in Italy (2–8-h shifts; samples collected after shifts) | $\uparrow$ 8-oxoGua ( $P = 0.0097$ )<br>$\uparrow$ 8-oxodG ( $P < 0.0001$ )<br>$\uparrow$ 8-oxoGuo ( $P < 0.0001$ )<br>in Saudi Arabia vs Italy<br>Italy: + correlations among the three oxidation end-points and between SPMA and 8-oxoGua | Smoking                                                            | Partial exposure assessment: SPMA, $13.97 \pm 12.93$ and $14.45 \pm 17.72$ $\mu\text{g/g}$ creatinine in smokers and non-smokers, respectively; cotinine, $1619.97 \pm 1057.26$ and $28.71 \pm 28.14$ $\mu\text{g/g}$ creatinine in smokers and non-smokers, respectively.<br>Variability in exposure to benzene.<br>Observational design. | <a href="#">Carrieri et al. (2019)</a> |

**Table 4.8 (continued)**

| End-point, assay                                    | Biological sample | Location, setting, study design                                         | Exposure duration or level<br>No. of study participants                                                                                                                                                                                                                                                                                                                                           | Response <sup>a</sup><br>(significance)                                                                                | Covariates controlled                      | Comments                                                                                                                                                                                                                                      | Reference                                |
|-----------------------------------------------------|-------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| 8-oxoGua<br>8-oxoGuo<br>8-oxodG,<br>by HPLC-MS/MS   | Urine             | Saudi Arabia and Italy<br>Cross-sectional<br>Service station attendants | Group 1: <i>n</i> = 29 service station attendants in Saudi Arabia (work shift, 10–13 h per day)<br>Group 2: <i>n</i> = 102 service station attendants in Italy<br>Group 3: <i>n</i> = 24 fibreglass-reinforced plastic workers<br>Group 4: <i>n</i> = 17 ship painters<br>Group 5: <i>n</i> = 6 divers<br>Group 6: <i>n</i> = 2 researchers<br>Group 7: <i>n</i> = 132 general population members | Compared with the general population group (7):<br>↑ 8-oxoGua (2, 3)<br>↑ 8-oxodG (1, 2, 3)<br>↑ 8-oxoGuo (1, 2, 3, 4) | Smoking, sex, ethnicity                    | Markers of exposure were assessed.<br>Multiple occupation groups.                                                                                                                                                                             | <a href="#">Tranfo et al. (2019)</a>     |
| 8-OHdG,<br>by commercial kit                        | Blood             | Thailand<br>Cross-sectional<br>Service station attendants               | <i>n</i> = 31 petrochemical laboratory workers<br><i>n</i> = 31 service station attendants<br><i>n</i> = 34 non-exposed control workers                                                                                                                                                                                                                                                           | ↑ 8-OHdG ( <i>P</i> < 0.001) in service station attendants                                                             | Sex, age, smoking                          | Markers of exposure were assessed.<br>Small sample size; no data about the years of service or working hours.<br>Air monitoring: 207.94 µg/m <sup>3</sup> (0.065 ppm <sup>b</sup> ) benzene in the workplace.<br>Multiple exposure scenarios. | <a href="#">Ruchirawat et al. (2010)</a> |
| T-SOD<br>GSH<br>MDA<br>8-OHdG,<br>by commercial kit | Blood             | China<br>Cross-sectional<br>Service station attendants                  | Workers exposed to fuel for an average of 8.7 yr<br><i>n</i> = 200 service station attendants<br><i>n</i> = 52 service station office workers                                                                                                                                                                                                                                                     | ↓ T-SOD ( <i>P</i> < 0.001)<br>↓ GSH ( <i>P</i> < 0.001)<br>↑ MDA ( <i>P</i> < 0.001)<br>↑ 8-OHdG ( <i>P</i> < 0.001)  | Age, sex, smoking, and alcohol consumption | Multiple markers of exposure assessed.<br>Air monitoring: 60.03 µg/m <sup>3</sup> (0.019 ppm <sup>b</sup> ) benzene in the workplace.                                                                                                         | <a href="#">Xiong et al. (2016)</a>      |

**Table 4.8 (continued)**

| End-point, assay                                                                                                                                                                   | Biological sample | Location, setting, study design                            | Exposure duration or level<br>No. of study participants                                                                                                                                                                                                                           | Response <sup>a</sup><br>(significance)                                                                      | Covariates controlled                                                                 | Comments                                                                                                                                                                                                                                                                                                                                                      | Reference                                       |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| 8-OHdG<br>CAT<br>δ-ALA-D<br>TBARS<br>NPSH<br>Ascorbic acid,<br>by Aebi method<br>(1984),<br>Berlin and Schaller<br>method (1974),<br>Ohkawa method<br>(1979), or<br>commercial kit | Blood             | Brazil<br>Cross-sectional<br>Service station<br>attendants | Workers mostly on a<br>6–8 h per day work<br>shift, exposed to fuel<br>for 1 to > 15 yr<br><i>n</i> = 17 service station<br>attendants<br><i>n</i> = 12 painters<br><i>n</i> = 16 non-exposed<br>controls                                                                         | ↑ 8-OHdG ( <i>P</i> < 0.001)<br>No change for other<br>oxidative stress end-<br>points                       | Age, sex,<br>smoking,<br>and alcohol<br>consumption                                   | No exposure<br>assessment. Small<br>sample size.                                                                                                                                                                                                                                                                                                              | <a href="#">Brum et al.<br/>(2021)</a>          |
| 8-oxodG,<br>by ELISA                                                                                                                                                               | Urine             | India<br>Cross-sectional<br>Service station<br>attendants  | Workers mostly on a<br>8 h per day work shift,<br>exposed to fuel for an<br>average of 7.30 ± 7.98 yr<br>or not for 8.76 ± 6.24 yr<br><i>n</i> = 79 service station<br>attendants<br><i>n</i> = 21 cashiers (non-<br>exposed control group)                                       | ↑ 8-oxodG<br>+ Correlation between<br>working hours (8–11) per<br>day and 8-oxodG                            | Age,<br>smoking,<br>alcohol<br>consumption,<br>eating habits,<br>physical<br>activity | No exposure<br>assessment. Conflicting<br>results.                                                                                                                                                                                                                                                                                                            | <a href="#">Beerappa<br/>et al.<br/>(2013)</a>  |
| SOD<br>GPx<br>CAT<br>MDA,<br>by biochemical<br>analysis                                                                                                                            | Blood             | India<br>Cross-sectional<br>Service station<br>attendants  | Workers exposed to<br>fuel for ~5 h per day,<br>workers dispensed<br>~665 L of fuel<br>per day for ≥ 5 yr<br>(10.66 ± 3.58 yr)<br><i>n</i> = 200 service station<br>attendants, with<br>fuelling tasks<br><i>n</i> = 200 controls,<br>with no history of<br>occupational exposure | ↓ SOD ( <i>P</i> < 0.05)<br>↓ GPx ( <i>P</i> < 0.05)<br>↑ CAT ( <i>P</i> < 0.05)<br>↑ MDA ( <i>P</i> < 0.05) | Sex, age,<br>smoking,<br>alcohol<br>consumption                                       | Markers of exposure<br>were assessed.<br>Combination of<br>markers of exposure:<br>↑ benzene, toluene, and<br>xylene in ambient air;<br>↑ benzene, toluene, and<br>xylene in breathing-<br>zone air; ↑ benzene and<br>xylene in blood.<br>Benzene in ambient<br>air: 1322 µg/m <sup>3</sup><br>(0.414 ppm <sup>b</sup> ).<br>Multiple end-points<br>assessed. | <a href="#">Rekhadevi<br/>et al.<br/>(2010)</a> |

**Table 4.8 (continued)**

| End-point, assay                             | Biological sample | Location, setting, study design                         | Exposure duration or level<br>No. of study participants                                                                                                                                                                                                                                                                                           | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                | Covariates controlled                               | Comments                                                                                                                                                                                    | Reference                             |
|----------------------------------------------|-------------------|---------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| mtDNACn, by qPCR                             | Blood             | Italy<br>Cross-sectional<br>Service station attendants  | Median personal benzene exposure:<br>Gas station attendants, 60.9 µg/m <sup>3</sup><br>Police officers, 21.8 µg/m <sup>3</sup><br>Controls, 6.3 µg/m <sup>3</sup><br><i>n</i> = 153 bus drivers<br><i>n</i> = 78 service station attendants<br><i>n</i> = 77 police officers<br><i>n</i> = 33 petrochemical workers<br>Referents in the same area | ↑ mtDNACn ( <i>P</i> < 0.05)<br>+ Correlation with airborne benzene concentration                                                                                                                                                      | Age, sex, smoking, and number of cigarettes per day | Markers of exposure were assessed.<br>Multicentre study. Low-level exposure to benzene (> 100 times lower than the Occupational Safety and Health Administration/European Union standards). | <a href="#">Carugno et al. (2012)</a> |
| PCO<br>GST,<br>by ELISA, Habig method (1975) | Blood             | Brazil<br>Cross-sectional<br>Service station attendants | <i>n</i> = 38 service station attendants<br><i>n</i> = 28 non-exposed, non-smoking participants                                                                                                                                                                                                                                                   | ↑ PCO ( <i>P</i> < 0.001)<br>↓ GST ( <i>P</i> < 0.05)<br>BTX levels correlated positively with PCO and negatively with GST ( <i>P</i> < 0.001)<br>PCO and GST correlated significantly with inflammatory biomarkers ( <i>P</i> < 0.05) | Age, smoking                                        | Markers of exposure were assessed.<br>Small sample size.                                                                                                                                    | <a href="#">Moro et al. (2019)</a>    |



**Table 4.8 (continued)**

| End-point, assay                                                                                                                                      | Biological sample | Location, setting, study design                                       | Exposure duration or level<br>No. of study participants                                                                              | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                                               | Covariates controlled | Comments                                                                                                                                      | Reference                               |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| SOD<br>GPx<br>MDA<br>Hsp70,<br>by commercial kit<br>ELISA                                                                                             | Blood             | China<br>Cross-sectional<br>Service station attendants                | <i>n</i> = 96 service station attendants with fuelling tasks<br><i>n</i> = 48 service station attendants with office cashier's tasks | ↓ SOD ( <i>P</i> < 0.001)<br>↓ GPx ( <i>P</i> = 0.001)<br>↑ MDA ( <i>P</i> = 0.021)<br>↑ Hsp70 ( <i>P</i> = 0.002)<br>+ Correlations between BTEX exposure and SOD and GPx levels<br>+ Correlations between BTEX exposure and MDA and Hsp70<br>Only in exposed workers:<br>+ Correlations between Hsp70, GPx, and MDA |                       | Markers of exposure were assessed. Demographic characteristics were reported without statistical analyses.                                    | <a href="#">Xia et al. (2017)</a>       |
| MDA<br>AOPP<br>CAT,<br>by colorimetric detection                                                                                                      | Blood             | Syrian Arab Republic<br>Cross-sectional<br>Service station attendants | <i>n</i> = 30 service station attendants<br><i>n</i> = 30 non-exposed controls                                                       | ↑ MDA ( <i>P</i> = 0.0001)<br>↑ AOPP ( <i>P</i> = 0.020)<br>↑ CAT ( <i>P</i> = 0.002)<br>Alcohol consumption increased MDA ( <i>P</i> = 0.021) and CAT ( <i>P</i> = 0.036) levels<br>No effects of smoking or work duration                                                                                           |                       | Small sample size. No markers of exposure were assessed. Demographic characteristics reported without statistical analyses.                   | <a href="#">Alses and Alzeer (2021)</a> |
| SOD<br>CAT<br>GSH<br>MDA<br>TAC<br>NO,<br>by Nishikimi method (1972),<br>Sinha method (1972), Beutler method (1963),<br>MDA assay, and commercial kit | Blood (serum)     | Egypt<br>Cross-sectional<br>Service station attendants                | <i>n</i> = 62 service station male attendants<br><i>n</i> = 29 non-exposed controls                                                  | ↓ CAT ( <i>P</i> < 0.01)<br>↓ GSH ( <i>P</i> < 0.001)<br>↑ NO ( <i>P</i> < 0.05)<br>↑ MDA ( <i>P</i> < 0.05)<br>↑ NO/AMCase ( <i>P</i> < 0.05)<br>No change in SOD or TAC                                                                                                                                             | Age, sex              | Various oxidative stress and immune response markers were measured. Limited adjustment for covariates. No markers of exposure were evaluated. | <a href="#">Toson et al. (2022)</a>     |

**Table 4.8 (continued)**

| End-point, assay                                                                                                                 | Biological sample | Location, setting, study design                                           | Exposure duration or level<br>No. of study participants                                                                                                                                                                                                           | Response <sup>a</sup><br>(significance)                                                                                                                                                        | Covariates controlled | Comments                                                                                                          | Reference                            |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| Oxy-Hb<br>Met-Hb<br>Hemichrome<br>FRAP<br>Protein carbonyl, by spectrophotometry, Juybari method, (2018), and colorimetric assay | Blood             | Islamic Republic of Iran<br>Cross-sectional<br>Service station attendants | <i>n</i> = 40 service station attendants<br><i>n</i> = 39 non-attendants (non-exposed control group)                                                                                                                                                              | ↓ Oxy-Hb ( <i>P</i> < 0.001)<br>↑ Met-Hb ( <i>P</i> < 0.001)<br>↑ Hemichrome ( <i>P</i> < 0.001)<br>↓ FRAP ( <i>P</i> < 0.001)<br>↑ Protein carbonyl ( <i>P</i> < 0.001)                       | Age, sex, smoking     | No markers of exposure were assessed.                                                                             | <a href="#">Ahmadi et al. (2019)</a> |
| ROS<br>MDA<br>T-SOD<br>GSH<br>by DCFH-DA, TBARS, RANSOD, and DTNB                                                                | Blood (serum)     | India<br>Cross-sectional<br>Service station attendants                    | Workers exposed to fuel for < 10 yr (group I, <i>n</i> = 282; mean age, 28 ± 8 yr) or > 10 yr (group II, <i>n</i> = 146; mean age, 34 ± 14 yr), with a 12-h work shift per day<br><i>n</i> = 428 service station attendants<br><i>n</i> = 78 non-exposed controls | ↑ ROS ( <i>P</i> < 0.01)<br>↑ MDA ( <i>P</i> < 0.001)<br>↓ T-SOD ( <i>P</i> < 0.05)<br>↓ GSH ( <i>P</i> < 0.001)<br>Concentration of benzene in blood correlated with ROS, MDA, GSH, and T-SOD | Smoking               | Markers of exposure were assessed. Confounders were not controlled for; participants were limited to non-smokers. | <a href="#">Uzma et al. (2010)</a>   |

**Table 4.8 (continued)**

| End-point, assay                                                                                                                     | Biological sample  | Location, setting, study design                                           | Exposure duration or level<br>No. of study participants                                                                                                                                                                                                                                                                                                 | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Covariates controlled | Comments                                                                                                                                                                                                                                           | Reference                                      |
|--------------------------------------------------------------------------------------------------------------------------------------|--------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| PCO<br>GSH<br>GST<br>3-NT<br>NO<br>SOD<br>CAT,<br>by ELISA,<br>spectrophotometry,<br>Weber method<br>(2012), Griess<br>method (2011) | Blood              | Rio Grande,<br>Brazil<br>Cross-sectional<br>Service station<br>attendants | Workers on 8-h work<br>shifts, exposed to<br>fuel for an average of<br>9.7 ± 1.1 yr (range,<br>0.5–32 yr)<br><i>n</i> = 43 service station<br>attendants<br><i>n</i> = 28 non-exposed<br>controls                                                                                                                                                       | ↑ PCO ( <i>P</i> < 0.001)<br>↓ GSH ( <i>P</i> < 0.001)<br>↓ GST ( <i>P</i> < 0.01)<br>↑ 3-NT ( <i>P</i> < 0.001)<br>↑ NO ( <i>P</i> < 0.001)<br>No change in SOD or<br>CAT activities<br>+ Associations of PCO<br>and 3-NT with personal<br>benzene exposure<br>– Associations of GSH<br>and GST with personal<br>benzene exposure<br>Duration of benzene<br>exposure (years) was<br>significantly associated<br>with protein damage,<br>GSH ( <i>P</i> < 0.05), and GST<br>activity ( <i>P</i> < 0.05) | Sex                   | Combination of<br>markers of exposure<br>assessed.<br>↑ Urinary <i>t,t</i> -MA in the<br>exposed group.<br>Personal benzene<br>exposure:<br>Exposed, 76.20 µg/m <sup>3</sup> ;<br>Controls, 42.01 µg/m <sup>3</sup> .                              | <a href="#">Moro et al.<br/>(2013)</a>         |
| GSH,<br>by Cereser method<br>(2001), Barch<br>method (1997),<br>comet assay                                                          | Urine and<br>blood | Italy<br>Cross-sectional<br>Service station<br>attendants                 | Workers on 8-h work<br>shifts, exposed to<br>fuel for 14.2 ± 14.1 yr<br>(attendants) or<br>10.7 ± 10.4 yr (pump<br>workers)<br><i>n</i> = 28 service station<br>attendants<br><i>n</i> = 33 petrochemical<br>industry operators<br><i>n</i> = 21 gasoline pump<br>maintenance workers<br>(GPMW)<br><i>n</i> = 51 non-exposed<br>workers (control group) | ↑ GSH level in<br>petrochemical industry<br>operators ( <i>P</i> < 0.0001)                                                                                                                                                                                                                                                                                                                                                                                                                              | Smoking               | Markers of exposure<br>were assessed.<br>Biological monitoring:<br>(↑) <i>t,t</i> -MA, (↑) SPMA.<br>Air monitoring:<br>40 µg/m <sup>3</sup> (0.013 ppm <sup>b</sup> )<br>benzene in the<br>workplace.<br>Small subgroups for<br>analyses in blood. | <a href="#">Fracasso<br/>et al.<br/>(2010)</a> |

**Table 4.8 (continued)**

| End-point, assay                                                    | Biological sample | Location, setting, study design                                        | Exposure duration or level<br>No. of study participants                                                                                                                                                                                                                   | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                  | Covariates controlled | Comments                                                                                                                                                     | Reference                                   |
|---------------------------------------------------------------------|-------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| SOD<br>GPx,<br>by atomic<br>absorption<br>spectroscopy              | Blood             | Egypt<br>Cross-sectional<br>Service station<br>attendants              | Half of the group were refuelling workers, working 8-h shifts on 6 days per week, exposed to fuel for > 15 yr<br><i>n</i> = 50 service station attendants<br><i>n</i> = 50 office workers (non-exposed control group)                                                     | ↓ SOD ( <i>P</i> = 0.04)<br>↓ GPx ( <i>P</i> = 0.011)<br>+ Correlation between trace metals and SOD activity in exposed workers. Hygienic effect of the BTEX mixture did not show significant correlations with oxidative stress markers | Age                   | Markers of exposure were assessed.<br>Air BTEX levels were measured.<br>Small sample size.                                                                   | <a href="#">Rizk et al. (2020)</a>          |
| CAT<br>SOD<br>GSH<br>GPx<br>NO<br>MPO<br>MDA,<br>by commercial kits | Blood             | Egypt<br>Prospective observational study<br>Service station attendants | Workers exposed to fuel during 8-h work shifts; subgroups created according to the number of years working in the station: 2, 4, 6, or > 6 yr<br><i>n</i> = 40 service station attendants from 10 different areas<br><i>n</i> = 10 volunteers (non-exposed control group) | ↓ CAT ( <i>P</i> < 0.05)<br>↓ SOD ( <i>P</i> < 0.05)<br>↓ GPx ( <i>P</i> < 0.05)<br>↓ GSH ( <i>P</i> < 0.05)<br>↓ NO ( <i>P</i> < 0.05)<br>↑ MPO ( <i>P</i> < 0.05)<br>↑ MDA ( <i>P</i> < 0.05)                                          |                       | No markers of exposure were assessed. Two blood samples were collected for each individual. Cohort characteristics and exposure parameters poorly described. | <a href="#">Abdel Maksoud et al. (2019)</a> |
| AOPP<br>ROMs<br>AGE,<br>by Kocak method (2009) and d-ROMS test      | Urine             | Sicily, Italy<br>Cross-sectional<br>Service station attendants         | Workers exposed to fuel during 8-h work shifts, for an average of 14.06 ± 6.23 yr<br><i>n</i> = 91 service station attendants<br><i>n</i> = 63 office workers, (control group with no history of occupational exposure)                                                   | ↑ AOPP ( <i>P</i> < 0.001)<br>↑ ROMs ( <i>P</i> < 0.001)<br>No change in AGE<br>AOPP and ROMs negatively correlated with fruit and vegetable consumption                                                                                 | Sex                   | No markers of exposure were evaluated. Dietary influence.<br>+ Association of <i>t,t</i> -MA level with ROMs ( <i>P</i> < 0.01).                             | <a href="#">Costa et al. (2016)</a>         |

**Table 4.8 (continued)**

| End-point, assay                | Biological sample | Location, setting, study design                            | Exposure duration or level<br>No. of study participants                                                                               | Response <sup>a</sup><br>(significance)                                                             | Covariates controlled | Comments                              | Reference                           |
|---------------------------------|-------------------|------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-----------------------|---------------------------------------|-------------------------------------|
| MDA, by ELISA                   | Blood             | Iraq<br>Cross-sectional<br>Service station attendants      | Workers exposed to fuel for $\geq 1$ yr<br>$n = 26$ non-obese male service station attendants<br>$n = 26$ non-exposed controls        | $\uparrow$ MDA ( $P < 0.001$ )                                                                      | Sex                   | No markers of exposure were assessed. | <a href="#">Numan et al. (2024)</a> |
| TAS, by Koracevic method (2001) | Blood             | Nigeria<br>Cross-sectional<br>Roadside gasoline dispensers | Workers exposed to fuel for $\geq 1$ yr<br>$n = 90$ service station attendants (roadside dispensers)<br>$n = 90$ non-exposed controls | $\downarrow$ TAS ( $P < 0.01$ )<br>Significant correlation between the duration of exposure and TAS | Sex, smoking          | No markers of exposure were assessed. | <a href="#">Adamu et al. (2018)</a> |

$\delta$ -ALA-D, delta-aminolevulinic acid dehydratase; AMCase, acidic mammalian chitinase; AOPP, advanced oxidation protein products; AGE, advanced glycation end-products; BMI, body mass index; BTEX, benzene, toluene, ethylbenzene, and xylene; BTX, benzene, toluene, and xylene; CAT, catalase; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); d-ROMs, derivatives of reactive oxygen metabolites; DSB, DNA strand break; ELISA, enzyme-linked immunosorbent assay; FRAP, fluorescence recovery after photobleaching; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione S-transferase; h, hour(s); HPLC, high-performance liquid chromatography; Hsp70, heat shock protein 70; MDA, malondialdehyde; met-Hb, methaemoglobin; MN, micronucleus; MPO, myeloperoxidase; MS, mass spectrometry; MS/MS, tandem mass spectrometry; mtDNAcn, mitochondrial DNA copy number; NO, nitric oxide; NPSH, non-protein thiols; 3-NT, 3-nitrotyrosine; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; 1-OHPyr, 1-hydroxypyrene (1-pyrenol),  $\alpha$ -naphthol ( $\alpha$ -naphthol or 1-hydroxynaphthalene), and  $\beta$ -naphthol ( $\beta$ -naphthol or 2-hydroxynaphthalene); 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGua, 8-oxoguanine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; oxy-Hb, oxyhaemoglobin; PAH, polycyclic aromatic hydrocarbon; PCO, protein carbonyl; ppm, parts per million; qPCR, quantitative polymerase chain reaction; RANSOD, Radox superoxide dismutase kit; ROMs, serum reactive oxygen metabolites; SOD, superoxide dismutase; SPMA, S-phenylmercapturic acid; TAC, total antioxidant capacity; TAS, total antioxidant status; TBARS, thiobarbituric acid-reactive substances; *t,t*-MA, *trans,trans*-muconic acid; T-SOD, total superoxide dismutase; yr, year(s).

<sup>a</sup> +, positive; –, negative;  $\downarrow$ , decrease;  $\uparrow$ , increase; or no change.

<sup>b</sup> The conversion of benzene concentrations from  $\text{mg}/\text{m}^3$  to ppm was performed using the formula  $\text{ppm} = [(\text{mg}/\text{m}^3) \times 24.45]/78.11$ , where 24.45 is the molar volume of an ideal gas under standard temperature and pressure conditions, and 78.11 is the molar mass of benzene. This approach ensured consistent transformations, on the basis that that 1 ppm of benzene is approximately equivalent to  $3.19 \text{ mg}/\text{m}^3$ .

Similarly, the levels of end-points of oxidative damage to proteins, such as plasma protein carbonyls, were significantly higher in service station attendants than in controls ([Ahmadi et al., 2019](#); [Moro et al., 2019](#)).

Service station attendants exhibited markedly elevated levels of ROS, which are key contributors to oxidative stress, compared with control individuals ([Uzma et al., 2010](#); [Fenga et al., 2016](#)). Furthermore, a positive correlation was identified between the concentration of benzene in the blood and the level of ROS in male service station attendants who worked 12-hour shifts ([Uzma et al., 2010](#)).

The levels or activities of antioxidant defence components, including GSH, and the antioxidant enzymes glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD), were found to be notably reduced in service station attendants, indicating impairment of the antioxidant defence system ([Fracasso et al., 2010](#); [Uzma et al., 2010](#); [Kisin et al., 2015](#); [Fenga et al., 2016](#); [Xiong et al., 2016](#); [Abdel Maksoud et al., 2019](#); [Moro et al., 2019](#); [Rizk et al., 2020](#); [Toson et al., 2022](#)). However, higher levels and activities of the antioxidant enzymes SOD, GPx, and CAT have been reported in a few studies ([Rekhadevi et al., 2010](#); [Kamal et al., 2016](#); and [Rizk et al., 2020](#)), indicating an oxidative stress response or a compensatory response to heightened oxidative stress.

In workers exposed to gasoline, a significant reduction in total antioxidant capacity has also been shown through the assessment of ferric reducing antioxidant power or of the total antioxidant status ([Adamu et al., 2018](#); [Ahmadi et al., 2019](#); [Toson et al., 2022](#)).

[Xia et al. \(2017\)](#) reported an inverse correlation between the cumulative exposure to gasoline and the levels of antioxidant enzymes (SOD, GSH, and CAT) ([Xia et al., 2017](#)). [The Working Group considered this finding to be an indication of increased oxidative stress. Additionally, the SOD and GSH levels were found to correlate

with heat shock protein Hsp70 levels, suggesting a stress response to gasoline ([Xia et al., 2017](#)).]

[Costa et al. \(2016\)](#) found higher levels of advanced oxidation protein products, advanced glycation end-products, and reactive oxygen metabolites in serum samples collected from service station attendants than in samples from a control group of office workers. [Abdel Maksoud et al. \(2019\)](#), in a prospective observational study of 40 service station attendants, observed significant alterations in blood antioxidant defence markers, including the concentrations of the metals cadmium, lead, iron, copper, and zinc, over time, as measured in serum samples collected after 0, 2, 4, and 6 months of the study ([Abdel Maksoud et al., 2019](#)).

Service station attendants had significantly lower serum levels of trace metals (copper, zinc, iron, and manganese) than control office workers ([Rizk et al., 2020](#)) or service station attendants with a longer length of employment ([Abdel Maksoud et al., 2019](#)). These decreases positively correlated with the decrease in SOD activity ([Rizk et al., 2020](#)). [The Working Group suggested that the low metal levels could have been the result of their consumption during the heightened antioxidant response. The Working Group noted that the imbalance in oxidative status could be also linked to disruption of the STAT3 and SAPK/JNK signalling pathways, as shown by [Fenga et al. \(2016\)](#) after chronic exposure to a low dose of benzene.]

[The findings described above were provided mainly by cross-sectional studies. These showed that service station attendants were typically exposed to significantly higher concentrations of BTEX compounds than were control individuals ([Rekhadevi et al., 2010](#); [Ruchirawat et al., 2010](#); [Uzma et al., 2010](#); [Moro et al., 2013](#); [Carrieri et al., 2019](#); [Tranfo et al., 2019](#)). The exposures were assessed using individual dosimeters in combination with GC, as well as biomarkers in blood and/or urine samples, including benzene metabolites, such as SPMA, and end-products of



benzene metabolism. The levels of BTEX compounds in the air sampled from refuelling areas have been reported to be significantly higher than those in office areas (Xiong et al., 2016). In two studies, an observational study design was used: the authors compared service attendants from different regions, and no comparison with a non-exposed control group was made (Lagorio et al., 1994b; Carrieri et al., 2019).]

Adamu et al. (2018) found a positive correlation between the duration of exposure to gasoline and the total antioxidant status in the blood of service station attendants, suggesting a cumulative effect over time, and Fenga et al. (2017) found a positive correlation between the urinary level of 8-OHdG and job seniority. The authors suggested that both the intensity and the duration of the exposure contributed to the observed increase in oxidative stress. In addition, Toson et al. (2022) reported significantly higher serum levels of nitric oxide in individuals exposed to gasoline vapour, especially in those with longer durations (> 10 years) of exposure.

Xiong et al. (2016) performed a multiple linear regression analysis that showed that exposure and the length of the exposure were significant factors influencing antioxidant capacity and the potential oxidative damage to DNA assessed in blood samples.

Additional studies by Hassan et al. (2013), Al-Helaly and Ahmed (2014), Awodele et al. (2014), Malini and Maithily (2017), Ibrahim et al. (2021), and Eworo et al. (2023) also showed that occupational exposure to gasoline was associated with increased oxidative stress in workers. The studies showed higher levels of LPO markers (particularly MDA) and lower levels of antioxidant vitamins and enzymes (including vitamins E and C, SOD, CAT, and GSH) in exposed workers than in controls (Hassan et al., 2013; Al-Helaly and Ahmed, 2014; Awodele et al., 2014; Malini and Maithily, 2017; Ibrahim et al., 2021). Some studies (Hassan et al., 2013; Al-Helaly and Ahmed, 2014; Ibrahim et al., 2021) identified

a positive correlation between the duration of exposure and end-points related to oxidative stress, suggesting cumulative effects, and one study (Eworo et al., 2023) showed an increase in oxidative damage to DNA (urinary levels of 8-OHdG) and reduced lung function in service station attendants. [The Working Group noted some limitations in these additional studies with respect to the study design, sample size, lack of exposure assessment and control for confounding, and poor statistical analysis. However, overall, the evidence was in line with an increase in oxidative stress, altered antioxidant enzyme activity, and in some cases DNA damage, after occupational exposure to gasoline. The Working Group also noted that prolonged exposure may exacerbate oxidative stress.]

#### (b) *Experimental systems*

See Table 4.9.

##### (i) *Non-human mammalian systems in vivo*

In rats, exposure to gasoline via inhalation caused mild, reversible changes in several biochemical, haematological, and histological parameters. Male rats exposed to gasoline showed elevated hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity, which returned to normal after the exposure ceased. However, no significant alterations were observed in female rats (Chu et al., 2005). Daily exposure to gasoline fumes for 8 hours over 60 or 90 days via inhalation led to significant decreases in the activities of SOD, GPx, and GST in the liver and the level of GSH in excised rat lung, compared with the control group. In addition, there were increases in the levels of LPO, nitric oxide, and xanthine oxidase activity, indicating tissue damage and oxidative stress (Owumi et al., 2021a). Extended exposure also caused time-dependent reductions in blood cell counts and haemoglobin levels (Owumi et al., 2021a), and increases in tissue pro-inflammatory cytokines, and reactive oxygen and nitrogen species (Owumi et al., 2021b)

**Table 4.9 End-points relevant to oxidative stress in non-human mammalian systems in vivo exposed to automotive gasoline**

| End-point              | Assay                                                                                                             | Species, strain (sex)         | Biological sample, tissue | Results <sup>a</sup>    | Dose (LED or HID)                                                                                                                              | Route, duration, dosing regimen                                                       | Comments                         | Reference                            |
|------------------------|-------------------------------------------------------------------------------------------------------------------|-------------------------------|---------------------------|-------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|----------------------------------|--------------------------------------|
| ROS, GSSG, and MDA     | Fluorescence spectrophotometry, colorimetric assay using DTNB, and ELISA, respectively                            | Rat, Wistar (M)               | Blood                     | ↑                       | 4 × 500 mL of unleaded gasoline were placed in beakers and positioned in the chamber                                                           | Inhalation (saturated exposure chamber), for 6 h per day on 6 days per week for 11 wk |                                  | <a href="#">Ghahri et al. (2024)</a> |
| GSH                    | Colorimetric assay using DTNB                                                                                     |                               |                           | ↓                       |                                                                                                                                                |                                                                                       |                                  |                                      |
| GSH, GPx, SOD, and GST | Colorimetric assay using DTNB, oxidation of NADPH, inhibition of epinephrine autoxidation, and CDNB, respectively | Rat, Wistar (M)               | Liver                     | ↓                       | 500 mL of gasoline was placed in a container and positioned in the chamber                                                                     | Inhalation (saturated exposure chamber), 8 h per day for 60 or 90 days                | The effects were time-dependent. | <a href="#">Owumi et al. (2021a)</a> |
| LPO, NO, and XO        | TBARS, colorimetric assay, and spectrophotometry, respectively                                                    |                               |                           | ↑                       |                                                                                                                                                |                                                                                       |                                  |                                      |
| EROD                   | Fluorescence assay                                                                                                | Rat, Sprague-Dawley (M and F) | Liver                     | ↑ (M only)              | 500 ppm gasoline vapour concentration                                                                                                          | Inhalation (saturated exposure chamber), 6 h per day on 5 days per week for 4 wk      | The effects were reversible.     | <a href="#">Chu et al. (2005)</a>    |
| Ascorbic acid          | HPLC                                                                                                              |                               | Urine                     | ↑                       |                                                                                                                                                |                                                                                       |                                  |                                      |
| PROD and BROD          | Fluorescence assay                                                                                                | Rat, Sprague-Dawley (F)       | Liver                     | ↑ for HD but not for MD | MD: gasoline/ethanol 160/18 mg/kg bw; HD: gasoline/ethanol 1600/180 mg/kg on the first day, then 800/90 mg/kg for the rest of the study period | Gavage in corn oil daily for 4 wk                                                     | The effects were dose-dependent. | <a href="#">Poon et al. (2001)</a>   |
| EROD, TBARS, and GSH   | Fluorescence, TBARS, and colorimetric assays, respectively                                                        |                               |                           | No change               |                                                                                                                                                |                                                                                       |                                  |                                      |
| Ascorbic acid          | HPLC                                                                                                              |                               | Urine                     | ↑                       |                                                                                                                                                |                                                                                       |                                  |                                      |
| Aldehydes              | PURPALD                                                                                                           |                               |                           | ↑                       |                                                                                                                                                |                                                                                       |                                  |                                      |

**Table 4.9 (continued)**

| End-point              | Assay                                                           | Species, strain (sex)   | Biological sample, tissue | Results <sup>a</sup> | Dose (LED or HID)                                                                                                                                                                       | Route, duration, dosing regimen                                                                          | Comments                                                                                                                                   | Reference                              |
|------------------------|-----------------------------------------------------------------|-------------------------|---------------------------|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| MDA, SOD, and GPx      | Colorimetric assay                                              | Rat, Sprague-Dawley (M) | Blood                     | No change            | The average vapour dose was 11.13 ± 1.1 cm <sup>3</sup> /h                                                                                                                              | Inhalation (saturated exposure chamber) for 6 h per day on 6 days per week for 11 wk                     |                                                                                                                                            | <a href="#">Abubakar et al. (2015)</a> |
| RONS                   | Colorimetric assay using DCFH-DA                                | Rat, Wistar (M)         | Lung                      | ↑                    | 500 mL of gasoline was placed in a container and positioned in the chamber                                                                                                              | Inhalation (saturated exposure chambers) for 8 h per day for 60 or 90 days                               |                                                                                                                                            | <a href="#">Owumi et al. (2021b)</a>   |
| GSH                    | Colorimetric assay using DTNB                                   |                         |                           | ↓                    |                                                                                                                                                                                         |                                                                                                          |                                                                                                                                            |                                        |
| NO and XO              | Colorimetric assay and spectrophotometry, respectively          |                         |                           | ↑                    |                                                                                                                                                                                         |                                                                                                          |                                                                                                                                            |                                        |
| GPx                    | Colorimetric assay using inhibition of epinephrine autoxidation |                         |                           | ↑                    |                                                                                                                                                                                         |                                                                                                          |                                                                                                                                            |                                        |
| GST                    | Colorimetric assay using CDNB                                   |                         |                           | ↓ (at 90 days)       |                                                                                                                                                                                         |                                                                                                          |                                                                                                                                            |                                        |
| SOD                    | Colorimetric assay using oxidation of NADPH                     |                         |                           | No change            |                                                                                                                                                                                         |                                                                                                          |                                                                                                                                            |                                        |
| MDA, SOD, CAT, and GSH | TBARS Spectrophotometry                                         | Rat, Sprague-Dawley     | Kidney                    | ↑<br>↓               | Inhalation: (the average vapour dose was 0.5 ± 0.08 cm <sup>3</sup> /min per kg/m <sup>3</sup> per day)<br>Food: 100 mL in 150 g of food;<br>Water: 2.5 mL/kg bw in 5 mL water per feed | Exposure involved inhalation (5 min per day) and ingestion of contaminated food and water daily for 8 wk | The effects on MDA and GSH were not route-dependent; however, ingestion caused significantly greater reductions in SOD and CAT activities. | <a href="#">Azeez et al. (2013)</a>    |

**Table 4.9 (continued)**

| End-point                                         | Assay                                                                                              | Species, strain (sex)      | Biological sample, tissue | Results <sup>a</sup> | Dose (LED or HID)                                                                         | Route, duration, dosing regimen                                                     | Comments | Reference                           |
|---------------------------------------------------|----------------------------------------------------------------------------------------------------|----------------------------|---------------------------|----------------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------|-------------------------------------|
| MDA<br>SOD, CAT,<br>and GSH                       | TBARS<br>Spectrophotometry                                                                         | Rat,<br>Sprague-<br>Dawley | Lung                      | ↑<br>↓               | The average vapour dose was $0.5 \pm 0.08$ cm <sup>3</sup> /min/kg/m <sup>3</sup> per day | Inhalation (saturated exposure chamber) for 5 min per day for 8 wk                  |          | <a href="#">Azeez et al. (2012)</a> |
| GSH, GST, MDA, SOD, and CAT                       | GSH, GST, SOD, and CAT were measured using spectrophotometry; MDA was measured using the TBA assay | Rat, Wistar (M)            | Testis                    | ↑                    | 600 mL of gasoline was placed in a container and positioned in the chamber                | Inhalation (saturated exposure chamber) for 6 h per day, 5 times per week for 10 wk |          | <a href="#">Ezim et al. (2024)</a>  |
| H <sub>2</sub> O <sub>2</sub> and NO <sub>2</sub> | Colorimetric assay using xylenol orange and Griess assay, respectively                             |                            |                           | ↑                    |                                                                                           |                                                                                     |          |                                     |
| Ascorbic acid                                     | Colorimetric assay                                                                                 |                            |                           | No change            |                                                                                           |                                                                                     |          |                                     |

BROD, benzoylresorufin-*O*-dealkylase; bw, body weight; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; EROD, ethoxyresorufin-*O*-deethylase; F, female(s); GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; h, hour(s); HD, high dose; HID, highest ineffective concentration; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; HPLC, high-performance liquid chromatography; LED, lowest effective concentration; LPO, lipid peroxidation; M, male(s); MD, medium dose; MDA, malondialdehyde; min, minute(s); NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NO<sub>2</sub>, nitrogen dioxide; ppm, parts per million; PROD, pentoxyresorufin-*O*-deethylase; PURPALD, 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole; RONS, reactive oxygen and nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; wk, week(s); XO, xanthine oxidase.

<sup>a</sup> ↑, increase; ↓, decrease; or no change.

(see also Sections 4.2.6 and 4.2.7). Similarly, rats exposed to gasoline vapour via inhalation, in drinking-water, or in food showed elevated levels of MDA, an index of LPO, and low levels of SOD, CAT, and GSH in kidney tissue homogenates ([Azeez et al., 2013](#)). [The Working Group noted that the article did not specify the sex of the animals.] Furthermore, gasoline vapour exposure reduced the activities of the antioxidant enzymes SOD, CAT, GST, and GPx, and reduced the GSH levels, indicative of oxidative stress ([Azeez et al., 2012](#); [Owumi et al., 2021b](#)). The plasma levels of ROS, oxidized glutathione, and MDA were also found to be higher whereas the content of GSH was lower in male Wistar rats exposed to unleaded gasoline for 6 hours per day on 6 days per week for 11 weeks, compared with the control group ([Ghahri et al., 2024](#)). [Ezim et al. \(2024\)](#) found significant decreases in antioxidant enzyme activities (SOD, CAT, GSH) and increases in MDA and hydrogen peroxide ( $H_2O_2$ ) in the testes of male Wistar rats exposed to gasoline vapour for 6 hours per day on 5 days per week for a period of 10 weeks.

#### (ii) Other species

*Astyanax altiparanae* exposed to WSFG (1.5%, v/v) for 96 hours in a semi-static system, with 25% gasoline test solution renewal every 24 hours, showed lower GST levels in the liver, and higher activities of SOD and GPx in both the liver and kidney, compared with controls ([Bettim et al., 2016](#)).

When *Prochilodus lineatus* (a neotropical freshwater fish) was exposed to a 5% WSFG solution for 6, 24, or 96 hours, increases in EROD and the GST activity in the liver and gills were observed after 24 and 96 hours, indicating the biotransformation of WSFG ([Simonato et al., 2011](#)). The authors also observed increases in the activities of CAT and GPx after 96 hours of exposure, and increases in the GSH content and glutathione reductase activity after 24 and 96 hours. Oxidative damage was also evidenced

by increased levels of LPO in the liver after 6 hours of exposure and in the gills after 96 hours of exposure ([Simonato et al., 2011](#)). However, the inhalation of gasoline did not cause significant changes in oxidative markers in male rats after 11 weeks ([Abubakar et al., 2015](#)).

[The Working Group noted that in these in vivo experiments, factors such as the duration of exposure, concentration, route of administration, and type of measurement made were crucial.]

#### Synopsis

[Overall, the Working Group noted that automotive gasoline exposure in occupational settings, mainly in service station attendants, induced oxidative stress markers in urine and blood. Oxidative stress end-points were significantly altered, often in combination with alterations in genotoxicity or pro-inflammation end-points. The evidence was confirmed in several tissues (i.e. liver, lungs, kidney and blood) in experimental systems in both mammalian and non-mammalian species.]

### 4.2.6 Induces chronic inflammation

#### (a) Exposed humans

See [Table 4.10](#).

[Moro et al. \(2019\)](#) observed significantly higher levels of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF $\alpha$ , and interferon gamma, IFN $\gamma$ ) in serum samples from 38 male service station attendants in Brazil than in controls who were not exposed to either benzene or other xenobiotics. The service station attendants had elevated urinary levels of BTX, assessed by quantification of concentrations of *t,t*-MA, hippuric acid, and methylhippuric acid, respectively. The authors also observed lower expression of the cytokines CD80 and CD86 in blood monocytes, measured using flow cytometry, and higher blood protein carbonyl and GST contents. [The Working Group noted that the findings indicated a pronounced

**Table 4.10 End-points relevant to chronic inflammation in humans exposed to automotive gasoline**

| End-point                                                                                      | Biological sample             | Location, study design                                  | Exposure duration<br>No. of study participants                                                                                                                                                                 | Response <sup>a</sup><br>(significance)                                                                                                                                  | Covariates controlled                      | Comments                                                                                                                                                                                                                                                                                                     | Reference                           |
|------------------------------------------------------------------------------------------------|-------------------------------|---------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| IL-1 $\beta$<br>IL-6<br>IL-10<br>TNF $\alpha$<br>IFN $\gamma$<br>CD80<br>CD86                  | Blood                         | Brazil<br>Cross-sectional<br>Service station attendants | Exposure: $\geq 6$ mo working in the job; mean, $8.5 \pm 1.6$ yr<br>$n = 38$ service station attendants<br>$n = 22$ non-exposed controls (no history of occupational exposure to benzene or other xenobiotics) | $\uparrow$ IL-1 $\beta$<br>$\uparrow$ IL-6<br>$\downarrow$ IL-10<br>$\uparrow$ TNF $\alpha$<br>$\uparrow$ IFN $\gamma$<br>$\downarrow$ CD80<br>$\downarrow$ CD86         | Age, smoking, general health, lifestyle    | Clear inflammation (alterations in cytokines and cells). Reductions in anti-inflammatory responses.<br>Markers of exposure assessed: urinary benzene, xylene, and toluene metabolites ( <i>t,t</i> -MA, HA, and MHA).<br>Strengths of the study: exposure assessments and a duration between 6 mo and years. | <a href="#">Moro et al. (2019)</a>  |
| IL-1 $\beta$<br>IL-6<br>TNF $\alpha$<br>IFN $\gamma$<br>IL-10<br>Monocytes (%)                 | Blood (plasma EDTA)           | Brazil<br>Cross-sectional<br>Service station attendants | Exposure: $\geq 1$ yr working in the job<br>$n = 17$ service station attendants<br>$n = 16$ non-exposed controls from a university                                                                             | $\uparrow$ IL-1 $\beta$<br>$\uparrow$ IL-6<br>$\uparrow$ TNF $\alpha$<br>$\uparrow$ IFN $\gamma$<br>$\downarrow$ IL-10<br>$\uparrow$ NO $_x$<br>No change in % monocytes | Age, sex, smoking, and alcohol consumption | Imbalance between the levels of pro-inflammatory and anti-inflammatory cytokines. Small but well-defined cohort.<br>Exposure duration: Workers mostly on a 6–8-h per day work shift, exposed to fuel for 1 to > 15 yr.                                                                                       | <a href="#">Brum et al. (2021)</a>  |
| TNF $\alpha$<br>WBC and differential counts<br>M1 macrophage inflammatory-related polarization | Blood (plasma EDTA and serum) | Egypt<br>Cross-sectional<br>Service station attendants  | Exposure: < 10 yr and > 10 yr of working<br>$n = 62$ male service station attendants<br>$n = 29$ non-exposed controls                                                                                          | $\uparrow$ TNF $\alpha$<br>$\uparrow$ Leukocyte count<br>$\uparrow$ Lymphocyte count<br>$\uparrow$ M1 macrophage inflammatory-related polarization                       | Age, sex                                   | Increased TNF $\alpha$ level and leukocyte count.<br>M1 inflammation-related polarization.<br>Pro-inflammatory effects associated with the exposure duration and oxidative stress biomarkers.<br>Cohort not well described.<br>Two categories of exposure duration.                                          | <a href="#">Toson et al. (2022)</a> |



**Table 4.10 (continued)**

| End-point                                                                     | Biological sample             | Location, study design                                                                                    | Exposure duration<br>No. of study participants                                                                                                                                                  | Response <sup>a</sup><br>(significance)                                                                                                                                                                                                       | Covariates controlled                                               | Comments                                                                                                                                                                                                                                                                                                                                                                                  | Reference                                   |
|-------------------------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| IL-1 $\beta$                                                                  | Blood (serum)                 | Iraq<br>Cross-sectional<br>Service station attendants                                                     | Exposure: participants worked an average of 81.4 $\pm$ 6.4 h weekly; most workers had been working for $\geq$ 1 yr<br>$n$ = 26 service station attendants<br>$n$ = 26 male non-exposed controls | $\uparrow$ IL-1 $\beta$                                                                                                                                                                                                                       | Age, sex, BMI, smoking, educational level, monthly income           | Positive association between blood lead and IL-1 $\beta$ level.<br>Small sample size.<br>Lead level measured for occupational exposures of $\geq$ 1 yr, 82 h per week.                                                                                                                                                                                                                    | <a href="#">Numan et al. (2024)</a>         |
| CRP<br>IL-12<br>IFN $\gamma$<br>TNF $\alpha$<br>IL-17<br>TGF $\beta$<br>IL-10 | Blood (serum)                 | Islamic Republic of Iran<br>Cross-sectional, randomized, double-blind trial<br>Service station attendants | Exposure: $\geq$ 1 yr, average 4.1 $\pm$ 2.7 yr of working<br>$n$ = 24 service station attendants treated with placebo<br>$n$ = 29 service station attendants treated with omega-3 fatty acids  | $\downarrow$ CRP (trend)<br>$\downarrow$ IL-12 (trend)<br>$\downarrow$ IFN $\gamma$<br>$\downarrow$ TNF $\alpha$ (trend)<br>$\downarrow$ IL-17<br>$\uparrow$ TGF $\beta$<br>$\uparrow$ IL-10 (after supplementation with omega-3 fatty acids) | Age, smoking, duration of work, chronic diseases                    | Antioxidant supplementation reduced inflammatory cytokine levels and increased anti-inflammatory mediators.<br>Mean durations of exposure for the workers treated with omega-3 fatty acids or placebo, 5 yr and 4 yr, respectively, after $\geq$ 1 yr.<br>Blood collection conducted after 2 mo of treatment with placebo or omega-3 fatty acids.<br>No control group. Small sample size. | <a href="#">Barkhordari et al. (2020)</a>   |
| IL-6<br>CRP<br>WBC and differential cell count                                | Blood (plasma EDTA and serum) | Iraq<br>Cross-sectional<br>Service station attendants                                                     | Exposure: > 5 yr, 12-h shift each day<br>$n$ = 72 service station attendants (fuelling workers)<br>$n$ = 75 non-exposed controls                                                                | $\uparrow$ IL-6<br>$\uparrow$ CRP<br>$\uparrow$ WBC and differential cell counts                                                                                                                                                              | Age, smoking, drug, exposure duration; hereditary diseases excluded | Increased level of IL-6.<br>Decline in blood leukocyte count. No link with exposure duration.                                                                                                                                                                                                                                                                                             | <a href="#">Sajid Jabbar and Ali (2020)</a> |
| IL-1 $\beta$<br>IL-6<br>TNF $\alpha$                                          | Blood (plasma EDTA)           | Pakistan<br>Cross-sectional<br>Service station attendants                                                 | $n$ = 34 service station attendants<br>$n$ = 112 office workers and students (non-exposed control group)                                                                                        | Pro-inflammatory markers:<br>$\uparrow$ IL-1 $\beta$<br>$\uparrow$ IL-6<br>$\uparrow$ TNF $\alpha$                                                                                                                                            | NR                                                                  | Elevated blood levels of pro-inflammatory cytokines.<br>No information on exposure. Cohort poorly described.                                                                                                                                                                                                                                                                              | <a href="#">Khan et al. (2023a)</a>         |

**Table 4.10 (continued)**

| End-point                                | Biological sample   | Location, study design                                                    | Exposure duration<br>No. of study participants                                                                                                                                                   | Response <sup>a</sup><br>(significance)               | Covariates controlled                              | Comments                                                                                                                                                                                  | Reference                              |
|------------------------------------------|---------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| WBC and RBC and differential cell counts | Blood (plasma EDTA) | Saudi Arabia<br>Cross-sectional<br>Service station attendants             | Exposure: < 10 yr or > 10 yr of working<br><i>n</i> = 50 service station attendants<br><i>n</i> = 49 non-exposed controls                                                                        | ↓ RBC count<br>↑ WBC count                            | Age                                                | Increase in the leukocyte counts of short-term-exposed workers. Exposure duration investigated. Cohort was poorly described. Two categories of exposure duration: > 1 to < 10 or > 10 yr. | <a href="#">Binsaleh et al. (2024)</a> |
| WBC and differential cell counts         | Blood (plasma EDTA) | Ethiopia<br>Cross-sectional<br>Service station attendants                 | Exposure duration: ≥ 1 yr, 2–5 yr, or > 5 yr of working<br><i>n</i> = 60 (56 men, 4 women) service station attendants<br><i>n</i> = 120 non-exposed controls (92 men and 28 women staff members) | ↓ RBC count<br>↑ WBC count                            | Age, marital status                                | Increased absolute eosinophil count in workers, not related to exposure duration. Cohort poorly described. Three categories of exposure duration: 1, 2–5, and > 5 yr.                     | <a href="#">Kebamo et al. (2024)</a>   |
| WBC and differential cell count          | Blood               | Ethiopia<br>Cross-sectional<br>Service station attendants                 | Exposure 1–14 yr of working in the job<br><i>n</i> = 55 (41 men, 14 women) service station attendants<br><i>n</i> = 55 (41 men, 14 women) non-exposed academic staff as controls                 | ↑ WBC count                                           | Age                                                | Increased absolute lymphocyte count in workers, not related to exposure duration.                                                                                                         | <a href="#">Getu et al. (2020)</a>     |
| WBC and differential cell count          | Blood (plasma EDTA) | Islamic Republic of Iran<br>Cross-sectional<br>Service station attendants | Exposure: ≥ 4 yr of working in the job<br><i>n</i> = 80 service station attendants<br><i>n</i> = 80 non-exposed controls                                                                         | ↑ Neutrophils, lymphocytes, monocytes<br>↓ Leukocytes | Age, sex, BMI, weight, smoking; no chronic disease | Increased granulocyte and monocyte counts. Cohort poorly described.                                                                                                                       | <a href="#">Zamanian et al. (2018)</a> |

**Table 4.10 (continued)**

| End-point                        | Biological sample   | Location, study design                                                    | Exposure duration<br>No. of study participants                                                                                                                                           | Response <sup>a</sup><br>(significance) | Covariates controlled | Comments                                                                                                                                               | Reference                            |
|----------------------------------|---------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| WBC and differential cell count  | Blood (plasma EDTA) | Islamic Republic of Iran<br>Cross-sectional<br>Service station attendants | Exposure: > 5 yr of working in the job; > 10 h per day<br><i>n</i> = 80 service station attendants<br><i>n</i> = 80 non-exposed controls                                                 | ↑ WBC count                             | Sex, smoking          | Increased neutrophil count. Cohort poorly described.                                                                                                   | <a href="#">Tiu and Dubey (2017)</a> |
| WBC and differential cell count  | Blood (plasma EDTA) | Nigeria<br>Cross-sectional<br>Service station attendants                  | Exposure: ≥ 6 mo of working in the job, (< 2 yr vs = 2 yr)<br><i>n</i> = 40 service station attendants<br><i>n</i> = 40 non-exposed controls                                             | ↑ WBC count                             | Sex                   | Increased granulocyte count in workers, as a function of exposure duration.                                                                            | <a href="#">Tijani et al. (2024)</a> |
| WBC count                        | Blood               | Iraq<br>Cross-sectional<br>Service station attendants                     | Exposure: average, 4 ± 4.7 yr<br><i>n</i> = 34 service station attendants<br><i>n</i> = 16 non-exposed controls                                                                          | No change                               | Age, sex              | No effect on WBC count. No association with exposure duration (lead). Cohort was poorly described. Both women and men were considered in the analysis. | <a href="#">Mohammed (2016)</a>      |
| WBC and differential cell counts | Blood (plasma EDTA) | Ethiopia<br>Cross-sectional<br>Service station attendants                 | Exposure: ≥ 8 mo, 3–7 yr, or > 8 yr of working<br><i>n</i> = 43 (28 men, 15 women) service station attendants<br><i>n</i> = 77 (49 men, 28 women) non-exposed academic staff as controls | No change                               | Age                   | No association with exposure duration. Both women and men were considered in the analysis. Three categories of exposure duration < 1, 3–7, and > 5 yr. | <a href="#">Teklu et al. (2021)</a>  |

BMI, body mass index; CD, cluster of differentiation; CRP, C-reactive protein; EDTA, ethylenediaminetetraacetic acid; HA, hippuric acid; IFN $\gamma$ , interferon gamma; IL, interleukin; M1, classically activated macrophage; MHA, methylhippuric acid; mo, month(s); NO $_x$ , nitrogen oxides; NR, not reported; RBC, red blood cell; TGF $\beta$ , transforming growth factor beta; TNF $\alpha$ , tumour necrosis factor alpha; *t,t*-MA, *trans-trans*-muconic acid; yr, year(s); WBC, white blood cell.

<sup>a</sup> ↑, increase; ↓, decrease; or no change.

inflammatory response, along with an oxidative stress response (see also Section 4.2.5).] This was accompanied by a parallel reduction in anti-inflammatory potential, as shown by lower levels of IL-10 ([Moro et al., 2019](#)). [The Working Group noted that a strength of the study was the analysis of markers of exposure.]

[Brum et al. \(2021\)](#) also observed increased levels of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF $\alpha$ , and IFN $\gamma$ ) in serum samples from service station attendants in Brazil, when compared with the levels in samples from office-based control participants. This was accompanied by lower levels of the anti-inflammatory cytokine IL-10. The pro-inflammatory response was associated with an increase in oxidative stress and oxidative damage to DNA, identified as high 8-OHdG levels in the blood (see also Sections 4.2.2 and 4.2.5). [The Working Group noted the link between the increases in inflammation and oxidative stress and the exposure to gasoline.]

[Toson et al. \(2022\)](#) also observed significantly higher TNF $\alpha$  concentrations, as well as higher leukocyte and lymphocyte counts, in blood samples from male service station attendants in Egypt compared with control participants. These effects were positively associated with the duration of exposure (the number of years of working) and the increase in oxidative stress. In addition, the authors observed elevated levels of acidic mammalian chitinase and nitric oxide, possibly indicative of inflammatory polarization of macrophages (M1) ([Toson et al., 2022](#)).

A population of workers from a service station was compared with healthy control participants in Iraq ([Numan et al., 2024](#)). The levels of the key pro-inflammatory cytokine IL-1 $\beta$  were found to be significantly elevated in the service station attendants compared with the controls. There was a positive association between the blood lead level, reflecting the extent of exposure, and the concentration of this cytokine. [The Working Group noted that, although lead is currently

banned from gasoline, this very recent study still showed high lead levels in workers exposed to gasoline vapour.]

End-points of inflammation (C-reactive protein (CRP), IL-12, IFN $\gamma$ , TNF $\alpha$ , and IL-17) and immunosuppression (transforming growth factor beta, TGF $\beta$ ; and IL-10) were also measured in service station attendants in Iran who had received an omega-3 fatty acid supplement as an antioxidant treatment or a placebo for 60 days. After supplementation, the levels of IFN $\gamma$  and IL-17 were significantly lower, whereas the TGF $\beta$  and IL-10 levels were higher. The authors concluded that the inflammatory responses after gasoline exposure were driven by oxidative stress, which impaired the body's anti-inflammatory capacity ([Barkhordari et al., 2020](#)). [The Working Group noted that the informativeness of the study was limited by the comparison of two groups of equally exposed service station attendants, who received omega-3 supplementation or a placebo, and therefore there was no non-exposed control group. In addition, the sample size was small.]

[The Working Group reviewed several other studies, which are described below, and showed an induction of the inflammatory response after occupational exposure to automotive gasoline in workers in various regions worldwide. However, the studies were considered to be less informative, because of the poor level of detail on the exposure assessments and the cohorts analysed.]

[Sajid Jabbar and Ali \(2020\)](#) observed significant increases in the serum concentrations of IL-6 and high-sensitivity CRP in service station attendants in Iraq who were exposed to gasoline vapours, compared with non-exposed control participants. In addition, the authors reported significant reductions in leukocyte counts, including the lymphocyte, monocyte, and neutrophil counts. [Khan et al. \(2023a\)](#) observed higher levels of TNF $\alpha$ , IL-1, and IL-6 in plasma samples from service station attendants in Pakistan, versus non-exposed office

workers (mainly university students and faculty members). [Binsaleh et al. \(2024\)](#) observed significantly higher white blood cell, neutrophil, and lymphocyte counts in samples from a cohort of service station attendants in Saudi Arabia, versus those from non-exposed control participants. These differences were more pronounced in workers with shorter durations of exposure (1–10 years of working in the job) than in those with prolonged exposure (> 10 years of working in the job). Significant increases in the absolute eosinophil and lymphocyte counts in blood samples from service station attendants in Ethiopia, compared with those from non-exposed controls, were observed by [Kebamo et al. \(2024\)](#) and [Getu et al. \(2020\)](#), respectively. Higher neutrophil, monocyte, or lymphocyte counts were also reported by other authors ([Tiu and Dubey, 2017](#); [Zamanian et al., 2018](#); [Tijani et al., 2024](#)). However, [Sirwan \(2013\)](#) and [Teklu et al. \(2021\)](#) observed no alterations in the white blood cell, neutrophil, or lymphocyte counts of service station attendants.

(b) *Non-human mammalian experimental systems*

See [Table 4.11](#).

[Abdrabouh \(2019, 2022\)](#) studied male albino Wistar rats that were exposed to gasoline fumes for 10 weeks, and observed inflammation in their lungs, indicated by high levels of TNF $\alpha$  and the pro-apoptotic marker Bax, along with fibrosis, indicated by high levels of fibrotic markers, such as TGF $\beta$ , fibroblast growth factor-1, and collagen-1, and altered tissue histology. Co-exposure with fenugreek seed supplement significantly alleviated the identified oxidative and inflammatory defects. Similar results were observed in the liver.

[Owumi et al. \(2021b\)](#) examined the effects of the inhalation of petroleum product fumes for 60 or 90 days in male albino Wistar rats, and observed significant increases in pro-inflammatory cytokine production, oxidative stress, and

tissue damage in the lungs, as a function of the duration of the exposure. Similar findings were reported for the liver in a follow-up article that described the same protocol, in which the authors also describe lower white blood cell counts, including those for lymphocytes and monocytes ([Owumi et al., 2021b](#)). [Nour-Eldine et al. \(2022\)](#) showed that exposure to gasoline vapour via inhalation for 7 weeks in mice caused inflammation in the lungs, characterized by T-helper 2 polarization. [Alabi et al. \(2017\)](#) exposed mice intraperitoneally to leachates of soil from service stations for 5 days and found increases in blood leukocyte counts as a function of the injected dose, and as compared with control animals.

*Synopsis*

[Overall, the Working Group noted that exposure to automotive gasoline in humans induced systemic oxido-inflammatory responses, with activation of pro-inflammatory cytokines and cells, which appeared to correlate with the duration of exposure. Several studies indicated positive associations between the exposure to gasoline vapour and end-points relevant to chronic inflammation, including blood cytokine concentrations and leukocyte counts, in service station attendants. The mechanism of inflammation also involved compromised anti-inflammatory and antioxidant capacities. The findings in humans were confirmed in experimental systems in vivo.]

4.2.7 *Is immunosuppressive*

See [Table 4.12](#).

(a) *Humans*

*Exposed humans*

[Uzma et al. \(2008\)](#) reported that a cohort of service station attendants from India exhibited reduced lung volume (FEV<sub>1</sub>, forced expiratory volume in the first second) and capacity (FVC, forced vital capacity), along with lower total

**Table 4.11 End-points relevant to chronic inflammation in non-human mammalian systems in vivo exposed to automotive gasoline**

| End-points, assays                                                                                                                                                                                       | Species, strain (sex)     | Biological sample   | Results <sup>a</sup>                                                                                                                                                                                                                     | Dose (LED or HID) | Route, duration, dosing regimen                                                              | Comments                                                                                                                           | Reference                            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| TNFα<br>Fibrosis markers<br>WBC count<br>RBC count<br>by H&E staining, ELISA, Sysmex, and electron microscopy                                                                                            | Rat, Albino<br>Wistar (M) | Lung tissue, plasma | ↑ TNFα<br>↑ Lung fibrosis markers (TGFβ1, COL-1, and Hyp)<br>↓ WBC and RBC counts<br>(Other results: the antioxidant supplement fenugreek seeds reduced oxidative stress and inflammation)                                               | Not determined    | Crude gasoline 500 mL, via inhalation for 6 h per day on 6 days per week for 10 wk           | Well-designed mechanistic study. No quantification of exposure and only one time point analysed.                                   | <a href="#">Abdrabouh (2022)</a>     |
| IL-6<br>IL-β1<br>TGFβ1<br>FGF1<br>COL-1<br>Liver toxicity serum markers: ALT, ALP, AST, GGT, total bilirubin, total protein<br>Tissue histopathology by H&E staining, ELISA, Sysmex, electron microscopy | Rat, Albino<br>Wistar (M) | Liver, serum        | ↑ IL-6<br>↑ IL-β1<br>↑ Fibrotic markers (TGFβ1, FGF1, COL-1)<br>↑ Serum biochemical parameters (all)<br>Altered liver histology<br>(Other results: the antioxidant supplement fenugreek seeds reduced oxidative stress and inflammation) | Not determined    | Crude gasoline 500 mL, via inhalation for 6 h per day on 6 days per week for 10 wk           | Well-designed mechanistic study. No quantification of exposure and only one time point analysed.                                   | <a href="#">Abdrabouh (2019)</a>     |
| TNFα<br>IL-1β<br>Tissue histopathology by H&E staining, ELISA, DCFH-DA, Bradford method                                                                                                                  | Rat, Albino<br>Wistar (M) | Lung tissue         | ↑ TNFα<br>↑ IL-1β<br>Lung: diffuse interstitial congestion and proliferation of pneumocytes<br>(Other results: imbalance between oxidative stress and antioxidant capacity)                                                              | Not determined    | Crude gasoline, volatile compounds 500 mL, via inhalation for 8 h per day, for 60 or 90 days | Well-designed study. Effects were exposure duration-dependent. No quantification of exposure. Oxido-inflammatory process observed. | <a href="#">Owumi et al. (2021b)</a> |



**Table 4.11 (continued)**

| End-points, assays                                                                                                                                                                                 | Species, strain (sex)   | Biological sample   | Results <sup>a</sup>                                                                                                                                                                                        | Dose (LED or HID)                                                                                                                                                                                       | Route, duration, dosing regimen                                                             | Comments                                                                                                                                         | Reference                                 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| Leukocyte count<br>Liver toxicity serum markers (ALT, AST, GGT, LDH)<br>Tissue histopathology<br>LPO, XO, and NO levels<br>by H&E staining, Sysmex, uric acid level (for XO), Greiss method for NO | Rat, Albino Wistar (M)  | Liver tissue, serum | ↓ Leukocyte count<br>↑ Serum liver parameters<br>↑ Cellular infiltration, fibroplasia, hypertrophy<br>(Other results: imbalance between oxidative stress, LPO, XO, and NO levels, and antioxidant capacity) | Not determined                                                                                                                                                                                          | Crude petrol, volatile compounds<br>500 mL, via inhalation on 8 h per day for 60 or 90 days | Well-designed study. Effects in an exposure-duration dependent manner. No quantification of exposure. Oxido-inflammatory process observed.       | <a href="#">Owumi et al. (2021a)</a>      |
| Leukocyte count by automatic blood analyser                                                                                                                                                        | Mouse, Albino Swiss (M) | Blood (plasma)      | ↑ Leukocyte count                                                                                                                                                                                           | 1–5% of extracted leachate from soil of service stations (v/v, stimulated leachate/distilled water). Test soil contained metals, PAH (1.26 mg/kg), benzene (3.25 mg/kg); not determined in control soil | 500 µL of 1–5% leachate given intraperitoneally, one injection per day for 5 days           | Short-term, descriptive study. Exposure quantification. Only one time point was investigated.                                                    | <a href="#">Alabi et al. (2017)</a>       |
| IL-4<br>IL-6<br>IL-10<br>IL-12<br>TNFα,<br>by H&E staining, PCR                                                                                                                                    | Mouse, C57BL/6 (F)      | Lung tissue         | ↑ IL-4<br>↑ IL-6<br>↑ IL-10<br>↓ IL-12<br>↓ TNFα                                                                                                                                                            | Generated gasoline vapour, one dose (0.86 g/animal per 90 min)                                                                                                                                          | Crude gasoline<br>100 mL, via inhalation for 90 min per day on 5 days per week for 7 wk     | Well-designed study, but only one time point analysed. Gasoline vapour analysis by HS-GC-MS/MS. Data suggested a predominant Th2 cell phenotype. | <a href="#">Nour-Eldine et al. (2022)</a> |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COL-1, collagen type 1; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; ELISA, enzyme-linked immunosorbent assay; F, female(s); FGF1, fibroblast growth factor 1; GGT, gamma-glutamyl transferase; h, hour(s); H&E, haematoxylin-eosin; HID, highest ineffective dose; HS-GC-MS/MS, headspace-gas chromatography-tandem mass spectrometry; Hyp, hydroxyproline; IL, interleukin; LDH, lactate dehydrogenase; LED, lowest effective dose; LPO, lipid peroxidation; M, male(s); min, minute(s); ND, not detected; NO, nitric oxide; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; RBC, red blood cell; TGFβ1, transforming growth factor beta 1; Th2, T-helper 2; TNFα, tumour necrosis factor alpha; v/v, volume per volume; WBC, white blood cell; wk, week(s); XO xanthine oxide.

<sup>a</sup> ↑, increase; ↓, decrease.

**Table 4.12 End-points relevant to immunosuppression in humans exposed to automotive gasoline**

| End-point                                                                                                                                                          | Biological sample    | Location, setting, study design                                                            | Exposure duration no. of study participants                                                                                                                                       | Response <sup>a</sup> (significance)                                                                                                                    | Covariates controlled | Comments                                                                                                                                                                                     | Reference                               |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| Parameters reflecting pulmonary function: FEV <sub>1</sub> , FVC<br>WBC, neutrophil, monocyte, and lymphocyte counts                                               | Blood (plasma EDTA)  | India<br>Cross-sectional<br>Service station attendants                                     | Workers exposed to fuel for an average 1–5, 5–10, 10–15, or > 15 yr, with shifts of 8 h per day<br><i>n</i> = 42 service station attendants<br><i>n</i> = 36 non-exposed controls | ↓ Lung FEV <sub>1</sub><br>↓ Lung FVC<br>↓ WBC, neutrophil, monocyte, and lymphocyte counts                                                             | Age, BMI              | Well-organized study with tests of function.                                                                                                                                                 | <a href="#">Uzma et al. (2008)</a>      |
| Parameters reflecting pulmonary function: FEV <sub>1</sub> , FVC<br>Leukocyte count<br>IL-4<br>IL-5<br>IL-10<br>IL-12P70<br>IFN $\gamma$<br>MIP-1 $\alpha$<br>IL-3 | Lung, induced sputum | Brazil<br>Cross-sectional<br>Service station attendants                                    | Workers exposed to fuel for $\geq$ 1 yr<br><i>n</i> = 52 (42 men; mean age, 36 yr) service station attendants<br><i>n</i> = 22 non-exposed controls (11 women; mean age, 31 yr)   | ↓ Lung FEV <sub>1</sub><br>↓ Lung FVC<br>↓ Leukocyte count<br>↓ IL-4<br>↓ IL-5<br>↓ IL-10<br>↓ IL-12P70<br>↓ IFN $\gamma$<br>↓ MIP-1 $\alpha$<br>↑ IL-3 | Smoking               | Well-conducted study in which clinical and pulmonary parameters were analysed.<br>No clear information concerning exposure assessment.                                                       | <a href="#">da Silva et al. (2022)</a>  |
| Tregs<br>Parameters reflecting pulmonary function: PIF, FIVC, FIV <sub>1</sub> , FVC                                                                               | Blood, heparinized   | Croatia<br>Participants from an industrial area, from rural fields, and healthy volunteers | <i>n</i> = 88 inhabitants                                                                                                                                                         | ↑ Tregs<br>↓ Pulmonary parameters (all)                                                                                                                 | No                    | Increase in blood Tregs in the industrial area.<br>No demographic information about the exposed group.<br>The variability in the pollutants near gasoline-related industry was a limitation. | <a href="#">Mićović et al. (2009)</a>   |
| Parameters reflecting pulmonary function: FEV <sub>1</sub> and FVC<br>WBC proportions and counts (lymphocytes, neutrophils, and monocytes)                         | Blood, heparinized   | India<br>Cross-sectional<br>Service station attendants<br>Traffic police personnel         | <i>n</i> = 82 male service station attendants working three different 8-h shifts<br><i>n</i> = 57 male traffic police personnel<br><i>n</i> = 20 male non-exposed controls        | ↓ Lung FEV <sub>1</sub><br>↓ Lung FVC<br>WBC proportions and counts: no change                                                                          | Sex                   | Well-designed study. Exposure to benzene, toluene, and xylene determined. External (VOCs) and internal ( <i>t,t</i> -MA, SPMA, mandelic acid, HA, MHA, and PM <sub>10</sub> ) exposures.     | <a href="#">Mukherjee et al. (2016)</a> |

**Table 4.12 (continued)**

| End-point                                                      | Biological sample   | Location, setting, study design                         | Exposure duration no. of study participants                                                                                                                                        | Response <sup>a</sup> (significance)                                                                                                                                                      | Covariates controlled                                                                                                                                                                             | Comments                                                                                                                                                                         | Reference                              |
|----------------------------------------------------------------|---------------------|---------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| Lymphocyte cell subtype analysis                               | Blood (plasma EDTA) | India<br>Cross-sectional<br>Service station attendants  | <i>n</i> = 25 service station attendants<br><i>n</i> = 25 automobile service station workers<br><i>n</i> = 35 non-exposed controls                                                 | ↓ CD4 <sup>+</sup><br>↓ CD8 <sup>+</sup><br>↓ CD9 <sup>+</sup><br>↓ CD19 <sup>+</sup><br>↓ CD4 <sup>+</sup> /CD8 <sup>+</sup><br>↑ CD16 <sup>+</sup><br>↑ CD56 <sup>+</sup><br>↑ NK cells | Age, height, weight, sex, BMI, years of schooling, smoking/tobacco chewing habits, alcohol consumption, cooking fuel use at home, use of mosquito repellents, food habits, average monthly income | Well-defined cohort and study. Exposure was characterized. Small sample size. <i>t,t</i> -MA for benzene exposure. Attendants worked 8–10 h per day on 6 days per week for 7 yr. | <a href="#">Ray et al. (2007)</a>      |
| Complete immunophenotyping                                     | Blood (plasma EDTA) | Brazil<br>Case study<br>Service station attendants      | Workers exposed to fuel for ≥ 4 yr<br><i>n</i> = 2 female service station attendants                                                                                               | ↓ NK cell function<br>Abnormal CD16 expression and neutropenia                                                                                                                            | NR                                                                                                                                                                                                | Case study.                                                                                                                                                                      | <a href="#">Santiago et al. (2017)</a> |
| NK cells<br>CD56 <sup>+</sup> Th cells<br>CD4/CD8 cells        | Blood (plasma EDTA) | Brazil<br>Cross-sectional<br>Service station attendants | <i>n</i> = 132 service station attendants<br><i>n</i> = 68 convenience store workers<br><i>n</i> = 23 non-exposed controls                                                         | ↑ NK cells<br>↓ Naive Th lymphocytes<br>↑ CD56 <sup>+</sup> Th cells<br>↓ CD4/CD8 cells (data for service station attendants)                                                             | Alcohol consumption, smoking                                                                                                                                                                      | Well-designed study and strong data. Benzene exposure was assessed using <i>t,t</i> -MA measurement.                                                                             | <a href="#">Poça et al. (2021)</a>     |
| WBC count and differential count<br>CD membrane immune markers | Blood (plasma EDTA) | Brazil<br>Cross-sectional<br>Service station attendants | High (9.31 ± 7.94 yr) and low (7.17 ± 6.27 yr) exposure groups, <i>n</i> = 49 (7 men, 42 women) service station attendants<br><i>n</i> = 26 (8 men, 18 women) non-exposed controls | ↑ Lymphocyte and monocyte proportions<br>↓ CD4/CD8 and CD10<br>↑ CD16 <sup>+</sup> CD56<br>↑ CD25                                                                                         | Age, sex, smoking, drug and alcohol consumption                                                                                                                                                   | Good phenotyping. No clear conclusions were drawn by the authors. Two categories of exposure duration: low and high risk of benzene poisoning.                                   | <a href="#">Santiago et al. (2019)</a> |

**Table 4.12 (continued)**

| End-point                                             | Biological sample    | Location, setting, study design                                        | Exposure duration no. of study participants                                                                                                                                                                                                 | Response <sup>a</sup> (significance)    | Covariates controlled | Comments                                                                                                                            | Reference                                   |
|-------------------------------------------------------|----------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| CD80/CD86 counts in monocytes and lymphocytes<br>IL-8 | Blood, (plasma EDTA) | Brazil<br>Cross-sectional<br>Service station attendants                | Workers exposed to fuelling for $\geq 6$ mo; on average, $119.8 \pm 12.8$ mo<br>$n = 60$ service station attendants<br>$n = 28$ non-exposed controls                                                                                        | ↓ CD80/CD86<br>↑ IL-8                   | Sex                   | Well-designed study and clear data. Gasoline exposure was confirmed by measurement of <i>t,t</i> -MA, HA, and MHA metabolites.      | <a href="#">Moro et al. (2015)</a>          |
| CD4/CD8 count<br>IgG1<br>IgG2                         | Blood, (plasma EDTA) | India<br>Cross-sectional<br>Service station attendants                 | Workers exposed to fuel for $< 10$ yr ( $n = 282$ ; mean age, $28 \pm 8$ yr) or $> 10$ yr ( $n = 146$ ; mean age, $34 \pm 14$ yr), with a 12-h work shift per day)<br>$n = 428$ service station attendants<br>$n = 78$ non-exposed controls | ↓ CD4/CD8<br>↓ CD4T<br>↓ IgG1<br>↓ IgG2 | Smoking               | Also identified oxidative stress and reduced antioxidant capacity, associated with the level of benzene. The sample size was large. | <a href="#">Uzma et al. (2010)</a>          |
| IgA<br>IgG<br>IgM<br>IgE                              | Blood, heparinized   | Egypt<br>Prospective observational study<br>Service station attendants | $n = 40$ service station attendants from 10 different areas, with subgroups based on the number of years working in the station: 2, 4, 6 or, $> 6$ yr)<br>$n = 10$ volunteers (non-exposed control group)                                   | ↓ IgA<br>↓ IgG<br>↓ IgM<br>↓ IgE        | None                  | Imbalance between ROS and antioxidants. Cohort characteristics and exposure parameters were poorly described.                       | <a href="#">Abdel Maksoud et al. (2019)</a> |

**Table 4.12 (continued)**

| End-point                              | Biological sample   | Location, setting, study design                                           | Exposure duration no. of study participants                                                                                                                                               | Response <sup>a</sup> (significance)                                                                | Covariates controlled                                                                                                                          | Comments                                                                                                                           | Reference                                 |
|----------------------------------------|---------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| WBC, neutrophil, and lymphocyte counts | Blood (plasma EDTA) | Brazil<br>Cross-sectional<br>Service station attendants                   | Workers exposed to fuel for $\geq 6$ mo: three categories of exposure duration: $< 10$ , $10-20$ , or $> 20$ yr<br>$n = 324$ service station attendants<br>$n = 218$ non-exposed controls | ( $\uparrow$ ) Leukocyte count<br>( $\downarrow$ ) Lymphocyte count                                 | A linear regression analysis was performed that considered smoking, alcohol consumption, and processed food consumption as confounding factors | Well-characterized cohort and exposure. Gasoline exposure was confirmed by measurement of <i>t,t</i> -MA, HA, and MHA metabolites. | <a href="#">Giardini et al. (2023)</a>    |
| WBC, neutrophil, and lymphocyte counts | Blood (plasma EDTA) | Islamic Republic of Iran<br>Cross-sectional<br>Service station attendants | Workers exposed to fuel for $\geq 5.9$ yr<br>Three categories of exposure duration: $< 1$ , $1-5$ , or $> 5$ yr<br>$n = 41$ service station attendants<br>$n = 27$ non-exposed controls   | $\downarrow$ WBC count<br>$\downarrow$ Neutrophil count<br>$\downarrow$ Lymphocyte count            | Sex, smoking                                                                                                                                   | The service station attendants carried only one functional <i>GST</i> allele. Cohort was poorly described.                         | <a href="#">Ansari-Lari et al. (2003)</a> |
| WBC, lymphocyte, and neutrophil counts | Blood               | Brazil<br>Cross-sectional<br>Service station attendants                   | 114 service station attendants; workers classified according to clinical findings suggestive of benzene exposure                                                                          | $\downarrow$ Lymphocyte count<br>$\uparrow$ Neutrophil count in the group without clinical findings | Demographic characteristics described but statistical analysis not performed                                                                   | No control group was included in the analysis. Only the blood cell count was reported.                                             | <a href="#">Mitri et al. (2015)</a>       |

**Table 4.12 (continued)**

| End-point                              | Biological sample   | Location, setting, study design                          | Exposure duration no. of study participants                                                                                                                                                                                              | Response <sup>a</sup> (significance) | Covariates controlled | Comments                                                                                                           | Reference                              |
|----------------------------------------|---------------------|----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| WBC and neutrophil counts              | Blood (plasma EDTA) | Nigeria<br>Cross-sectional<br>Service station attendants | Total of 213 exposed participants:<br><i>n</i> = 72 vendors of heavy oils<br><i>n</i> = 46 vendors of gasoline alone<br><i>n</i> = 57 motor mechanics<br><i>n</i> = 38 service station attendants<br><i>n</i> = 129 non-exposed controls | ↓ WBC count<br>↓ neutrophil count    | None                  | Large cohort with various types of exposure. Cohort was poorly described. No exposure data.                        | <a href="#">Niazi et al. (1989)</a>    |
| WBC, lymphocyte, and neutrophil counts | Blood (plasma EDTA) | Nigeria<br>Cross-sectional<br>Service station attendants | <i>n</i> = 50 service station attendants<br><i>n</i> = 50 non-exposed controls                                                                                                                                                           | No change                            | None                  | No details of the exposure were reported. Cohort was poorly described. The analysis was of men and women combined. | <a href="#">Babatope et al. (2024)</a> |

BMI, body mass index; CD, cluster of differentiation; EDTA, ethylenediaminetetraacetic acid; FEV<sub>1</sub>, forced expiratory volume in the first second; FVC, forced vital capacity; FIVC, forced inspiratory vital capacity; FIV<sub>1</sub>, volume inspired in the first second of the test; GST, glutathione S-transferase; h, hour(s); HA, hippuric acid; IFN $\gamma$ , interferon gamma; Ig, immunoglobulin; IL, interleukin; MHA, methylhippuric acid; MIP-1 $\alpha$ , macrophage inflammatory protein 1 $\alpha$ ; mo, month(s); NK, natural killer; NR, not reported; PIF, peak inspiratory flow; PM<sub>10</sub>, particulate matter with a diameter of  $\leq 10$   $\mu$ m; ROS, reactive oxygen species; Th, T-helper; Tregs, regulatory T cells; *t,t*-MA, *trans-trans*-muconic acid; SPMA, S-phenylmercapturic acid; VOC, volatile organic compound; WBC, white blood cell; wk, week(s); yr, year(s).

<sup>a</sup> ↑, increase; ↓, decrease; (†) or (↓) findings in a study of limited quality, or no change.



leukocyte, lymphocyte, neutrophil, and monocyte counts, compared with control participants. These effects were more pronounced with a prolonged duration of exposure, although there was no detailed description of the exposure assessment.

The impact of gasoline exposure on the lungs and the immune response was further explored by [da Silva et al. \(2022\)](#), who compared non-smoking service station attendants in Brazil with non-exposed controls. The analysis of induced sputum (induced by saline inhalation) showed a significantly lower proportion of lymphocytes in the workers, suggesting immune dysfunction within the airways. The FVC and FEV<sub>1</sub> values for the exposed workers were significantly lower than those for the controls, despite their mean lung spirometry values being within the normal range. In addition, the workers had lower levels of several cytokines (IL-4, IL-5, IL-10, macrophage inflammatory protein MIP-1 $\alpha$ , and IFN $\gamma$ ) in the respiratory tract, indicative of impaired T-helper lymphocyte activity.

[Mićović et al. \(2009\)](#) quantified the regulatory T cells (Tregs) in residents living close to gasoline-related industrial area in Croatia, and noted an increase in Tregs associated with chronic exposure to gasoline, but also an impairment of pulmonary function, which would normally be associated with a decline in Treg numbers. [The Working Group noted that the study did not include an exposure assessment.] In another study, [Mukherjee et al. \(2016\)](#) observed an impairment of lung function in service station attendants in Kolkata, India, versus traffic policemen and occupationally non-exposed control participants. The level of impairment increased with the duration of exposure, inferred by the difference between groups of age < 35 years and > 35 years. The authors did not observe significant alterations in the total or differential leukocyte counts (including of neutrophils, lymphocytes, monocytes, and eosinophils) between the two exposed groups, but there was a significant difference in

the total leukocyte count when compared with the non-exposed group.

[The Working Group noted that several studies indicated that occupational exposure to gasoline vapour is associated with immunological alterations, and particularly with a reduction in blood lymphocytes.]

In a well-designed study, the differential counts of CD4<sup>+</sup>, CD8<sup>+</sup>, and CD19<sup>+</sup>-expressing cells were significantly decreased in service station attendants in India ([Ray et al., 2007](#)). In contrast, there was a 20% increase in the CD16<sup>+</sup> CD56<sup>+</sup> NK cell count.

In a case study of two service station attendants in Brazil, [Santiago et al. \(2017\)](#) observed clinical symptoms of acute benzene intoxication, along with impaired function of NK cells and abnormal CD16 expression ([Santiago et al., 2017](#)).

In another study, [Poça et al. \(2021\)](#) identified a significant decrease in the percentage of circulating naive CD3<sup>+</sup>CD4<sup>+</sup> T-helper cells and significant increases in memory CD3<sup>+</sup>CD4<sup>+</sup>CD45RO<sup>+</sup> T-helper cells and CD56<sup>+</sup> NK cells in a cohort of service station attendants in Brazil.

[Santiago et al. \(2019\)](#) also reported alterations in the CD4<sup>+</sup> T-helper/CD8<sup>+</sup> T-suppressor cell ratio, the downregulation of cells expressing CD2, CD8, CD10, or CD8<sup>low</sup>, an increase in CD16<sup>+</sup>CD56<sup>+</sup> NK cells, and increases in the lymphocyte and monocyte counts.

[Moro et al. \(2015\)](#) reported lower CD86 protein expression in both lymphocytes and monocytes, along with higher serum IL-8 levels, in workers exposed to gasoline in Brazil. [The Working Group noted that the immunological alterations correlated with the measured levels of benzene.]

[Uzma et al. \(2010\)](#) reported lower immunoglobulin levels, CD4<sup>+</sup> T-cell count, CD4<sup>+</sup>/CD8<sup>+</sup> ratio, and antioxidant capacity, which were associated with higher levels of benzene and its metabolites, in service station attendants in India (see also Sections 4.2.5 and 4.2.6). [Abdel Maksoud et al. \(2019\)](#) noted that significantly

reduced serum levels of immunoglobulin (Ig) IgA and IgG and increased levels of IgM and IgE, alongside decreased antioxidant capacity and elevated oxidative stress, were associated with gasoline exposure in service station attendants from various areas of Egypt, compared with non-exposed controls.

[The Working Group noted that alterations in the immune response were also observed in other studies, described below, with a more limited characterization of blood immune cell subpopulations and of the exposure.] [Giardini et al. \(2023\)](#) identified a lower lymphocyte count and a higher total leukocyte count in service station attendants in Brazil, compared with office workers, consistent with lymphopenia and leukocytosis ([Giardini et al., 2023](#)).

[Ansari-Lari et al. \(2003\)](#) identified significant lower white blood cell, lymphocyte, and neutrophil counts in service station attendants in Iran who carried only one functional *GSTT1/GSTM1* allele.

The study of [Mitri et al. \(2015\)](#) identified significantly lower white blood cell and lymphocyte counts, but a higher neutrophil count, in service station attendants in Brazil. The authors did not report any clinical findings associated with exposure to benzene. [Niazi et al. \(1989\)](#) noted a lower neutrophil count, consistent with neutropenia, in a cohort of vendors of gasoline and heavy oil in Nigeria. However, [Babatope et al. \(2024\)](#) did not observe alterations in the leukocyte or CD4<sup>+</sup> cell counts of service station attendants in Nigeria.

#### (b) *Experimental systems*

##### *Non-human mammalian systems in vivo*

See [Table 4.13](#).

[White et al. \(2014\)](#) observed that the exposure of female Sprague-Dawley rats to gasoline vapour condensates for 4 weeks did not result in alterations to their humoral immune response, assessed using the IgM antibody-forming cell

response to T-dependent sheep erythrocyte antigen. Furthermore, [Bushnell et al. \(2015\)](#) did not observe alterations to immune system function (cell-mediated and humoral immune responses) of the rat offspring aged 6 weeks that were exposed to gasoline vapour during days 9–20 of gestation.

[Yestemirova et al. \(2024\)](#) identified a lower white blood cell count in rats that were chronically exposed (for 90 days) to gasoline vapour. This effect was mitigated by oral vitamin D3 supplementation starting from day 0. In contrast, [Uboh et al. \(2010\)](#) observed an increase in white blood cell count in rats after prolonged exposure to gasoline vapour (for 20 weeks), an effect that was alleviated by oral supplementation with vitamins A and E during the last 2 weeks of exposure.

#### *Synopsis*

[Overall, the Working Group noted that there was some evidence in exposed humans that exposure to automotive gasoline may induce immunosuppression, in particular a reduction in the T-cell response. The studies highlighted localized immunosuppression in the respiratory tract (airways) of exposed individuals, along with a deficit in NK cell activity. However, the activation of cell-mediated immunity (granulocytes and pro-inflammatory cytokines), as reported in some of the studies, could be attributed to compensatory immune activation in response to immunosuppression. The Working Group considered that these effects could be associated with occupational exposure to gasoline vapours, including benzene as a primary contributor. Altogether, well-conducted animal studies do not provide compelling evidence that chronic gasoline exposure induces immunosuppressive effects.]

**Table 4.13 End-points relevant to immunosuppression in non-human mammalian systems in vivo exposed to automotive gasoline**

| End-point                           | Assay                                                                                                                             | Species, strain (sex) or cells                  | Tissue, cells                 | Results <sup>a</sup> | Dose (LED or HID)        | Route, duration, dosing regimen                                                                                  | Comments                                                                                                                                 | Reference                                 |
|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|-------------------------------|----------------------|--------------------------|------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| IgM response, humoral immunity      | IgM antibody-forming cell response to T cell-dependent antigen sheep erythrocytes                                                 | Rat, Sprague-Dawley (F)                         | Splenocytes                   | No change            | 20 000 mg/m <sup>3</sup> | Gasoline vapour condensate, via inhalation, at 0, 2000, 10 000, or 20 000 mg/m <sup>3</sup> for 4 wk             | Well-designed and documented study (for exposure and immune readouts). Only one time point analysed.                                     | <a href="#">White et al. (2014)</a>       |
| Humoral- and cell-mediated immunity | Injection of sheep red blood cell antigen, IgM measurements (ELISA) and sensitization with bovine albumin (thickness of the feet) | Rat, Long-Evans, dams and offspring (age, 6 wk) | Serum and feet of pelvic limb | No change            | 9000 ppm                 | Gasoline vapour condensate, via inhalation, at 0, 3000, 6000, or 9000 ppm, exposed on days 9–20 of gestation     | Well-designed and documented study (exposure and immune readouts). Only one time point analysed.                                         | <a href="#">Bushnell et al. (2015)</a>    |
| Leukocyte count                     | Advia-2120i analyser                                                                                                              | Rat, inbred albino (M)                          | Blood                         | ↓ Leukocyte count    | Not determined           | Crude petrol volatile compounds (500 mL), via inhalation, for 8 h per day for 60 or 90 days 15 animals per group | Well-designed study. Administration of vitamin D3 reduced the immune response. The exposure was poorly documented.                       | <a href="#">Yestemirova et al. (2024)</a> |
| Leukocyte count                     | Microscopy                                                                                                                        | Rat, Wistar (F)                                 | Blood                         | ↑ Leukocyte count    | Not determined           | Crude petrol volatile compounds, via inhalation, for 6 h per day on 6 days per week for 20 wk                    | Few parameters studied. Cell counts and exposure were poorly documented. Administration of vitamins A and E reduced the immune response. | <a href="#">Uboh et al. (2010)</a>        |

F, female(s); h, hour(s); HID, highest ineffective dose; Ig, immunoglobulin; LED, lowest effective dose; M, male(s); NA, not applicable; ppm, parts per million; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; (↑) or (↓) findings in a study of limited quality; or no change.

#### 4.2.8 Modulates receptor-mediated effects

##### (a) Exposed humans

See [Table 4.14](#).

[Uzma et al. \(2008\)](#) observed higher serum levels of total thyroxine (T4) and free T4, and lower serum levels of triiodothyronine (T3) and thyroid-stimulating hormone (TSH), in 42 service station attendants in Hyderabad, India, than in 36 non-exposed individuals. Notably, the T3 and TSH levels inversely correlated with the duration of exposure. [The Working Group noted that an assessment of markers of exposure to automotive gasoline was not performed; the authors measured only air carbon monoxide levels in service stations and residential areas.]

When concentrations of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured, lower levels of testosterone were found for 11 service station attendants (the exposed group) than for 112 non-exposed office workers in Shiraz, Iran ([Saadat and Monzavi, 2008](#)). When the relation between the hormone levels and the *GSTT1* (encoding a phase II enzyme) null polymorphism was assessed, no differences were observed between exposed and non-exposed individuals with respect to the presence of the *GSTT1* active genotype. In individuals carrying the null variant, lower testosterone and higher LH concentrations were detected in exposed versus non-exposed individuals. [The Working Group noted that no markers of exposure were measured.]

In the same cohort in Shiraz, Iran, the testosterone, FSH, and LH levels of exposed and non-exposed individuals were compared according to the *GSTM1* genotype ([Saadat et al., 2013](#)). In the *GSTM1*-positive group, hormone concentrations were not affected by the exposure. However, among *GSTM1*-null individuals, the exposed group had lower testosterone levels than did the non-exposed group. In addition, analysis of the combination of *GSTM1* and

*GSTT1* genotypes showed that exposed “null *GSTM1*/positive *GSTT1*” or “null *GSTM1*/null *GSTT1*” individuals had lower testosterone concentrations than did the non-exposed group. Other genotype combinations did not show significant differences in hormone concentrations according to exposure. [The Working Group noted that no markers of exposure were measured.]

Fifty-eight service station attendants and automobile-repair workers, used as a lead-exposed group, in Chandigarh, India, had higher TSH concentrations than did 35 non-exposed individuals ([Singh et al., 2000](#)). The TSH concentrations of exposed individuals who had high lead concentrations in the blood were higher than those of controls and individuals with moderate blood concentrations of lead. When the lead-exposed group was subdivided according to the duration of lead exposure, the T3 concentrations of those individuals with the longest exposures were significantly lower. No differences were observed in T4 concentration in any of the analyses. [The Working Group noted that the study focused on lead exposure. The exposed group was composed of both gasoline-filling and automobile-repair workers; therefore, the impact of automotive gasoline exposure on the findings was unclear. Additionally, confounding factors were not accounted for.]

**Table 4.14 End-points relevant to modulation of receptor-mediated effects in humans exposed to automotive gasoline**

| End-point                                                  | Biological sample | Location setting, study design                                                  | Exposure duration, no. of study participants                                                                                                                                        | Response <sup>a</sup>                                                                                                                                                                    | Covariates controlled                                | Comments                                                                                                                           | Reference                                 |
|------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| Thyroid hormone levels: T3, T4, T4F, TSH                   | Blood             | India<br>Cross-sectional study<br>Service station attendants                    | Workers exposed to fuel for 1–5, 5–10, 10–15, or > 15 yr during 8-h daily work shifts<br><i>n</i> = 42 service station attendants<br><i>n</i> = 36 non-exposed non-smokers          | ↑ T4 and T4F<br>↓ T3 and TSH<br>(T3 and TSH levels inversely correlated with the years of exposure)                                                                                      | Age, sex, and smoking history                        | <i>Limitations:</i> No exposure assessment, air carbon monoxide levels alone were measured in fuel stations and residential areas. | <a href="#">Uzma et al. (2008)</a>        |
| Testosterone, FSH, LH                                      | Blood             | Islamic Republic of Iran<br>Cross-sectional study<br>Service station attendants | Workers exposed to fuel for an average of 6.9 yr (range: 6 mo to 35 yr)<br><i>n</i> = 114 service station attendants;<br><i>n</i> = 112 non-exposed age-matched male office workers | ↓ Testosterone levels in the exposed group<br>(↓ Testosterone and ↑ LH levels in <i>GSTM1</i> -null exposed participants.)                                                               | Age and sex                                          | <i>Limitation:</i> No exposure assessment.                                                                                         | <a href="#">Saadat and Monzavi (2008)</a> |
| Testosterone, FSH, LH (according to <i>GSTM1</i> genotype) | Blood             | Islamic Republic of Iran<br>Cross-sectional study<br>Service station attendants | Workers exposed to fuel for an average of 6.9 yr (range: 6 mo to 35 yr)<br><i>n</i> = 114 service station attendants<br><i>n</i> = 100 non-exposed age-matched male office workers  | In <i>GSTM1</i> -null exposed participants:<br>↓ Testosterone levels<br>No change in FSH or LH<br>In <i>GSTM1</i> -active exposed participants:<br>No change in testosterone, FSH, or LH | Age, sex, cigarette smoking, and alcohol consumption | <i>Limitation:</i> No exposure assessment.                                                                                         | <a href="#">Saadat et al. (2013)</a>      |

**Table 4.14 (continued)**

| End-point   | Biological sample | Location setting, study design                               | Exposure duration, no. of study participants                                                                                                                                                                                                                                                                                       | Response <sup>a</sup>                                                         | Covariates controlled | Comments                                                                                                                                                                                | Reference                           |
|-------------|-------------------|--------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| T3, T4, TSH | Blood             | India<br>Cross-sectional study<br>Service station attendants | Workers were exposed to fuel for an average of $156.9 \pm 122.7$ mo. They were divided into two groups: $29.3 \pm 15.4$ mo, and $210 \pm 108$ mo<br>$n = 58$ exposed to lead while engaged in petrol filling and automobile-repair jobs<br>$n = 35$ non-exposed healthy men, with no history of organic or inorganic lead exposure | ↑ TSH levels<br>↓ T3 levels in participants with longer durations of exposure | Sex                   | <i>Limitation:</i> No adjustment for confounding. The study focused on lead exposure; therefore, the exposed group was composed of both gasoline-filling and automobile-repair workers. | <a href="#">Singh et al. (2000)</a> |

FSH, follicle-stimulating hormone; h, hour(s); LH, luteinizing hormone; mo, month(s); T3, triiodothyronine; T4, thyroxine; T4F, free thyroxine; TSH, thyroid-stimulating hormone; yr, year(s).

<sup>a</sup> ↑, increase; ↓, decrease; (↑) or (↓) findings in a study of limited quality; or no change.



*(b) Experimental systems*

See [Table 4.15](#).

In addition to studies in exposed humans, the effects of automotive gasoline on hormone levels have also been assessed in experimental models. Male Wistar rats that were exposed to 2 g/m<sup>3</sup> petrol vapour for 8 hours per day on 5 days per week for 60 days had increased serum corticosterone and adrenal catecholamine concentrations and lower hypothalamic noradrenaline concentrations than did the controls, but there was no difference in serum T4 concentrations ([Vyskocil et al., 1988](#)). [The Working Group noted that the exposure dose might be considered to be low, which strengthened the findings.]

Rats exposed to gasoline fumes for 12 weeks had lower testosterone levels when exposed for 3, 5, or 9 hours per day, but not for 1 hour per day, compared with non-exposed rats ([Owagboriaye et al., 2018](#)). In addition, FSH, LH, and prolactin levels were significantly increased in all exposed groups in a time-dependent manner. [The Working Group noted that in this study, the levels of exposure were not precisely measured, because a saturated chamber was used.]

[Standeven et al. \(1994a\)](#) showed reduced relative uterine and ovarian weight in female B6C3F<sub>1</sub> mice treated with 2056 ppm of the PS-6 blend unleaded gasoline vapour for 6 hours per day on 5 days per week for 16 weeks. Treatment with dietary ethinyl estradiol (EE2) at 1 ppm increased the relative uterine weights and reduced the relative ovarian weights of the mice, and when the PS-6 unleaded gasoline and EE2 treatments were combined, the effects were similar to those of EE2 alone. The effect of PS-6 unleaded gasoline on the serum 17 $\beta$ -estradiol (E2) concentration was assessed, but neither of the doses used (292 or 2056 ppm) had a significant effect. In contrast, EE2 treatment reduced the E2 concentration, and this reduction was not affected by the addition of unleaded gasoline to this treatment. To further assess the anti-estrogenic

effects of unleaded gasoline, uterine peroxidase activity was measured. Whereas PS-6 unleaded gasoline did not affect uterine peroxidase activity, EE2 alone or in combination with PS-6 unleaded gasoline significantly reduced the enzyme activity. Finally, the uterine unoccupied cytosolic estrogen receptor level was measured, and again there was no effect of PS-6 unleaded gasoline alone, whereas EE2 alone or in combination with PS-6 unleaded gasoline reduced receptor levels in mice that were initiated with N-nitrosodiethylamine (DEN; 5 mg/kg). [The Working Group noted that the study did not show statistically significant alterations in E2 levels, uterine peroxidase activity, or uterine unoccupied cytosolic estrogen receptor levels after exposure to PS-6 unleaded gasoline, but that treatment with EE2 (a positive control) affected all these parameters. These null results suggested a lack of effect of unleaded gasoline on the analysed end-points.]

The anti-estrogenic effects of unleaded gasoline have also been analysed in ovariectomized (OVEX) female B6C3F<sub>1</sub> mice treated by intragastric intubation on 3 consecutive days with 200, 600, 1800, or 3000 mg/kg per day PS-6 unleaded gasoline ([Standeven et al., 1994b](#)). PS-6 unleaded gasoline did not compete with [<sup>3</sup>H]E2 for estrogen receptor binding in uterine cytosol. However, the conversion of E2 and EE2 to water-soluble metabolites by hepatocytes was accelerated in mice treated with 1800 mg/kg per day PS-6 unleaded gasoline; the effect was not associated with the hepatomegaly. E2 metabolism was also accelerated in mice treated with 600 or 3000 mg/kg per day, but not 200 mg/kg per day of PS-6 unleaded gasoline. The ability of PS-6 unleaded gasoline to counteract the effect of E2 on uterine growth in OVEX mice was also assessed. The induction of uterine growth by E2 was not blocked either when the treatments were concomitant or when mice were pre-treated with PS-6 unleaded gasoline.

**Table 4.15 End-points relevant to the modulation of receptor-mediated effects in non-human mammalian systems in vivo exposed to automotive gasoline**

| End-point                                                                                                                                                                               | Species, strain (sex)         | Biological sample                   | Results <sup>a</sup>                                                                                                           | Dose (LED or HID)                                                               | Route, duration, dosing regimen                                                                                       | Comments                                                                                                                                    | Reference                                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| Serum corticosterone, competitive protein-binding<br>Adrenal adrenaline and noradrenaline<br>Hypothalamic noradrenaline<br>Serum thyroxine, RIA-test-T4<br>hypothalamic, by fluorometry | Rats, Velaz Wistar (M)        | Blood, hypothalamus, adrenal glands | ↑ Corticosterone<br>↑ Adrenaline and noradrenaline in adrenal glands<br>↓ Hypothalamic noradrenaline<br>No change in thyroxine | LED: 2 g/m <sup>3</sup> gasoline for 8 h per day on 5 days per week for 60 days | Gasoline vapour diluted in ambient air (2 g/m <sup>3</sup> gasoline) for 8 h per day on 5 days per week for ≤ 60 days | Strength: Findings made at low exposure levels.                                                                                             | <a href="#">Vyskocil et al. (1988)</a>    |
| Testosterone<br>FSH<br>LH<br>Prolactin, by ELISA                                                                                                                                        | Rat, albino (M)               | Serum                               | ↓ Testosterone after exposure for 3, 5, or 9 h per day<br>↑ FSH, LH, and prolactin                                             | Not specified                                                                   | Exposure in a chamber saturated with gasoline vapour for 1, 3, 5, or 9 h per day for 12 wk                            | Exposure levels were not precisely measured in the saturated chamber. There was a time-dependent effect.                                    | <a href="#">Owagboriaye et al. (2018)</a> |
| 17β-estradiol, solid-phase radioimmunoassay<br>Uterine peroxidase activity, by the method of Lyttle and DeSombre (1977)<br>Unoccupied uterine cytosolic estrogen receptor level         | Mouse, B6C3F <sub>1</sub> (F) | Uterus, serum                       | No change (17β-estradiol level, uterine peroxidase activity, unoccupied uterine cytosolic estrogen receptor level)             | HID: 2056 ppm                                                                   | PS-6 blend UG vapour at 0, 292, or 2056 ppm via inhalation for 6 h per day on 5 days per week for 16 wk               | The null results suggest a lack of effect of UG on the analysed end-points, because the positive control (EE2) affected all the parameters. | <a href="#">Standeven et al. (1994a)</a>  |

**Table 4.15 (continued)**

| End-point                                                                                                                                            | Species, strain (sex)                         | Biological sample | Results <sup>a</sup>                                                                      | Dose (LED or HID)                                                                                                                                                                                                                       | Route, duration, dosing regimen                                                                     | Comments                                                                                                              | Reference                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|-------------------|-------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| Competitive binding to estrogen receptor<br>Conversion of [ <sup>3</sup> H]E2 and EE2 to water-soluble metabolites, by liquid scintillation counting | Mice, B6C3F <sub>1</sub> , ovariectomized (F) | Uterus, liver     | No competitive binding<br>↑ 17β-estradiol and EE2 conversion to water-soluble metabolites | HID: 3000 mg/kg per day for competitive binding to estrogen receptors<br>LED: 1800 mg/kg per day for the conversion of EE2 to water-soluble metabolites<br>LED: 600 mg/kg per day for the conversion of E2 to water-soluble metabolites | PS-6 UG at 200, 600, 1800 or 3000 mg/kg per day or corn oil, via intragastric intubation for 3 days | Acute exposure, which might not reflect what is observed in humans (occupational or environmental exposure).          | <a href="#">Standeven et al. (1994b)</a> |
| Rate of conversion of [ <sup>3</sup> H]17β-estradiol to water-soluble metabolites                                                                    | Mouse, B6C3F <sub>1</sub> (F)                 | Liver             | ↑ estrogen metabolism                                                                     | LED: 1800 mg/kg bw                                                                                                                                                                                                                      | 91-01 UG or PS-6 UG injection at 1800 mg/kg bw per day for 3 days                                   | Hormone levels were not measured. This was an acute treatment, not reflecting occupational or environmental exposure. | <a href="#">Moser et al. (1996b)</a>     |

bw, body weight; E2, 17β-estradiol; EE2, ethinyl estradiol; ELISA, enzyme-linked immunosorbent assay; F, female(s); FSH, follicle-stimulating hormone; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; LH, luteinizing hormone; M, male(s); ppm, parts per million; RIA, radioimmunoassay; UG, unleaded gasoline; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; (↑) or (↓) findings in a study of limited quality; or no change.

Based on these results, the authors proposed that the previously observed effects of PS-6 unleaded gasoline on the uterus might not be linked to an anti-estrogenic mechanism. [The Working Group highlighted that the study was performed in OVEX mice, which might make it difficult to directly compare the findings with those of other studies. In addition, the exposure was acute and therefore did not reflect occupational or environmental exposure in humans.]

In a study conducted in female B6C3F<sub>1</sub> mice, exposure to unleaded gasoline vapour (91-01 unleaded gasoline or PS-6 unleaded gasoline) for 3 days significantly reduced uterine weight, relative to that of controls. In addition, exposure to 91-01 unleaded gasoline vapour for 21 days led to a reduction in relative uterine weight ([Moser et al., 1996b](#)). The authors stated that such a reduction was also observed after acute exposure to unleaded gasoline and they used intragastric administration to assess the effect of unleaded gasoline on the rate of conversion of [<sup>3</sup>H]17- $\beta$ -estradiol ([<sup>3</sup>H]E2) to water-soluble metabolites. The injection of 91-01 at 1800 mg/kg bw or PS-6 at 1800 mg/kg bw per day for 3 days resulted in a 3.1 and 3.4-fold increase, respectively, in the rate of estrogen metabolism. This was in accordance with the hypothesis that a high level of exposure to unleaded gasoline may have anti-estrogenic effects in female mice. [The Working Group noted that hormone levels were not measured in the study. In addition, E2 metabolism was only assessed after the acute treatment (3 days), limiting the relevance of the findings.]

### Synopsis

[Overall, the Working Group noted that in the two studies from the same cohort in which occupational exposure in humans has been assessed, the most consistent effect on hormone levels was a reduction in testosterone concentration. This result has also been confirmed in experimental systems in vivo. This effect seemed to be dependent on polymorphisms that

impaired the activity of phase II biotransformation enzymes. The results for thyroid hormones were inconsistent: studies in humans occupationally exposed to gasoline showed the opposite effects to those in rodents. This was true for TSH, for example. Stress hormones might be affected by exposure, but only a few studies have been performed in animal models. Finally, the results for female sex hormones were scarce and inconsistent. Estrogen metabolism seemed to be accelerated by exposure to automotive gasoline in animal models, but the variety of experimental models (ovariectomized versus non-ovariectomized mice) used, and of the methods used to assess the anti-estrogenic effects, hampered the interpretation of the results.]

### 4.2.9 Alters cell proliferation, cell death, or nutrient supply

#### Experimental systems

See [Table 4.16](#).

Experimental models have been used to assess the effects of unleaded gasoline on cell proliferation, cell death, or nutrient supply. [Standeven and Goldsworthy \(1993\)](#) showed that treatment of female B6C3F<sub>1</sub> mice with PS-6 unleaded gasoline or API 91-1 unleaded gasoline by intragastric intubation for 3 days increased hepatocyte proliferation, as measured by BrdU labelling, and the relative liver weight. The authors also assessed the effects of unleaded gasoline in an initiation–promotion study (see Section 3). No effect on total body weight, as well as a lack of hepatic necrosis, were observed after 13 weeks of exposure to PS-6 unleaded gasoline vapour, after initiation with DEN or saline ([Standeven and Goldsworthy, 1993](#)). [The Working Group noted that hepatocyte proliferation was assessed only after acute exposure. In addition, only female mice were analysed.]

**Table 4.16 End-points relevant to altered cell proliferation, cell death, or nutrient supply in non-human mammalian systems in vivo exposed to automotive gasoline**

| End-point                                                                                                                                                         | Species, strain (sex)         | Tissue | Results <sup>a</sup>                                                                                                                                                  | Dose (LED or HID)                    | Route, duration, dosing regimen                                                                                      | Comments                                                                                                                                                                                                                | Reference                                        |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| Cell proliferation, by BrdU labelling index                                                                                                                       | Mouse, B6C3F <sub>1</sub> (F) | Liver  | ↑ Hepatocyte labelling index in PS-6 or API 91-1 blend UG, no alteration in body weight;<br>No hepatic necrosis;<br>↑ Relative liver weight;<br>↑ Hepatic hypertrophy | LED: 2056 ppm or 1800 mg/kg          | PS-6 or API 91-1 blend UG in corn oil at 1800 mg/kg per day, or corn oil, by gavage, for 3 days                      | <i>Limitations:</i> Hepatocyte proliferation was only assessed after acute exposure; only females analysed. Initiation–promotion study, 13 wk. Exposure to PS-6 blend UG caused the formation of preneoplastic lesions. | <a href="#">Standeven and Goldsworthy (1993)</a> |
| Cell proliferation, by BrdU labelling<br>TGFβ, M6P/IGF-II R, by immunohistochemistry                                                                              | Mouse, B6C3F <sub>1</sub> (F) | Liver  | ↑ BrdU labelling index in focal hepatocytes<br>(↑) TGFβ1 immunoreactivity in non-focal hepatocytes                                                                    | LED: 2038 ppm                        | PS-6 UG + DEN (DEN, 5 mg/kg, 7.1 mL/kg bw), or saline by i.p. injection for 6 h per day on 5 days per week for 16 wk | <i>Limitation:</i> Immunohistochemistry results were not clear.                                                                                                                                                         | <a href="#">Moser et al. (1996a)</a>             |
| Cell proliferation, by [ <sup>3</sup> H]thymidine labelling and histopathology<br>CPN lesions, by histopathology, α <sub>2u</sub> -globulin, immunohistochemistry | Rat, F344 (M, F)              | Kidney | Males:<br>↑ Proliferation of proximal tubule cells<br>↑ Number of CPN foci<br>↑ Polyangular deposits of α <sub>2u</sub> -globulin<br>Females: no changes              | LED: 300 ppm (M)<br>HID: 300 ppm (F) | UG (PS-6 UG vapour) at 0, 10, 70, or 300 ppm                                                                         | Sex-dependent differences in the evaluated end-points might be linked to accumulation of α <sub>2u</sub> -globulin.                                                                                                     | <a href="#">Short et al. (1989b)</a>             |

**Table 4.16 (continued)**

| End-point                                   | Species, strain (sex)                                                   | Tissue | Results <sup>a</sup>                                                                                                    | Dose (LED or HID) | Route, duration, dosing regimen                                                                                                                                                                                                                                                         | Comments                                                                                           | Reference                               |
|---------------------------------------------|-------------------------------------------------------------------------|--------|-------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|-----------------------------------------|
| Cell proliferation, by BrdU labelling index | Mice, B6C3F <sub>1</sub> Ovariectomized (OVEX) or ovaries-preserved (F) | Liver  | No change in hepatic labelling index<br>↑ relative liver weight                                                         | HID: 2038 ppm     | 91-01 UG at 2038 ppm or saline injection daily for 4 mo (Mice were initiated with a single i.p. injection of DEN or saline at 12 days of age; then, ovariectomized or had the ovaries preserved at 28 days. At age 8 wk, mice were exposed to control air or a target concentration UG) | The tumour-promoting activity of UG was attenuated, but hepatocyte proliferation was not affected. | <a href="#">Moser et al. (1997a)</a>    |
| Cell proliferation, by BrdU labelling       | Mice, B6C3F <sub>1</sub> (M)                                            | Liver  | ↑ Hepatic labelling index                                                                                               | LED: 2046 ppm     | 2046 ppm of vapourized PS-6 blend UG + 5.0 mg/kg DEN, or saline, via i.p. injection for 6 h per day on 5 days per week for 16 wk                                                                                                                                                        |                                                                                                    | <a href="#">Standeven et al. (1995)</a> |
| Cell proliferation, by BrdU labelling       | Mice B6C3F <sub>1</sub> (M, F)                                          | Liver  | ↑ Hepatic labelling index in week 1 in both males and females; this had returned to control levels at week 3, 6, and 13 | LED: 2056 ppm     | PS-6 UG vapour at 67, 292, or 2056 ppm via inhalation for 6 h per day on 5 days per week for 13 wk                                                                                                                                                                                      |                                                                                                    | <a href="#">Tilbury et al. (1993)</a>   |
| Liver histopathology                        |                                                                         |        | No sign of liver necrosis                                                                                               |                   |                                                                                                                                                                                                                                                                                         |                                                                                                    |                                         |
| AST and ALT enzyme activities               |                                                                         |        | No change in AST or ALT                                                                                                 |                   |                                                                                                                                                                                                                                                                                         |                                                                                                    |                                         |
| Organ weights                               |                                                                         |        | ↑ Absolute and relative liver weights                                                                                   |                   |                                                                                                                                                                                                                                                                                         |                                                                                                    |                                         |



**Table 4.16 (continued)**

| End-point                                                                                                               | Species, strain (sex)         | Tissue | Results <sup>a</sup>                                                                                                                                                     | Dose (LED or HID)                    | Route, duration, dosing regimen                                                                                                                                                         | Comments                                                                                                                                                                                                | Reference                                        |
|-------------------------------------------------------------------------------------------------------------------------|-------------------------------|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| Cell proliferation, by BrdU labelling<br><br>Organ weights                                                              | Mouse, B6C3F <sub>1</sub> (F) | Liver  | ↑ Hepatocyte proliferation (total UG or heavy UG)<br>↑ Relative liver weight                                                                                             | LED: 3000 mg/kg per day              | Light UG, heavy UG, or total UG at 3000 mg/kg per day, or corn oil, via intragastric intubation for 3 days                                                                              | The heavy fraction of UG was further fractionated according to boiling-point, which showed that specific components, and probably TMP, were responsible for the observed effects on cell proliferation. | <a href="#">Standeven and Goldsworthy (1994)</a> |
| Cell proliferation, by BrdU labelling                                                                                   | Mouse, B6C3F <sub>1</sub> (F) | Liver  | ↑ Hepatocyte proliferation (after PS-6 UG inhalation for 3 days or injection of 91-01 UG for 3 days) (Hepatocyte proliferation returned to control levels after 21 days) | LED: PS-6 UG or 91-01 UG: 1800 mg/kg | 91-01 UG vapour at 2014 ppm or PS-6 UG at 2028 ppm) via inhalation for 3 or 21 days, 6 h per day, 5 days per week, or 91-01 UG or PS-6 UG injection at 1800 mg/kg bw per day for 3 days | Hepatocyte proliferation returned to control levels after 21 days.                                                                                                                                      | <a href="#">Moser et al. (1996b)</a>             |
| Cell proliferation, by [ <sup>3</sup> H]thymidine labelling<br>Kidney histopathology examination                        | Rat, F344 (M)                 | Kidney | ↑ Kidney cell (nephron) proliferation                                                                                                                                    | LED: 200 ppm                         | Exposure to UG vapour (0, 2, 20, 200, or 2000 ppm) for 6 h per day on 5 days per week for 3 wk                                                                                          | Effect was dose-dependent in the P2 nephron segment but was also observed in P3 at the highest dose.                                                                                                    | <a href="#">Short et al. (1987)</a>              |
| RNA synthesis, by [ <sup>14</sup> C]orotate incorporation<br>DNA synthesis, by [ <sup>3</sup> H]thymidine incorporation | Rat, Wistar (F)               | Lung   | No change                                                                                                                                                                | HID: 100 ppm                         | Gasoline vapour at 100 ppm, via inhalation, 8 h per day for ≤ 50 days                                                                                                                   | Although no effects on DNA and RNA synthesis were observed, UG exposure reduced surfactant levels.                                                                                                      | <a href="#">Stewart et al. (1979)</a>            |

**Table 4.16 (continued)**

| End-point                                                                                                                                         | Species, strain (sex)           | Tissue | Results <sup>a</sup>                                                                              | Dose (LED or HID)                  | Route, duration, dosing regimen                                                                                                                                                                                                   | Comments                                                                                                                                                                           | Reference                              |
|---------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|--------|---------------------------------------------------------------------------------------------------|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| DNA synthesis, by [ <sup>3</sup> H]-thymidine labelling<br>Kidney $\alpha_{2u}$ -globulin concentration, by ELISA<br>Kidney protein droplet score | Rats, F344 (M)                  | Kidney | ↑ Cell proliferation<br>↑ $\alpha_{2u}$ -Globulin concentration<br>↑ Kidney protein droplet score | LED: EHT 500 mg/kg;<br>UG 16 mg/kg | EHT 50–500 mg/kg, PS-6 UG 16–500 mg/kg, or 2,2,4-TMP 0.95–30 mg/kg by gavage for 10 days                                                                                                                                          | All the chemicals induced DNA synthesis, but UG induced this at lower doses. The induction was dose-dependent. Results might be linked to accumulation of $\alpha_{2u}$ -globulin. | <a href="#">Borghoff et al. (1992)</a> |
| Histopathology                                                                                                                                    | Mice, C57BL (sex not specified) | Lung   | ↑ Epithelial hyperplasia and mitotic figures                                                      | Not specified                      | Exposure to an atmosphere of ozonized gasoline or a washed air chamber for ≤ 68 wk                                                                                                                                                | Study description lacks important details, such as the sex of the animals and the dose of the exposure.                                                                            | <a href="#">Kotin et al. (1958)</a>    |
| Atypical cell foci<br><br>CPN score                                                                                                               | Rats, F344 (M, F)               | Kidney | ↑ Atypical cell foci (increasing linear trend with increasing doses)<br>↑ CPN score               | LED: 300 ppm                       | EHEN at 170 ppm in the drinking-water for 2 wk, followed by a 4-wk holding period, then inhalation of UG at doses of 10, 70, or 300 ppm for 24 wk, then held until 65–67 wk after the start of the experiment<br>30 rats per dose | UG and TMP had similar effects on nephrosis, atypical cell foci, and renal tubular cell tumours. Results might be linked to accumulation of $\alpha_{2u}$ -globulin.               | <a href="#">Short et al. (1989a)</a>   |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BrdU, bromodeoxyuridine; CPN, chronic progressive nephropathy; DEN, *N*-nitrosodiethylamine; EHEN, *N*-ethyl-*N*-hydroxyethylnitrosamine; EHT, European High Test gasoline; ELISA, enzyme-linked immunosorbent assay; F, female(s); h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male(s); mo, month(s); ppm, parts per million; M6P/IGF-II R, mannose 6-phosphate/insulin-like growth factor II receptor; OVEX, ovariectomized; TGF $\beta$ , transforming growth factor beta; TMP, trimethylpentane; UG, unleaded gasoline; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; (↑) or (↓) findings in a study of limited quality; or no change.

In a short-term study, female B6C3F<sub>1</sub> mice were initiated with an injection of DEN, followed by exposure to PS-6 unleaded gasoline vapour for 16 weeks. There was a significant increase in the BrdU labelling index in altered hepatocyte foci of PS-6 unleaded gasoline-treated and DEN-initiated mice, when compared with control animals. The hepatic labelling index of the tissue surrounding the foci was similar for the exposed and control groups ([Moser et al., 1996a](#)). [The Working Group noted that only female mice were evaluated.]

Female and male F344 rats were exposed to various concentrations of PS-6 unleaded gasoline vapour (10, 70, or 300 ppm) for up to 50 weeks. In the male rats, the proliferation of kidney proximal tubule cells, and especially P2 cells, was induced by treatment with PS-6 unleaded gasoline at 300 ppm or with TMP at 50 ppm for 3, 10, or 22 weeks. A number of foci of CPN were induced at week 22 by treatment with PS-6 unleaded gasoline at 300 ppm, but only in the males. Large polyangular deposits of  $\alpha_{2u}$ -globulin were observed in the kidneys of male rats exposed to unleaded gasoline PS-6 at 300 ppm at all the time points. In contrast, no effects were observed in the females. In addition, there were no effects on body weight or relative kidney weight of the treated rats versus the controls throughout the experiment ([Short et al., 1989b](#)). [The Working Group noted that the study showed relevant sex-dependent differences in the evaluated end-points, which might be linked to accumulation of  $\alpha_{2u}$ -globulin in male rats. The Working Group noted that proliferation of the proximal tubule cells may be one of the morphological changes that occur during spontaneous progressive glomerulonephropathy in the rat. Preneoplastic proliferation, characterized by atypical cellular hyperplasia, was not observed in this study.]

The promoter effect of unleaded gasoline on DEN-induced carcinogenesis was also tested in ovariectomized (OVEX) B6C3F<sub>1</sub> mice ([Moser](#)

[et al., 1997a](#)) (see also Section 3). The hepatic labelling index was not affected. Treatment with 91-01 unleaded gasoline at 2038 ppm for 16 weeks, either after DEN initiation or after saline injection, resulted in an increase in relative liver weight in comparison to the respective controls, both in OVEX mice and in those with their ovaries preserved. Exposure to unleaded gasoline for 7–8 months induced an increase in the relative weight of the liver in all conditions tested (saline or DEN, ovaries preserved or OVEX). [The Working Group noted that estrogen has previously been shown to inhibit liver tumour development. The authors hypothesized that the tumour-promoting effect of unleaded gasoline could have been a consequence of the inhibition of estrogen activity. This was corroborated by previous studies that showed uterine changes after the administration of unleaded gasoline. In summary, in this OVEX mouse model, the tumour-promoting activity of unleaded gasoline was attenuated, whereas hepatocyte proliferation was not affected.]

In another study in male B6C3F<sub>1</sub> mice (see Section 3), the administration of PS-6 unleaded gasoline vapour, after DEN initiation, increased the hepatic BrdU labelling index in altered hepatic foci, but no difference in the body weights of the groups was observed at any time point ([Standeven et al., 1995](#)). [The Working Group noted that the increase in hepatocyte proliferation was confined to altered hepatic foci.]

[Tilbury et al. \(1993\)](#) also used an inhalation model: they exposed mice to PS-6 unleaded gasoline vapour at 67, 292, or 2056 ppm for up to 13 weeks. After 1 week, a significant increase in hepatocyte BrdU labelling was observed in the livers of male and female mice, but this had returned to control levels after 3, 6, and 13 weeks. There were no signs of liver necrosis, nor any differences in AST or ALT activities, in the male or female B6C3F<sub>1</sub> mice. The highest dose of PS-6 unleaded gasoline caused increases in the absolute and relative liver weights of both

males and females at all the time points (1, 3, 6, and 12 weeks). [The Working Group noted that, according to the authors, chronic exposure to unleaded gasoline vapour led to liver tumour development in female mice, but not male mice. However, the study showed a transient induction of hepatocyte proliferation in both sexes.]

The short-term effects of the intragastric administration of PS-6 unleaded gasoline or one of two fractions separated according to their boiling-point (light unleaded gasoline, boiling-point, < 100 °C; heavy unleaded gasoline, boiling-point, > 100 °C), all at 3000 mg/kg per day, for 3 days, have also been assessed in female B6C3F<sub>1</sub> mice ([Standeven and Goldsworthy, 1994](#)). The mice treated with unleaded gasoline or the heavy fraction had higher relative liver weights and hepatocyte BrdU labelling indices, but there was no difference from controls with respect to body weight. In addition, none of the groups showed hepatic necrosis or altered sorbitol dehydrogenase activity. To further explore the effects of specific fractions of PS-6 unleaded gasoline, the heavy unleaded gasoline fraction was separated in two further fractions according to boiling-point (fraction 3, 100–132 °C; and fraction 4, > 132 °C). The effects of these fractions, plus the original light unleaded gasoline fraction, were then tested at a lower dose (1800 mg/kg per day). These treatments did not result in total body weight loss or hepatic necrosis. However, the unleaded gasoline fractions 3 and 4 caused increases in relative liver weight, and but only fraction 3 significantly increased the hepatocyte BrdU labelling index. Specific constituents of the heavy unleaded gasoline fraction (toluene; 2,2,3-TMP; 2,2,4-TMP; and 2,3,4-TMP) were also tested. The relative liver weights of the mice were increased by treatment with 2,2,4-TMP or 2,3,4-TMP, and the hepatocyte incorporation index was increased by all the TMPs ([Standeven and Goldsworthy, 1994](#)).

Inhalation exposure of female B6C3F<sub>1</sub> mice to 91-01 unleaded gasoline or PS-6 unleaded gasoline vapour for 3 or 21 days resulted in an increase in relative liver weight after 21 days. Cell proliferation, assessed using hepatic BrdU labelling, was increased after 3 days of inhalation of PS-6 unleaded gasoline, but this returned to control levels after 21 days. In the same study, the effects of intragastric exposure to 91-01 unleaded gasoline for 3 days were also assessed, and this treatment resulted in increases in relative liver weight and hepatocyte proliferation ([Moser et al., 1996b](#)). [The Working Group noted that the induction of hepatocyte proliferation seemed to be independent of unleaded gasoline formulation, but dependent on the dose and route of administration.]

Unleaded gasoline was administered to male F344 rats by inhalation on 5 days per week for 3 weeks, and the effects on kidney morphology and [<sup>3</sup>H]thymidine labelling were assessed. An increase in the labelling index was observed in the P2 proximal tubule segment after treatment with unleaded gasoline at 200 or 2000 ppm and also in the P3 segment after treatment at 2000 ppm ([Short et al., 1987](#)).

[Stewart et al. \(1979\)](#) investigated the effects of gasoline vapour exposure on RNA and DNA synthesis in the lung tissue of female Wistar rats. The inhalation of gasoline vapour (100 ppm, 8 hours per day) for 10, 25, or 50 days did not affect [<sup>14</sup>C]orotate incorporation into pulmonary RNA or [<sup>3</sup>H]thymidine incorporation into DNA ([Stewart et al., 1979](#)). [The Working Group noted that although no effects on DNA and RNA synthesis were observed, unleaded gasoline exposure reduced surfactant levels.]

The exposure of male F344 rats to various doses of European High Test (EHT) gasoline, PS-6 unleaded gasoline, or 2,2,4-TMP by gavage for 10 days did not affect their relative kidney weights, compared with controls ([Borghoff et al., 1992](#)). All these treatments increased the kidney protein droplet scores,  $\alpha_{2u}$ -globulin

concentrations, and cell proliferation, measured using [ $^3\text{H}$ ]thymidine incorporation, in a dose-dependent manner, but unleaded gasoline had the most striking effects.

The effect of an atmosphere of ozonized gasoline on the respiratory epithelium was assessed in C57BL mice, by comparison with mice living in a washed air chamber ([Kotin et al., 1958](#)). The pulmonary epithelial hyperplasia of the mice was more marked in the exposed group, with evidence of cellular activity, such as mitotic figures. [The Working Group noted that this publication lacked important details, such as the sex of the mice and the exposure dose, limiting the conclusions that could be drawn.]

In an initiation–promotion model using *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) as the initiating agent (170 ppm for 2 weeks), there was a significant linear trend towards increases in the frequency of atypical cell foci with increases in the dose of unleaded gasoline (10, 70, or 300 ppm) in the kidneys of male F344 rats after 65 weeks, as well as a significant increase after TMP administration (50 ppm), relative to controls. The CPN score was increased in male rats exposed to unleaded gasoline at 300 ppm or TMP at 50 ppm, relative to controls ([Short et al., 1989a](#)). In the 24-week model, significant increases were also observed for atypical cell foci (linear trend with increasing unleaded gasoline dose) in rats treated with TMP, and nephropathy in rats treated with TMP alone or with EHEN followed by TMP. However, there were no effects in female rats. [The Working Group noted that in this study, both unleaded gasoline and TMP were used and had similar effects, not only on nephropathy, but also on atypical cell foci and renal tubular cell tumours. This suggests that TMP might be responsible, at least in part, for the tumour-promoting effects of unleaded gasoline.]

## Synopsis

[The Working Group noted that there was evidence pointing to an induction of cell proliferation in the livers of female mice and the kidneys of male rats after exposure to unleaded gasoline, independent of the route and duration of exposure. In male rats, kidney damage and the induction of cell proliferation seemed to involve the formation of protein droplets, which consisted of  $\alpha_{2u}$ -globulin.]

## 4.3 Other relevant evidence

### *Effects related to $\alpha_{2u}$ -globulin in the kidneys of male rats*

Several studies investigated  $\alpha_{2u}$ -globulin nephropathy that specifically develops in male rats and its role in renal tumorigenesis.

Male rats were treated with unleaded gasoline for 9 days (0.04–2.0 mL/kg bw, orally). At the high dose-levels, the rats showed an increase in number and size of hyaline droplets in the epithelial cells of the renal proximal convoluted tubules (PCT) and enhanced cellular exfoliation. No other treatment-related effects were observed in the glomeruli, distal tubules, or medulla. The renal content of  $\alpha_{2u}$ -globulin was 4.4-fold higher after the 1.0 mL/kg dose, with no further increase at the highest doses. Immunoperoxidase staining of kidney tissue sections for  $\alpha_{2u}$ -globulin revealed large accumulations of antigen localized in many of the PCT epithelial cells, which contained hyaline droplets. However, the hepatic content of  $\alpha_{2u}$ -globulin and its mRNA expression were not altered by gasoline administration ([Olson et al., 1987](#)). [Olson et al. \(1988\)](#) showed a marked increase in both the number and size of hyaline droplets (phagolysosomes) in epithelial cells of the PCTs, and these structures accumulated  $\alpha_{2u}$ -globulin specifically. Electron microscopy confirmed that the gasoline-induced disruption of renal lysosomal proteolysis led to

protein accumulation, including of  $\alpha_{2u}$ -globulin, in phagolysosomes.

In male rats given a daily oral dose of unleaded gasoline at 2 mL/kg, renal  $\alpha_{2u}$ -globulin deposition increased to 210% of the baseline level 18 hours after the first dose and to 320% of baseline level after three daily doses. The increase in  $\alpha_{2u}$ -globulin was accompanied by a proliferation of hyaline droplets. Within 3 days of terminating the exposure, the  $\alpha_{2u}$ -globulin level returned to baseline, whereas the number of renal hyaline droplets decreased more slowly. The administration of E2 (1 mg/kg subcutaneously for 4 days, starting on day 9 of gasoline treatment) during the post-exposure recovery period resulted in a dramatic reduction in the  $\alpha_{2u}$ -globulin level, compared with rats that did not undergo hormone treatment. The E2 treatment also appeared to increase the removal of the accumulated hyaline droplets from the renal cortex. The authors concluded that the accumulation of  $\alpha_{2u}$ -globulin-containing hyaline droplets was rapidly reversible, and that it depended on the continuous exposure to gasoline and the maintenance of a normal rate of hepatic  $\alpha_{2u}$ -globulin synthesis ([Garg et al., 1988](#)).

In another study, F344 rats were exposed to 10, 70, or 300 ppm unleaded gasoline or 50 ppm TMP for 3–50 weeks. The labelling index was measured by continuous labelling with tritiated thymidine during the last week of exposure. A dose-related, up to 11-fold, increase in cell turnover in the P2 segment of the proximal tubule was identified in the exposed male rats, an effect that persisted during chronic exposure. This proliferative response closely paralleled the extent and severity of immunohistochemically detectable  $\alpha_{2u}$ -globulin deposition in the P2 segment. Neither  $\alpha_{2u}$ -globulin deposition nor cytotoxicity was evident in the P1 or P3 segments, although the rate of cell proliferation was increased by up to 8-fold in the P3 segment compared with the P1 segment. An increase in the number of proximal tubules affected by CPN

was found in males exposed to gasoline or TMP for 22 or 48 weeks, compared with controls, and these lesions contained epithelial cells that were highly proliferative.  $\alpha_{2u}$ -Globulin nephropathy and increases in cell turnover were not seen in untreated male or treated female rats, and the occurrence of CPN was much lower than that in treated male rats ([Short et al., 1989b](#)).

[Garg et al. \(1989\)](#) used transmission electron microscopy to study the renal effects of oral administration of unleaded gasoline in male rats treated by gavage, once daily, for 9 days. Treatment with gasoline at 0.4–2.0 mL/kg increased the number and size of phagolysosomes in the epithelial cells of renal PCTs, whereas doses of  $\leq 0.4$  mL/kg had no effect. Subcellular analysis revealed that many of the phagolysosomes observed in rats exposed to doses of  $\geq 0.4$  mL/kg were angular, with diameters of 0.5–9  $\mu\text{m}$ , whereas in control rats, the majority of phagolysosomes were round and had diameters of 0.5–2.5  $\mu\text{m}$ . At the highest dose (2.0 mL/kg), a progressive increase in the number and size of the phagolysosomes in PCT epithelial cells occurred over the 9 days of treatment. Alterations in the number and morphology of phagolysosomes occurred within 20 hours of a single dose. Many of the enlarged phagolysosomes contained a condensed, crystalline core of greater electron density than the surrounding matrix. The authors suggested that the rapid increase in abnormal, condensed contents of the phagolysosomes indicated that a disruption of renal protein catabolism is the primary mechanism of the gasoline-induced hyaline droplet nephropathy in male rats ([Garg et al., 1989](#)).

The renal effects of unleaded gasoline and TMP were studied in male F344 rats exposed to unleaded gasoline at 2–2000 ppm by inhalation or to TMP at 0.2–50 mg/kg by oral gavage for 3 weeks. Tritiated thymidine was given by osmotic pumps during the third week of these treatments, and cell turnover was assessed by autoradiography after the exposure. In control



rats, the P2 segment of the proximal tubule had a higher rate of cell turnover than the P1 or P3 segments. In rats exposed to gasoline or TMP, the P2 segment showed additional dose-related increases in cell turnover, up to six times the control level. The extent and localization of cell proliferation closely paralleled the extent and severity of the accumulation of crystalloid protein droplets and necrosis (by single-cell analysis) ([Short et al., 1987](#)).

[Borghoff et al. \(1992\)](#) dosed male F344 rats with EHT gasoline (50–500 mg/kg), PS-6 unleaded gasoline (16–500 mg/kg), or TMP (0.95–30 mg/kg) by oral gavage for 10 days. Twenty-four hours after the final dose, the protein droplet accumulation,  $\alpha_{2u}$ -globulin concentration, and [ $^3\text{H}$ ]thymidine nuclear labelling index (an index of cell proliferation) were measured in the kidneys of control and treated rats. Dose-related increases in protein droplets,  $\alpha_{2u}$ -globulin concentration, and cell proliferation were detected in the kidneys of rats treated with any of the three agents, compared with untreated rats. The accumulation of protein droplets and the increase in  $\alpha_{2u}$ -globulin were greater in the rats treated with unleaded gasoline and with TMP than in those treated with EHT. The authors attributed these differences to the higher content of branched hydrocarbons in the unleaded gasoline. However, the level of renal cell proliferation was similar in the three treated groups.

[Clark et al. \(2014\)](#) exposed Sprague-Dawley rats for 6 hours per day on 5 days per week for 13 weeks, via inhalation to a vapour condensate of either gasoline alone or gasoline combined with various oxygenates, including TBA, with target concentrations of 0, 2000, 10 000, or 20 000 mg/m<sup>3</sup>. Increased kidney weight and nephropathy were observed in male rats in all the treated groups, and these were reversible, at least to some extent, after 4 weeks of no exposure. The no-observed-adverse-effect concentration (NOAEC) for gasoline was 2000 mg/m<sup>3</sup> ([Clark et al., 2014](#)).

[Charbonneau et al. \(1989\)](#), exposed F344 rats via gavage to repeated doses of unleaded gasoline metabolite, or TMP. Protein droplet formation and cell proliferation were measured in the kidney, histologically and biochemically. The rats were divided into exposure groups that received various doses of gasoline or TMP and a control group for comparison. The male rats exhibited increased renal protein droplet formation and cell proliferation, which was localized to the P2 segment of the nephron, whereas female rats showed no such effects.

Regarding non-neoplastic lesions, [Benson et al. \(2011\)](#) found in a study of gasoline (see Section 3) that chronic progressive nephropathy occurred in nearly all male and 50–60% of female F344 rats, including in the control groups. The unleaded gasoline vapour-exposed groups showed slightly more severe nephropathy than did the controls (see Section 3) ([Benson et al., 2011](#)). [The Working Group noted that other non-neoplastic lesions of the kidney, such as protein droplet accumulation, or identification of the presence of  $\alpha_{2u}$ -globulin, were not reported in this publication. In addition, histopathology was not evaluated or reported at early time points, which would have permitted assessment of the incidence of chronic progressive nephropathy, to give information on potential mechanisms.]

Similarly, in the study of carcinogenicity of gasoline plus MTBE (see Section 3), chronic progressive nephropathy was observed in nearly all the males and 80% of the females in all the groups ([Benson et al., 2011](#)). [The Working Group noted that the presence of nephropathy in the control group suggested that this was not related to the renal carcinogenicity of the tested agents.]

In a chronic inhalation study, mice and rats were exposed to gasoline vapour at 67, 292, or 2056 ppm for 6 hours per day on 5 days per week for 103–113 weeks. One female rat from the intermediate-dose group (292 ppm) was diagnosed with a renal sarcoma. Regarding preneoplastic lesions, exposure-related histopathological

changes were observed in the kidneys of the male rats at the 3-month interim time point ([MacFarland et al., 1984](#)). [The Working Group noted that the diagnostic terminology used in the histopathological component of this study differed slightly from the standard terminology used presently for kidney lesions, and particularly terminology that is relevant for  $\alpha_{2u}$ -globulin-mediated nephropathy, although the lesions were analogous to those used for mechanistic consideration.] The lesions observed were described as proteinaceous casts and chronic interstitial inflammation. Protein casts occurred within dilated renal tubules and were commonly located at the corticomedullary junction. After 3 and 6 months of exposure, male rats in the group at 2056 ppm also showed mineralization within the renal pelvis. Progressive glomerulonephrosis was initially observed in males at 2056 ppm at the 12-month interim time point. The lesions were described as atrophied or sclerosed glomeruli, dilated renal tubules containing proteinaceous casts, tubular damage with regeneration or scarring, and the presence of foci of chronic inflammatory cells. At the terminal time point (103–113 weeks), progressive glomerulonephrosis was observed in 95–100% of the males in all the groups. [The Working Group noted that the severity of this lesion was not assessed.] Gasoline vapour concentration-dependent mineralization was observed, with 5%, 63%, and 91% of male rats being affected in the groups at 67, 292, and 2056 ppm, respectively.

Several studies investigated if the effects on  $\alpha_{2u}$ -globulin concentration were associated with TMP. [Stonard et al. \(1986\)](#) studied renal hyaline droplet formation and  $\alpha_{2u}$ -globulin kinetics in Alderley Park Wistar rats exposed to TMP once at up to 12 mmol/kg by oral gavage in olive oil. Hyaline droplets were markedly increased in adult male, but not in female or pre-pubertal male, rats 24–48 hours after a single oral dose of 12 or 24 mmol/kg. The  $\alpha_{2u}$ -globulin concentration was measured by ELISA using an antiserum

raised in New Zealand White rabbits given  $\alpha_{2u}$ -globulin purified from rat urine. A dose-dependent increase in renal  $\alpha_{2u}$ -globulin content in the male rats was observed 24 hours after exposure to doses of 0.3–12.0 mmol/kg. The renal  $\alpha_{2u}$ -globulin content peaked 48 hours after exposure to the highest dose and returned to baseline after 7 days. Immunocytochemical staining for  $\alpha_{2u}$ -globulin showed that in untreated male rats the protein was localized primarily to the S2 segment of the renal proximal tubule, whereas rats that received TMP at 12 mmol/kg also showed greater staining intensity in adjacent segments of the renal cortex. The authors concluded that there was a strong association between the presence of renal hyaline droplets and that of renal  $\alpha_{2u}$ -globulin ([Stonard et al., 1986](#)).

F344 rats were exposed to a single oral dose of [ $^{14}$ C]TMP (4.4 mmol/kg). The level of radioactivity was determined in the kidney, liver, and plasma after 4, 8, 12, 24, and 48 hours of exposure. The maximum level of radioactivity in the three tissues was measured at the 12- and 8-hour time points in the males and females, respectively, and selective retention of the radiolabel occurred in the kidneys of the male rats ([Charbonneau et al., 1987](#)). The radiolabel concentration in the kidneys increased in a non-linear, dose-dependent manner, in that the kidney:plasma ratio was higher after low doses than after higher doses. This greater renal retention of radiolabel was accompanied by higher concentrations of  $\alpha_{2u}$ -globulin 24 and 48 hours after the TMP exposure. The total amount of radioactivity recovered in urine samples 48 hours after dosing was similar in the males and females (32% and 31% of the dose). The identification and quantitation of the urinary metabolites of TMP showed that the male and female rats metabolized TMP via the same pathway and at a similar rate. 2,4,4-Trimethyl-2-pentanol was the principal metabolite present in the kidneys of the male rats but was absent from the kidneys of the females. However, the females excreted more

2,4,4-trimethyl-2-pentanol conjugates in the urine. (Charbonneau et al., 1987). [The Working Group noted that these data supported the formation and accumulation of a 2,4,4-trimethyl-2-pentanol- $\alpha_{2u}$ -globulin complex in male rats but did not provide information on the stability or reversibility of the complex.]

Loury et al. (1987b) performed in vitro and in vivo experiments to study the ability of TMP and its metabolites to covalently bind to  $\alpha_{2u}$ -globulin. Protein analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that hepatocytes isolated from male F344 rats produced significant quantities of  $\alpha_{2u}$ -globulin, whereas hepatocytes from female rats did not. After 15 hours of exposure of metabolically competent, primary cultures of male rat hepatocytes to  $^{14}\text{C}$ -TMP (0.1 or 0.5%, v/v), there was no evidence of radiolabelled  $\alpha_{2u}$ -globulin accumulation by SDS-PAGE. Similarly, no TMP-derived radioactivity was identified in SDS-PAGE fractions containing  $\alpha_{2u}$ -globulin from the liver, blood, renal cortex, or urine from male F344 rats dosed with  $^{14}\text{C}$ -TMP (300 mg/kg by intragastric intubation). According to the authors, the absence of a detectable covalent interaction between TMP and  $\alpha_{2u}$ -globulin in either the in vitro or in vivo experiments suggested that a TMP- $\alpha_{2u}$ -globulin adduct was not responsible for the formation of protein droplets in male rats (Loury et al., 1987b).

The effects of daily administration of TMP (12 mmol/kg for 10 days) on hepatic and renal microsomal mono-oxygenase activity, peroxisomal  $\beta$ -oxidation, and the concentration of  $\alpha_{2u}$ -globulin has also been studied in male and female Alderley Park rats. TMP caused enlargement of the liver and, to a lesser extent, the kidney; the induction of CYP-mediated omega-oxidation and the peroxisomal  $\beta$ -oxidation of fatty acids; the proliferation of peroxisomes; and an increase in the  $\alpha_{2u}$ -globulin level in the kidney (the last only in male rats). The male rats showed more

marked responses than did the female rats (Lock et al., 1987a).

In a separate study, F344 rats were exposed to  $[^3\text{H}]$ TMP at 4.4 mmol/kg by oral gavage, then blood, liver, and kidney samples were collected at 8, 24, 48, and 72 hours of exposure, and fractionated by ultracentrifugation. After exposure for 24 hours, approximately 60% of the radiolabel was localized to the  $116\,000 \times g$  supernatant. Column chromatography of the supernatant showed that approximately 26% of the radiolabel co-eluted with  $\alpha_{2u}$ -globulin and cross-reacted with an antibody specific for this protein. The remaining portion was eluted in the low-molecular-weight range ( $< 1000$  Da) and was assumed to be TMP. Chromatography of the male rat urine also separated two components, and approximately 0.1% of the radiolabel co-eluted with  $\alpha_{2u}$ -globulin. A single component in the low-molecular-weight range was eluted from samples of plasma, male and female rat liver, and female rat kidney. According to the authors, the results suggested that the high-molecular-weight radioactive material was formed by the reversible binding of a radioactive component of TMP to a male rat-specific protein. GC-MS analysis of an ethyl acetate extract of the  $\alpha_{2u}$ -globulin-containing fraction of the TMP-treated male rat kidney  $116\,000 \times g$  supernatant identified 2,4,4-trimethyl-2-pentanol as the only metabolite that was bound to  $\alpha_{2u}$ -globulin (Lock et al., 1987b).

The authors tested for  $\alpha_{2u}$ -globulin in vitro using the pH 6.7 Syrian hamster embryo cell transformation assay. The  $\alpha_{2u}$ -globulin caused morphological transformation, whereas bovine serum albumin, *d*-limonene, and TMP did not. According to the authors, these results supported the hypothesis that an increase in the  $\alpha_{2u}$ -globulin content of PCTs may directly cause renal tumorigenesis in male rats (Oshiro et al., 1998).

[Overall, the Working Group considered the evidence supporting an association between renal tumours in male rats and the accumulation

of  $\alpha_{2u}$ -globulin to be compelling, supporting the involvement of a species-specific mechanism. However, for renal tubule tumours, an IARC Scientific Publication has presented seven criteria that need to be met in order to conclude that these specific tumours arising in male rats are only due to a mechanism that is indeed specific to male rats ([IARC, 1999](#)).

The Working Group concluded that not all seven criteria were met. In fact, in light of the most recent scientific literature, the Working Group noted that additional carcinogenic mechanisms ([IARC, 2019](#)) may operate alongside the male rat-specific  $\alpha_{2u}$ -globulin mechanism. In particular, based on new evidence for the KCs, the Working Group noted that exposure to automotive gasoline-induced oxidative stress (KC5), and chronic inflammation (KC6) in experimental animals. Furthermore, the Working Group noted that automotive gasoline is genotoxic (KC2), and induces oxidative stress (KC5) and chronic inflammation (KC6) in exposed humans (see Sections 4.2.2, 4.2.5, and 4.2.6). To conclude, the available evidence does not provide strong support for concluding that the mechanism(s) underlying automotive gasoline-induced kidney carcinogenicity in male rats are solely attributable to an accumulation of the male rat-specific  $\alpha_{2u}$ -globulin.]

## 5. Summary of Data Reported

### 5.1 Exposure characterization

“Automotive gasoline” is a generic term used to describe a commercial product that is a complex mixture of volatile hydrocarbons and is primarily used in internal combustion engines, mainly in passenger cars and other types of vehicle. The typical components of gasoline are volatile hydrocarbons derived from petroleum, including alkanes, alkenes and aromatics (primarily C5 to C10), which are blended with

various additives. The composition of gasoline has changed over time and is currently strictly regulated. Lead compounds, formerly present in virtually all gasoline formulations, have now been banned in all countries. The concentration of benzene is restricted to below 1% in most countries. To improve anti-knocking properties, oxygenated additives such as ethanol, methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), and others are added, but maximum levels vary worldwide.

Because of their high vapour pressure, most gasoline components are readily vapourized and are common air pollutants, with higher levels found near gasoline service stations.

In most settings, the main exposure route is expected to be inhalation of vapour, but skin absorption also occurs after direct contact with liquid gasoline. Exposure to gasoline has been assessed by measuring some components, mainly benzene, in air samples and in urine; biomarkers that are specific to benzene include urinary benzene and S-phenylmercapturic acid. These measurements are meaningful only in settings where gasoline exposure is expected.

Occupational exposure is expected mainly during the production and transport of gasoline and during the refuelling of cars, which includes occupations such as refinery workers, tanker drivers, service station attendants, and mechanics. Exposures to gasoline vapour components, including benzene and others, are significantly higher in service station attendants than in the general population. Exposures have declined significantly since the introduction of vapour recovery systems; however, these systems are not available everywhere. Many workers who are exposed to gasoline are also co-exposed to other agents, the most common of these being engine exhaust.

Exposure to gasoline in the general population can occur via evaporation during car refuelling; evaporative emissions from cars, especially if parked in a garage attached to home; emissions

from gasoline service stations for those living in their proximity; use of devices fuelled with gasoline (e.g. lawn mowers); and use of gasoline for degreasing and cleaning.

Occupational exposure limits have been established in many countries for gasoline and components of gasoline such as benzene and MTBE.

## 5.2 Cancer in humans

Since the previous evaluation of automotive gasoline in *IARC Monographs* Volume 45, numerous epidemiological studies have been conducted that provide information on cancer in relation to gasoline exposure. The most informative studies for most cancer types were case-control studies on occupational exposures to gasoline, as determined by use of population-level job-exposure matrices. Also considered to be informative were studies on cancer incidence or mortality conducted among occupationally exposed populations, including studies of service station attendants conducted in Canada, the United States of America (USA), and several European countries, and studies of gasoline distribution workers in Canada, the United Kingdom (UK), USA, and Australia. There was a single study of a population exposed to contaminated groundwater from a major gasoline spill in Pennsylvania, USA. For childhood cancers, several informative studies were conducted on prenatal or postnatal exposure via either parental occupational exposure or residential proximity to service stations.

There were more than 100 available studies covering a wide range of cancer types, including childhood cancers and, in adults, haematolymphoid neoplasms and cancers of the urinary tract, digestive tract, respiratory system, breast, and nervous system. The informativeness of these studies was evaluated on the basis of the exposure assessment methodology and the potential for bias and control for confounding, which were

crucial in determining the weight given to each study when interpreting the evidence.

Regarding exposure assessment quality, cohort studies defined by some occupations (distribution workers and service station attendants) were considered to provide a suitable proxy for gasoline exposure. Other population-based studies were able to assess exposure using job-exposure matrices or evaluation by industrial hygienists on the basis of details of work tasks obtained by questionnaire or interviews. Cohort studies in which exposure was defined by occupation were conducted either through census or employers' records; these studies were limited by the lack of full job history, quantitative measures of exposure, and information on co-exposures. Case-control studies that used questionnaires were able to collect job or exposure information covering a longer time period; these studies all had potential misclassification error from self-reports and average exposure on the basis of job codes. Studies on exposure to gasoline in residential areas relied on measures of geographical distance from the source of gasoline as surrogate measures of exposure.

For the cohort studies in which exposure information was obtained before cancer occurrence, or in retrospective studies in which exposure assessment was blind to case status, any exposure error would be likely to be nondifferential with respect to cancer outcome and lead to bias towards the null. In questionnaire-based case-control studies, differential exposure misclassification could bias the result either towards or away from the null, depending on the nature of the misclassification error. Steps were taken in some studies to reduce or check for potential differential exposure misclassification.

Studies that provided only ever-versus-never measures of exposure to gasoline lacked the ability to examine associations by level of exposure. Studies with only qualitative measures of gasoline exposure were deemed less informative, because associations with cancer could be



biased downwards owing to the crude exposure categorization in which workers were grouped into a single category. Estimates of cumulative exposure over time were deemed more informative than estimates based on a single time point. In the more informative studies, a cumulative measure of gasoline exposure combined the number of years in a job with the estimated intensity of exposure to gasoline in that job.

Potential confounding from socioeconomic status, lifestyle-related factors, or co-exposures at the workplace depended on the cancer under consideration. Whereas the case-control studies typically used questionnaire information on the major potential lifestyle-related confounders for the cancer of interest, retrospective occupational cohorts and record-linkage studies generally lacked information on non-occupational risk factors. Smoking can confound the association between an occupational factor and lung cancer but would be expected to have no effect in studies in which smoking is not associated with the cancer under study. For certain smoking-related cancers (e.g. bladder, kidney), in the absence of data on smoking in a given occupational group, the Working Group considered the lack of an elevated rate of lung cancer to provide convincing evidence against major confounding by tobacco smoking for that cancer type, because the magnitude of the association of smoking is much greater for lung cancer than for other cancer types.

The Working Group considered confounding by occupational exposures other than gasoline to be potentially more important and therefore restricted its review to studies of cancer risk in occupations in which exposure to gasoline was predominant and co-exposures to carcinogens were considered less likely. For example, the available literature suggested that the intensity of exposure to diesel and gasoline engine exhaust in service station workers is similar to that of ambient environmental levels. The degree of confounding by co-exposures varied by exposed

population and cancer type, as discussed within the relevant sections below.

Concerns about selection bias were present for both cohort and case-control studies. The occupational cohort studies showed evidence of a downward bias from the healthy-worker effect (HWE) when mortality or morbidity rates were compared with those of the general population. To avoid bias from the HWE, internal analyses were used in some of the retrospective cohort studies. However, a downward healthy-worker survivor bias may still occur in this situation if there is exposure-related dropout from the workforce among a susceptible population. This was a particular concern in some of the studies in gasoline distribution workers in which a minimum duration of employment of 1 year was required.

Live birth bias downwards was a concern in studies on occupational exposure during pregnancy and cancer risk in the offspring. If gasoline induces spontaneous abortion, it is possible that associations between gasoline and childhood cancer would be underestimated, especially in studies on maternal occupational exposures during pregnancy.

Case-control studies are also prone to selection biases, which may occur when the cases and controls are not representative of the same target population, for example, when the cases are selected from a specific hospital. Selection bias can also occur if the participation of cases and controls is low and if the reasons for non-participation differ between these groups. Some studies adjusted for socioeconomic status, reducing concern about this bias. In cancer studies, people with the cancer of interest who die before the data are collected may not be captured in the study sample, introducing a selection bias. To overcome this problem, some investigators collected information from the relatives of deceased patients, but this can lead to recall bias if the quality of the information collected differs from that of the controls. The participation rate and the proportion of next-of-kin interviews were considered by



the Working Group when assessing the quality of the studies.

The Working Group reviewed eight cohort and nine case-control studies on the association between automotive gasoline exposure and urinary bladder cancer. Exposure to gasoline was either inferred from employment as a petroleum distribution worker or service station attendant, self-reported in a questionnaire, assessed using a job-exposure matrix, or based on expert judgement using the description of the jobs performed by the study subjects. The Working Group conducted a meta-analysis of these studies and found that the pooled risk estimate for occupational exposure to automotive gasoline was 1.25 (95% confidence interval, CI, 1.08–1.44), which increased to 1.31 (95% CI, 1.14–1.51) for studies using incidence data as the end-point. The findings were considered to be largely consistent across studies with different potentials for confounding from exposure to other bladder carcinogens, e.g. distribution workers, service station attendants, and studies with measures of exposure to gasoline. The Working Group also considered the potential for confounding by tobacco smoking or co-exposure to diesel engine exhaust but considered that confounding was unlikely to explain the overall association because of a lack of an association seen in studies on lung cancer. The Working Group also concluded that chance and bias could be excluded with reasonable confidence.

For acute myeloid leukaemia (AML) in adults, five studies were available to the Working Group; these included large occupational cohorts of service station attendants and petroleum distribution workers, a cohort of an environmentally exposed population, and one case-control study on mainly occupational exposures. There was a strong consistency across the five evaluated studies, all finding a positive association, with a meta-estimate of 1.51 (95% CI, 1.06–2.16). In two of these studies, duration of employment was analysed and provided some evidence of

an exposure-response relation. In an additional study nested within a cohort of petroleum workers, some additional evidence was found for an exposure-response association. Smoking and socioeconomic status were adjusted for in the case-control study, showing a limited impact of potential confounding from these two factors. The results in the occupational cohort studies were possibly biased downwards by exposure misclassification and an HWE, but their findings still pointed towards a positive association. The results for AML were further supported by four of five studies on myelodysplastic syndrome (MDS), a haematopoietic stem cell neoplasm that often progresses to AML and may share common risk factors. The evidence for MDS was considered to support the evidence for AML but to be weaker for MDS itself. The Working Group concluded that a credible positive association between gasoline exposure and AML had been observed and that the role of chance, bias and confounding could be ruled out with reasonable confidence. However, for MDS, the Working Group concluded that although a positive association was credible, the role of chance, bias and confounding could not be ruled out with reasonable confidence.

Several occupational cohort studies, including one cohort study in the general population, one residential cohort study in an environmentally exposed population, and six case-control studies contributed to the evaluation for non-Hodgkin lymphoma (NHL). All except three of these studies had incidence data and were pooled in a meta-analysis showing a meta-estimate of 1.12 (95% CI, 0.99–1.26). The two most informative case-control studies showed a positive exposure-response relation; however, there were concerns about selection bias. Other case-control studies were considered to be less informative owing to the self-reported exposure assessment, with the possibility of both upward recall bias and exposure misclassification bias towards the null. In some studies, results were also reported for

chronic lymphocytic leukaemia (CLL), a subtype of NHL, and showed an overall positive association, consistent with the results for NHL. In summary, a causal interpretation of the positive association between gasoline exposure and NHL risk was supported by the consistent results, especially in the two most informative case-control studies. However, selection bias was a concern in these two studies, and also the role of chance could not be ruled out.

In eight studies, including four occupational cohorts, two cohorts in the general population, and two case-control studies, the authors examined exposure to gasoline according to occupation, self-report, or exposure matrices and the incidence or mortality of multiple myeloma. Selection bias was a concern for the case-control studies. Two studies considered to be the most informative by the Working Group included a register-based cohort study and a case-control study using expert review of exposure and adjustment for smoking. Both of these more informative studies found an increased incidence of multiple myeloma in individuals exposed to gasoline, with some evidence indicating an exposure-response relation, though the estimates were imprecise. However, not all studies identified a positive association, including both studies of service station attendants. In a meta-analysis carried out by the Working Group, excluding mortality studies, a modest, imprecise association was identified between gasoline and multiple myeloma (meta-estimate, 1.16; 95% CI, 0.88–1.54). The Working Group concluded that a causal interpretation of a positive association between gasoline exposure and multiple myeloma was credible considering the results of the most informative studies, but that chance and bias could not be ruled out with reasonable confidence. Specifically, the number of available studies was not large, the pooled positive association was weak, the occupational cohort studies did not find an association, and a role for

selection bias could not be ruled out in the case-control studies.

For childhood acute lymphoblastic leukaemia (ALL), studies showed positive associations for maternal pregnancy exposures; however, the number of studies was modest. Concerns remained regarding selection and recall biases in some studies, and the conflicting results of analyses of self-reported exposures in comparison with analyses reliant on industrial hygienist review. More consistent were the results for postnatal exposures occurring because of residential proximity to gasoline stations (meta-odds ratio, 1.82; 95% CI, 1.23–2.69). Two of the most informative studies supported a relation between postnatal residential exposure to gasoline stations and risk of ALL, and the most informative study on maternal occupational pregnancy exposures found an association with exposure to maternal leaded gasoline, but no association with maternal unleaded gasoline. Overall, the body of evidence suggested that an association with ALL was credible, but that chance and bias could not be eliminated as explanations.

Studies on kidney cancer reviewed in the present monograph included occupational cohorts defined by occupation or industry (including petroleum distribution workers, service station attendants, and tanker drivers in the refinery industry), a nationwide cohort of active workers, as well as case-control studies in the general population. Findings from cohort studies were inconsistent, showed no clear association, and there was an absence of information regarding an exposure-response trend. Exposure assessment was also crude. Among case-control studies, although odds ratios were generally above unity (meta-estimate, 1.19; 95% CI, 0.95–1.49), particularly for those using expert-based exposure assessment, limitations included the small sample sizes and numbers of exposed cases, low participation rates, and potential recall and selection bias. Overall, although the positive association between exposure to gasoline and

kidney cancer observed in some studies was credible, chance, bias, and/or confounding could not be ruled out with reasonable confidence.

Information about a possible association between exposure to automotive gasoline and stomach cancer derived from seven cohort studies, including one study of residents in a groundwater-contaminated area, and one case-control study. The meta-analysis conducted by the Working Group suggested an elevated risk for stomach cancer incidence with exposure to automotive gasoline (meta-estimate, 1.26; 95% CI, 0.99–1.60), and this was demonstrated for duration and concentration in one of the two studies that allowed assessment of an exposure-response relation. Triangulation with the lung cancer findings reported in the present monograph and a published meta-analysis on the association between asbestos exposure and stomach cancer offered reassurance about the magnitude of confounding by smoking and other co-exposures. The HWE was present in the cohort studies, in particular among the distribution workers, and estimates were likely to be biased downwards. Overall, the Working Group considered that the body of evidence supported a credible positive association between exposure to automotive gasoline and cancer of the stomach. However, because of conflicting results in the two most informative studies, the small number of exposed cases, and the crude exposure assessment for most studies, the Working Group concluded that chance, bias, and confounding could not be ruled out with reasonable confidence.

There were 11 studies on lung cancer in workers involved in gasoline distribution or service station attendants, of gasoline spills, or estimated gasoline exposure. The findings were inconsistent between studies and the meta-analysis conducted by the Working Group indicated a meta-estimate of 1.04 (95% CI, 0.89–1.22). Overall, the Working Group found little evidence that the risk of lung cancer is positively associated with exposure to automotive gasoline.

For other cancer types, including of the brain and nervous system, neuroblastoma, hepatoblastoma, Wilms tumour, oral cavity, nasal cavity, pleura, larynx, pharynx, breast (male), breast (female), oesophagus, colon, rectum, pancreas, thyroid gland, liver, bile duct or gallbladder, cervix, ovary, prostate gland, germ cell tumour, testis, and skin (melanoma), the Working Group concluded that the findings were either too inconsistent or too close to the null to permit a conclusion to be reached on the presence or absence of a causal association.

### 5.3 Cancer in experimental animals

Exposure to gasoline caused an increase in the incidence of malignant neoplasms in both sexes of two species (mouse and rat) in multiple studies, including one that complied with Good Laboratory Practice (GLP).

In a well-conducted study that complied with GLP in male and female Fischer 344 (CDF F344 CrIbR) rats treated by inhalation (whole-body), gasoline caused an increase in the incidence of adenoma or carcinoma (combined) of renal tubules and squamous cell carcinomas of the nasal cavity in males.

In a study in male and female Sprague-Dawley rats treated by oral administration (gavage), gasoline caused an increase in the incidence of total malignant tumours of the nasal cavity in males.

In a well-conducted study in male and female B6C3F<sub>1</sub> mice treated by inhalation (whole-body), gasoline caused an increase in the incidence of hepatocellular adenoma or carcinoma (combined) in females.

In a well-conducted study in male and female Fischer 344 rats treated by inhalation (whole-body), gasoline caused an increase in the number of renal carcinomas and the total number of renal carcinomas, adenomas, or sarcomas (combined) in males.

In a well-conducted study in male and female Sprague-Dawley rats treated by oral administration (gavage), gasoline caused an increase in the incidence of total malignant tumours of the uterus-vagina in females.

## 5.4 Mechanistic evidence

Regarding the absorption, distribution, metabolism, and excretion of automotive gasoline itself (a complex mixture), no studies were available in exposed humans. However, analyses on the individual components of gasoline, such as alkanes, benzene, toluene, and xylenes, indicated that these are absorbed via inhalation and the skin, redistributed to organs, metabolized, and excreted in the urine. There were some models in rats suggesting that exposure to automotive gasoline vapour decreases the metabolism of benzene and increases the metabolism of MTBE. Competitive inhibition may affect the metabolism of individual components during exposure. Overall, the components of gasoline are absorbed and distributed in the body at varying rates of metabolism and excretion.

The Working Group evaluated numerous studies on the mechanistic evidence of carcinogenicity related to occupational exposure among service station attendants, who are regularly exposed to both gasoline vapour and engine exhaust emissions. For most of these studies, the exposure was to unleaded gasoline, on the basis of the date and region where most of the studies were conducted and from information provided in the studies. The evaluation was based on the knowledge that the components of gasoline vapour represent the primary source of exposure in this work environment. In addition, the Working Group identified several studies in which service station attendants were compared with other occupations exposed to engine exhaust (such as traffic officers). For the studies in experimental systems, the Working Group

identified studies involving exposure to leaded and unleaded gasoline.

There is consistent and coherent evidence that automotive gasoline exhibits key characteristics of carcinogens.

Automotive gasoline is genotoxic. In exposed humans, several well-conducted studies have provided consistent and coherent evidence of genotoxicity using a range of relevant end-points. Significant increases in the frequency of micronucleus formation in peripheral blood lymphocytes and buccal exfoliated cells were observed in service station attendants, with correlations between these effects and exposure duration. Some studies showing elevated levels of single- and double-strand DNA breaks and reduced DNA repair capacity in exposed humans gave additional support to the evidence of genotoxicity of automotive gasoline. Notably, increased DNA damage, including micronucleus formation, was observed, with evidence of oxidative stress. The consistency of these findings across different populations and regions, including Brazil, China, India, Mexico, and Thailand, strengthens the evidence linking genotoxicity and exposure to automotive gasoline. In many of these studies, the authors controlled for confounders such as age, smoking, and alcohol consumption, and used a combination of exposure assessment methods, including personal air monitoring, biological monitoring, and task-based exposure assessment. There was a paucity of data in human primary cells, and the database in experimental systems was inconsistent.

Automotive gasoline induces oxidative stress. In exposed humans, several well-conducted studies showed consistent and coherent alterations in oxidative stress end-points. Elevated levels of oxidative DNA damage markers, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), were observed in studies controlling for confounders such as age, smoking, and alcohol consumption, and using a combination of exposure assessment methods. Other studies in service station

attendants showed evidence for a compromised antioxidant defence system, such as alterations in the levels of glutathione, catalase, and superoxide dismutase. Consistent and coherent evidence was also identified in experimental systems in vivo and in vitro. Levels of malondialdehyde and 4-hydroxynonenal were significantly elevated, indicating lipid peroxidation and oxidative damage. Studies in rodents and non-mammalian species revealed that exposure to automotive gasoline caused oxidative stress, associated with inflammatory reactions.

Automotive gasoline induces chronic inflammation. Studies in which inflammatory end-points were investigated in service station attendants revealed an inflammatory profile. Elevated levels of pro-inflammatory cytokines (interleukins IL-1 $\beta$  and IL-6, and tumour necrosis factor alpha, TNF $\alpha$ ) were observed, indicating inflammation. This was accompanied by a reduction in levels of anti-inflammatory cytokines such as IL-10. Studies also reported increased leukocyte counts, including of monocytes, neutrophils, and lymphocytes. In service station attendants, these effects were frequently linked to increased oxidative stress and persistent exposure to automotive gasoline. The evidence is supported by studies in experimental systems in vivo showing increased leukocyte counts and pro-inflammatory cytokine production, particularly in the lungs and liver, after exposure to automotive gasoline. Collectively, these studies show that chronic exposure to automotive gasoline induces a systemic inflammatory response, which correlates with the duration of exposure.

Automotive gasoline alters cell proliferation, cell death, or nutrient supply. There was a paucity of data in exposed humans and in human primary cells. There is consistent and coherent evidence in experimental systems in vivo. In several studies, automotive gasoline induced cell proliferation in the liver of female mice and in the kidney of male rats.

There is suggestive evidence that automotive gasoline induces epigenetic alterations. In four studies in exposed humans, decreased global methylation or decreased methylation of *LINE-1* (long interspersed nuclear element) repetitive elements and specific subfamilies was observed in the peripheral blood of service station attendants from the same country. In some of these studies, the methylation levels of these elements were inversely correlated with those of exposure markers, including air and biological monitoring. Additionally, increased DNA methylation of *p15* and *IL6* as well as increased expression of miR-221 have been reported in service station attendants. There was no evidence available in human primary cells and in experimental systems.

There is suggestive evidence that automotive gasoline is immunosuppressive. In exposed humans, several studies indicated that exposure to automotive gasoline exerts immunosuppressive effects; service station attendants exhibited alterations in T-cell count, including a reduction in CD4<sup>+</sup> T lymphocytes. Localized immune dysfunction in the airways has been reported, characterized by a lower proportion and activation of T lymphocytes in induced sputum. Alterations in the natural-killer cell phenotype have also been documented. Additionally, reductions in some immunoglobulin levels further suggest immunosuppressive responses. These effects in service station attendants were frequently linked to increased oxidative stress and benzene exposure. However, no data on the functional capacity of immune cells were available to clearly indicate immunosuppression in service station attendants. In addition, in experimental systems, the data available did not support the hypothesis that automotive gasoline induces immunosuppression.

There is suggestive evidence of modulation of receptor-mediated effects. In two studies in service station attendants, higher blood levels of thyroxine (T4) and free thyroxine (T4F), and



lower levels of triiodothyronine (T3) and thyroid-stimulating hormone (TSH) were detected. Levels of T3 and TSH inversely correlated with years of exposure. In addition, in other two studies service station attendants exhibited decreased levels of testosterone. Evidence in experimental systems *in vivo* suggested hormonal alterations. Increased serum levels of corticosterone, adrenal catecholamines, follicle-stimulating hormone, luteinizing hormone, and prolactin were observed. Hypothalamic noradrenaline and serum levels of testosterone were decreased.

For the other key characteristics of carcinogens, there was a paucity of data or no data were available.

## 6. Evaluation and Rationale

### 6.1 Cancer in humans

There is *sufficient* evidence in humans for the carcinogenicity of automotive gasoline. Automotive gasoline causes acute myeloid leukaemia and cancer of the urinary bladder in adults. Positive associations have been observed for acute lymphoblastic leukaemia in children, and (in adults) non-Hodgkin lymphoma (including chronic lymphocytic leukaemia), multiple myeloma, myelodysplastic syndrome, and cancers of the stomach and kidney.

### 6.2 Cancer in experimental animals

There is *sufficient* evidence in experimental animals for the carcinogenicity of automotive gasoline.

### 6.3 Mechanistic evidence

There is *strong* mechanistic evidence for the key characteristics of carcinogens in exposed humans and experimental systems.

## 6.4 Overall evaluation

Automotive gasoline is *carcinogenic to humans* (Group 1).

## 6.5 Rationale

The Group 1 evaluation for automotive gasoline is based on *sufficient* evidence for cancer in humans and also on the combination of *sufficient* evidence for cancer in experimental evidence and *strong* mechanistic evidence for key characteristics of carcinogens in exposed humans. There was *sufficient* evidence in humans that gasoline exposure causes acute myeloid leukaemia and bladder cancer in adults. This evidence derived mainly from studies in high-income countries on occupational exposure to gasoline and in workers (e.g. service station attendants) who had high gasoline exposure and for whom there was less concern about carcinogenic co-exposures in the workplace. There was also *limited* evidence in humans for non-Hodgkin lymphoma (including chronic lymphocytic leukaemia), multiple myeloma, myelodysplastic syndrome, and cancers of the stomach and kidney, based largely on the same types of study. There was also *limited* evidence for acute lymphoblastic leukaemia in children, on the basis of studies of parental exposure during pregnancy, exposure during childhood, or close residential proximity to service stations.

The *sufficient* evidence in experimental animals is based on an increase in the incidence of malignant neoplasms and a combination of benign and malignant neoplasms in both sexes of two species (mouse and rat) in multiple studies, including one GLP study. There is *strong* mechanistic evidence, on the basis of consistent and coherent evidence for the key characteristics of carcinogens, that automotive gasoline is genotoxic, induces oxidative stress, and induces chronic inflammation provided by studies in exposed humans, mainly service station attendants. Additionally, in experimental systems,



automotive gasoline induces oxidative stress; induces chronic inflammation; and alters cell proliferation, cell death, or nutrient supply. There is *suggestive* evidence that automotive gasoline induces epigenetic alterations, is immunosuppressive, and modulates receptor-mediated effects.

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# METHYL *TERT*-BUTYL ETHER

## 1. Exposure Characterization

### 1.1 Identification of the agent

#### 1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 1634-04-4 ([NCBI, 2025](#))

EC/List No.: 216-653-1 ([ECHA, 2025a](#))

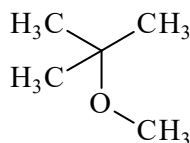
IUPAC systematic name: 2-methoxy-2-methylpropane ([NCBI, 2025](#))

Synonyms and registered trade names: methyl tertiary butyl ether; *tert*-butyl methyl ether; 2-methoxy-2-methylpropane; 2-methyl-2-methoxypropane; MTBE; methyl *t*-butyl ether; methyl 1,1-dimethylethyl ether; Driveron ([ECHA, 2025a](#)).

#### 1.1.2 Structural and molecular information

Molecular formula: C<sub>5</sub>H<sub>12</sub>O ([NCBI, 2025](#))

Molecular structure: ([NCBI, 2025](#))



Relative molecular mass: 88.15 ([NCBI, 2025](#)).

#### 1.1.3 Chemical and physical properties

*Description:* methyl *tert*-butyl ether (MTBE) is a colourless flammable liquid with a distinctive, disagreeable, anaesthetic-like odour ([ATSDR, 2024](#); [NCBI, 2025](#)). The odour threshold in water is 15–680 µg/L ([Prah et al., 1994](#); [Health Canada, 2006](#); [ATSDR, 2023](#))

*Boiling-point:* 55.3 °C at 101.3 kPa ([ECHA, 2025a](#))

*Melting point:* –108.6 °C at 101.3 kPa ([ECHA, 2025a](#))

*Density:* 0.74 g/mL at 20 °C ([ECHA, 2025a](#))

*Vapour pressure:* 33 kPa at 25 °C ([ECHA, 2025a](#))

*Solubility:* 41.85 g/L at 20 °C and pH 7 in water ([ECHA, 2025a](#)); soluble in ethanol and diethyl ether ([NCBI, 2025](#))

*Octanol/water partition coefficient (P):* log  $K_{ow}$  = 1.06 at 20 °C ([ECHA, 2025a](#))

*Organic carbon partition coefficient (P):* log  $K_{oc}$  = 2.13 ± 0.060 ([ATSDR, 2023](#))

*Henry's law constant:* 5.87 × 10<sup>–4</sup> atm m<sup>3</sup>/mol at 25 °C ([ATSDR, 2023](#))

*Autoignition temperature:* 460 °C at 101.3 kPa ([ECHA, 2025a](#))

*Flash point:* –28 °C at 101.3 kPa ([ECHA, 2025a](#))

*Explosive limits:* 1.65–8.4% in air ([ATSDR, 2023](#)).

### 1.1.4 Technical products and impurities

The typical purity of commercial MTBE is 98–99 wt% ([NCBI, 2025](#)). The typical impurities are alcohols (methanol, *tert*-butanol), 0.5–1.5 wt%; hydrocarbons (C5 and C6, diisobutenes), 0.1–1.0 wt%; water, 50–1500 mg/kg; total sulfur (maximum), 10 mg/kg; and residue on evaporation (maximum), 10 mg/kg. For use as an additive to gasoline ([Focus Technology Co., Ltd, 2025](#)) and special applications ([Scholz et al., 1990](#)), MTBE is sold with a purity of 99.95%.

## 1.2 Production and uses

### 1.2.1 Production

The common feedstocks for the manufacture of MTBE are methanol, derived from natural gas, and isobutylene, derived from natural gas or petroleum refining ([Concawe, 2012](#)). [The Working Group noted that few data were publicly available on the composition and production volumes of gasoline and its additives. Because independently verifiable data were lacking, data published by Concawe, the science division of a trade organization, are presented.] The chemical processes used to manufacture MTBE from these feedstocks vary ([NCBI, 2025](#)). In Europe, an advanced biofuel manufactured from agricultural waste, called bio-MTBE, is under development ([LyondellBasell, 2024](#)).

In the 1990s, the United States of America (USA) dominated production capacity and market demand for MTBE ([Di Girolamo et al., 2021](#)). The status of MTBE production at the end of the 20th century is described in *IARC Monographs Volume 73* ([IARC, 1999a](#)). After MTBE ceased being added to gasoline in the USA in 2006, MTBE production in the USA declined, but exports to other countries have

continued ([S&P Global, 2022](#); [US EIA, 2024](#)). Latin American countries, including Mexico and the Bolivarian Republic of Venezuela, import MTBE from the USA ([S&P Global, 2022](#)). Canada imported MTBE in the late 1980s and began production in 1992 ([Health Canada, 2006](#)).

Over time, demand for MTBE has increased worldwide, and many countries have expanded their production capacity to reduce reliance on foreign imports. By 2017, an estimated 270 MTBE-production plants existed throughout the world ([Di Girolamo et al., 2021](#)). The Organisation for Economic Co-operation and Development (OECD) classifies MTBE as a high-production-volume chemical ([OECD, 2024](#)). The global production capacity of MTBE was 25–35 million tonnes in 2018 ([Statista, 2023](#); [BlueQuark, 2024](#)). The global market for MTBE in 2022 was 17.6 million tonnes and was projected to continue to grow ([ChemAnalyst, 2024](#)).

In 2017, most of the world's MTBE-production plants were in China ([Di Girolamo et al., 2021](#)). In addition to China, MTBE suppliers were located in 32 other countries throughout the world, including India, the USA, Japan, and countries in Europe, south-eastern Asia, South America, and the Middle East ([ChemicalBook, 2024](#)). A new production facility in Algeria was scheduled to begin MTBE production in June 2025 ([Saada, 2024](#)).

In Europe, 55 facilities located in 30 countries produced MTBE and other gasoline oxygenates in 2010, and production capacity was greatest in Germany, France, and the Kingdom of the Netherlands (hereafter referred to as “the Netherlands”) ([Concawe, 2012](#)). Data from the European Chemicals Agency indicated that 74 companies had active registrations to import or manufacture 1 tonne or more of MTBE, and 21 registered companies were no longer active ([ECHA, 2025b](#)).

### 1.2.2 Uses

#### (a) Use in gasoline

An estimated 85% of the MTBE produced worldwide is used as an additive in gasoline blending ([ChemAnalyst, 2024](#)). When added to gasoline, MTBE leads to more efficient combustion in engines and reduces vehicle emissions of carbon monoxide and other pollutants (see Section 1.1.4 of the monograph on automotive gasoline in the present volume). MTBE also increases the octane rating, thus improving engine performance ([ChemAnalyst, 2024](#)).

In the USA, MTBE began to be added as an octane booster in 1979, to replace lead, initially up to 7% by volume and then increased to 11% by volume. Its use increased substantially in the 1990s following legislation aimed to reduce air pollution in certain areas. To meet new minimum oxygen standards, MTBE was blended in winter oxygenated fuels at 15% by weight (to reduce ambient levels of carbon monoxide) and in reformulated gasoline at 11% by weight (to reduce ozone and other air pollutants). Subsequently, from 2000 to 2009, many states enacted legislation to ban MTBE in automotive gasoline because of MTBE contamination of water sources. MTBE ceased being added to gasoline in the USA in 2006 after federal legislation repealed the minimum oxygen requirements for gasoline and provided tax incentives for gasoline blends that contained 10% ethanol by volume ([Stout and Healey, 2024](#)).

In the USA, MTBE and other oxygenates were measured in gasoline samples collected in states that had banned the use of MTBE (from 2004 to 2020) and in gasoline samples collected before the bans (from 1993–2002 or 2004). In samples collected before the bans, MTBE was detected in all 18 samples of oxyfuel or reformulated gasoline, at concentrations ranging from 60 000 to 133 000 mg/kg, and in 35 of 57 samples of conventional gasoline, at concentrations ranging from 95 to 59 600 mg/kg. In samples

collected after the ban, MTBE was detected in 26 out of 76 gasoline samples, at concentrations ranging from 17 to 980 mg/kg. These data indicated the incidental presence of MTBE years after the ban had been enacted ([Stout and Healey, 2024](#)).

In Europe, MTBE was added to gasoline in the mid-1970s to increase octane ratings and later to reduce air emissions. In 1997, average MTBE concentrations varied by country and ranged from 0.2% (Denmark) to 8.5% (Finland). Legislation in the European Union in 2009 increased the permitted concentration of MTBE from 15% to 22%. Denmark began to phase out MTBE before other countries did ([Kjølholt et al., 2014](#)). In 2010, the market share of ethyl *tert*-butyl ether (ETBE) was expected to further increase in Europe relative to that of MTBE ([Concawe, 2012](#)). In 2009, a directive by the European Parliament regarding biofuels led to the conversion of some MTBE-production plants to ETBE production ([Di Girolamo et al., 2021](#)).

In Europe, in a survey by the fuel industry from 2000 to 2009 on 1239 samples of European gasoline, a mean concentration of MTBE of 5.39 m/m% (wt%) and a median concentration of 4.25 wt% were calculated. MTBE concentrations varied by country and fuel type. The highest concentration measured was 20.43 wt% in a sample from Romania of super premium unleaded gasoline from summer 2001 ([Concawe, 2012](#)). These samples predated the subsequent shift from MTBE to ETBE in the fuel used in many European countries. Data collected by the fuel industry in 2010–2015 from 17 European countries showed a decline in average MTBE concentrations. In 2015, the mean MTBE content in 100 fuel samples was 1.63 wt%, and the median was 0.40 wt%. A maximum of 14.27 wt% was reported for premium gasoline in Italy ([Concawe, 2018](#)).

In Asia, increased urbanization and growth in the manufacture and use of motor vehicles, most notably in China and India, led to increased demand for MTBE ([ReportsInsights Consulting, 2024](#); [ChemAnalyst 2024](#)). In Bangladesh, analyses of 32 samples of gasoline from Khulna City showed mean concentrations of MTBE as 4.15% v/v in regular gasoline and 0.87% v/v in premium gasoline ([Mondal et al., 2024](#)). In 2017, China accounted for 40% of the global market demand for MTBE ([Di Girolamo et al., 2021](#)). In contrast, Japan stopped using MTBE in gasoline in 2010 ([S&P Global, 2022](#)).

[The Working Group noted that patterns in MTBE use varied greatly by geographical region. MTBE has not been completely replaced by ETBE in Europe, and its use appears to have expanded in other parts of the world. There is a lack of data on MTBE exposure in the warmer climates of some low- and middle-income countries (LMICs) where MTBE is now used, such as India.]

#### (b) Other uses

An estimated 10% of MTBE is used as a chemical intermediate ([ChemAnalyst, 2024](#); [ECHA, 2025c](#)). Other uses include laboratory supplies, herbicides, other motor fluids and additives, machine cleaning, automotive care, paints and coatings, and fragrances (NCBI, 2024; [ECHA, 2025c](#)). MTBE is also used in the synthesis of pharmaceutical compounds ([Nair et al., 2022](#)).

At the time of the previous evaluation of MTBE by the *IARC Monographs* programme, MTBE was being investigated for the dissolution of gallstones when surgery was not an option ([IARC, 1999a](#)). According to a review article by medical experts in Italy, this use of MTBE for gallbladder treatment was abandoned ([Portincasa et al., 2006](#)). Although later articles suggested that this limited use of MTBE continued in some countries ([Kim et al., 2015](#); [You et al., 2019](#)), current treatment options do not use MTBE ([Sobani et al., 2021](#)).

## 1.3 Analytical methods

Sampling and analytical methods for all the oxygenates used as additives to automotive gasoline being evaluated in the present volume, namely, MTBE, *tert*-butyl alcohol (TBA), ETBE, diisopropyl ether (DIPE), and *tert*-amyl methyl ether (TAME), in various matrices including air, water, sediment, soil, and biological samples are described below (see [Table 1.1](#) for details).

### 1.3.1 Air

Several methods have been described to measure MTBE, ETBE, TBA, DIPE, and TAME in air ([Table 1.1](#)). For ambient air samples, the United States Environmental Protection Agency (US EPA) method TO-12 uses adsorbent tubes for sampling, and gas chromatography with a flame ionization detector (GC-FID) for sample analysis ([US EPA, 1984](#)). Using this method, [Grosjean et al. \(1998\)](#) determined a sample size of about 1.5 µg by screening, and analytical limits of detection (LODs) corresponded to ambient air LODs of 0.36 µg/m<sup>3</sup> for MTBE ([US EPA, 1984](#)). The same sampling approach can be used and the analysis can also be performed by gas chromatography coupled with mass spectrometry (GC-MS), based on US EPA method TO-1 for the determination of volatile organic compounds (VOCs) in ambient air, using porous polymer sorbent desorption and GC-MS, with LODs of between [0.04 and 366 µg/m<sup>3</sup>] ([US EPA, 1999](#)).

A validated method for determining occupational exposure to MTBE by inhalation is the National Institute for Occupational Safety and Health (NIOSH) method 1615 ([NIOSH, 1994b](#)). This method uses a solid sorbent tube as a sampler (two charcoal tubes in series; front 400 mg; back 200 mg) and GC-FID for sample analysis, with an estimated LOD of 0.02 mg. There is also a validated NIOSH method (NIOSH method 1400) for TBA; a solid sorbent tube is used as a sampler (coconut shell charcoal), and GC-FID is

**Table 1.1 Analytical methods for the measurement of oxygenated additives (MTBE, ETBE, DIPE, TAME, and TBA) in various matrices**

| Sample matrix | Agent                       | Sample preparation                                                                                                              | Instrument (LOD or LOQ)                                           | Comments                                       | Reference                                                               |
|---------------|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|------------------------------------------------|-------------------------------------------------------------------------|
| <b>Air</b>    |                             |                                                                                                                                 |                                                                   |                                                |                                                                         |
| Ambient air   | DIPE                        | Coconut shell charcoal; 100 mg/50 mg                                                                                            | GC-FID (LOD, 0.2 µg/sample)                                       | NIOSH method 1618.                             | <a href="#">NIOSH (2003)</a>                                            |
| Ambient air   | MTBE                        | Cryogenic preconcentration/thermal desorption                                                                                   | GC-FID (LOD, 0.36 µg/m <sup>3</sup> )                             | US EPA method TO-12.                           | <a href="#">US EPA (1984)</a>                                           |
| Ambient air   | ETBE                        | Carbon adsorbent, thermal desorption                                                                                            | GC-FID (LOD, 0.01 ppb [0.05 µg/m <sup>3</sup> ])                  |                                                | <a href="#">Roukos et al. (2009)</a>                                    |
| Ambient air   | MTBE                        | Collection in specially prepared canisters, cryogenic preconcentration, thermal desorption                                      | GC-FID (LOD, 1.7 µg/m <sup>3</sup> )                              | US EPA method TO-14A.                          | <a href="#">Keprasertsup et al. (2003)</a>                              |
| Ambient air   | MTBE, ETBE, DIPE, TAME, TBA | Special canister, cold trap, thermal desorption                                                                                 | GC-MS/SIM (LOD, [0.62–0.85 µg/m <sup>3</sup> ])                   | Based on US EPA method TO-15, updated in 2009. | <a href="#">ThermoFisher Scientific (2020); Alpha Analytical (2025)</a> |
| Air           | TBA                         | Coconut shell charcoal tube, 100 mg/50 mg                                                                                       | GC-FID (LOD, 0.01 mg per sample)                                  | NIOSH method 1400.                             | <a href="#">NIOSH (1994a)</a>                                           |
| Air           | MTBE                        | Two charcoal tubes in series; front 400 mg; back 200 mg<br>Desorption with CS <sub>2</sub> , or 1% 2-butanol in CS <sub>2</sub> | GC-FID (LOD, 0.02 mg per sample)                                  | NIOSH method 1615, issue 2.                    | <a href="#">NIOSH (1994b)</a>                                           |
| Air           | MTBE<br>TAME                | The charcoal pads of the monitors were desorbed with benzene-free CS <sub>2</sub>                                               | GC-MS (LOQ, 0.02 µg/sample corresponding to 3 µg/m <sup>3</sup> ) |                                                | <a href="#">Vainiotalo et al. (2006)</a>                                |
| Air           | ETBE                        | Activated carbon tubes, extraction with CS <sub>2</sub>                                                                         | GC-FID (LOQ, 0.1 µg/mL; GC-MS LOQ, 4.2 µg/m <sup>3</sup> )        |                                                | <a href="#">Eitaki et al. (2011)</a>                                    |
| <b>Water</b>  |                             |                                                                                                                                 |                                                                   |                                                |                                                                         |
| Lake water    | MTBE                        | Purge-and-trap                                                                                                                  | GC-MS (LOD, 0.1 µ/L)                                              | US EPA method 524.2.                           | <a href="#">Reuter et al. (1998)</a>                                    |
| Water samples | TBA, TAME, DIPE, MTBE, ETBE | Purge-and-trap                                                                                                                  | GC-MS (LOD, 0.1 µ/L)                                              | US EPA method 5030C.                           | <a href="#">US EPA 2003</a>                                             |

**Table 1.1 (continued)**

| Sample matrix                              | Agent                       | Sample preparation                                                                      | Instrument (LOD or LOQ)                                                                     | Comments                                                                                   | Reference                                  |
|--------------------------------------------|-----------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|--------------------------------------------|
| Tap water, seawater, river water           | TBA, TAME, DIPE, MTBE, ETBE | HS                                                                                      | GC-MS (LODs, 0.006–0.5 µ/L)                                                                 |                                                                                            | <a href="#">Pérez Pavón et al. (2007)</a>  |
| Groundwater, surface water, drinking-water | MTBE<br>TBA                 | SPME/head space analysis                                                                | GC-MS (MTBE LOD, 0.03 µg/L; LOQ, 0.3 µg/L) (TBA LOQ, 4 µg/L)                                | Comparison with US EPA Method 5030/8260B.                                                  | <a href="#">Oh and Stringfellow (2003)</a> |
| Surface water, groundwater                 | MTBE                        | SPME and thermal desorption                                                             | IMS (LOD, 5 mg/L)                                                                           |                                                                                            | <a href="#">Nousiainen et al. (2011)</a>   |
| <b>Soil</b>                                |                             |                                                                                         |                                                                                             |                                                                                            |                                            |
| Solid waste, groundwater, soil             | MTBE<br>TBA                 | Purge-and-trap                                                                          | GC-MS (LOQ, ~5 µg/kg ww for soil/sediment; 0.5 mg/kg ww for wastes; 5 µg/L for groundwater) | US EPA method 8260B. Method 5030 (aqueous samples) and 5035 (solid and waste oil samples). | <a href="#">US EPA (1996)</a>              |
| Soil                                       | MTBE, TBA, ETBE, TAME, DIPE | Soil sample was spiked with 25 µL of the methanolic solution. Purge-and-trap technique. | GC-MS (LOD, 0.02–1.63 µg/kg)                                                                |                                                                                            | <a href="#">Rosell et al. (2006)</a>       |
| Soil                                       | ETBE, TAME                  | Extraction with CaCl <sub>2</sub> , centrifugation                                      | HS-GC-FID (NR)                                                                              |                                                                                            | <a href="#">Bartling et al. (2010)</a>     |
| <b>Biological specimens</b>                |                             |                                                                                         |                                                                                             |                                                                                            |                                            |
| Urine                                      | MTBE, TAME, TBA             | SPME                                                                                    | HS-GC-PID, ion trap (MTBE LOQ, [0.44 µg/L]; TAME LOQ, [0.51 µg/L]; TBA LOQ, [1.48 µg/L])    |                                                                                            | <a href="#">Vainiotalo et al. (2006)</a>   |
| Urine                                      | MTBE, ETBE                  | HS-SPME                                                                                 | GC-MS (MTBE LOQ, 10 ng/L; ETBE LOQ, 15 ng/L)                                                |                                                                                            | <a href="#">Fustinoni et al. (2010)</a>    |
| Blood                                      | MTBE, TBA                   | Purge-and-trap                                                                          | GC-MS (MTBE LOD, 0.05 µg/L; TBA LOD, 0.06 µg/L)                                             |                                                                                            | <a href="#">Prah et al. (2004)</a>         |
| Breath (exhaled)                           | MTBE                        | Stainless steel canisters, cryogenic concentration, thermal desorption                  | GC-MS ion trap (LOD, [0.37 µg/m <sup>3</sup> ]; SD, [0.18 µg/m <sup>3</sup> ])              | End-exhaled breath. Analysis derived from US EPA Method TO-14.                             | <a href="#">Prah et al. (2004)</a>         |



**Table 1.1 (continued)**

| Sample matrix | Agent                     | Sample preparation | Instrument<br>(LOD or LOQ)                                                                               | Comments     | Reference                            |
|---------------|---------------------------|--------------------|----------------------------------------------------------------------------------------------------------|--------------|--------------------------------------|
| Blood         | MTBE, TBA                 | HS-SPME            | GC-MS-SIM<br>(MTBE LOD, 0.03 µg/L;<br>TBA LOD, 0.05 µg/L;<br>MTBE LOQ, 0.09 µg/L;<br>TBA LOQ, 0.15 µg/L) | Blood serum. | <a href="#">Zhang et al. (2015a)</a> |
| Blood         | MTBE, TBA                 | Purge-and-trap     | GC-MS<br>(MTBE LOD, 0.01 µg/L;<br>TBA LOD, 0.06 µg/L)                                                    | Whole blood. | <a href="#">Bonin et al. (1995)</a>  |
| Blood         | MTBE, ETBE,<br>DIPE, TAME | HS-SPME            | GC-HRMS<br>(LOD, 0.6 ng/L)                                                                               | Whole blood. | <a href="#">Silva et al. (2008)</a>  |

CS<sub>2</sub>, carbon disulfide; DIPE, diisopropyl ether; ETBE, ethyl *tert*-butyl ether; GC-FID, gas chromatography-flame ionization detector; GC-HRMS, gas chromatography with high resolution mass spectrometry; GC-MS, gas chromatography-mass spectrometry; HS, headspace; IMS, ion mobility spectrometry; LOD, limit of detection; LOQ, limit of quantification; MTBE, methyl *tert*-butyl ether; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; PID, photoionization detection; ppb, parts per billion; SD, standard deviation; SIM, selected ion monitoring; SPME, solid-phase microextraction; TAME, *tert*-amyl methyl ether; TBA, *tert*-butyl alcohol; US EPA, United States Environmental Protection Agency; ww, wet weight.

used to measure TBA in the samples collected, with an estimated LOD of 0.01 mg per sample ([NIOSH, 1994a](#)). NIOSH method 1618 allows the measurement of DIPE in workplace air samples with the same features as method 1400 and with an estimated LOD of 0.2 µg per sample ([NIOSH, 2003](#)). For more methods, see [Table 1.1](#).

### 1.3.2 Water

Owing to the volatility of MTBE, analytical methods for measuring MTBE in drinking-water are based on purge-and-trap or headspace gas chromatography (GC) using photoionization detectors (PIDs) or mass spectrometry (MS) detection ([Rhodes and Verstuyft, 2001](#); [WHO, 2005](#)).

In the USA, there are two approved methods to measure MTBE in drinking-water, namely, US EPA methods 502.2 and 524.2. The former uses GC-PID and electrolytic conductivity detectors in series, and the latter uses purge-and-trap capillary GC-MS. These methods achieve an LOD of 0.5 µg/L, well below the odour threshold ([US EPA, 2001](#); [WHO, 2005](#)). Four other methods are considered equivalent to these two methods, namely, American Society for Testing and Materials (ASTM) standard method D5790-95 ([ASTM International, 1996, 1998](#)) and the American Public Health Association standard methods SM 6210D ([Greenberg et al., 1992](#); [Eaton et al., 1995](#)), SM 6200B ([Clesceri et al., 1998](#)), and SM 6200C ([Clesceri et al., 1998](#)).

In Canada, a technique based on US EPA methods 524 and 624 and using purge-and-trap followed by GC is used to measure MTBE and is able to reach an LOD of 0.06 µg/L ([Health Canada, 2006](#)).

An International Organization for Standardization (ISO) method is also available (ISO 20595:2018; [ISO, 2018](#)). This method specifies the determination of selected VOCs, including MTBE, TAME, DIPE, TBA and ETBE, in water. The method is suitable for drinking-water,

groundwater, surface water, and treated wastewater, with analyte concentrations of >0.1 µg/L. The lower application range depends on the individual compound, the amount of the blank value, and the matrix.

[Pérez Pavón et al. \(2007\)](#) developed a method to measure several analytes, including TBA, MTBE, DIPE, ETBE, and TAME, in water, using headspace-programmed temperature vapourization with GC-MS and achieving LODs of between 0.006 and 0.5 µg/L.

### 1.3.3 Soil

US EPA method 8260B and preparation technique 5030/5035 with the closed-system purge-and-trap instruments together is suitable for the determination of low-concentration (<200 µg/kg) VOCs, including fuel oxygenates such as MTBE, ETBE, TBA, DIPE, and TAME in soil samples ([US EPA, 1996](#); [Rosell et al., 2006](#)). The combination of closed-system purge-and-trap GC-MS can provide the best sensitivity and easy automation, and the approach does not require pretreatment of samples or produce flammable waste ([Rosell et al., 2006](#)).

The analysis of ETBE and TAME in soil samples using headspace gas chromatography with a flame ionization detector (GC-FID) is highly sensitive, enabling the detection of these additives at concentrations as low as 0.08 and 0.43 mg/g dry mass, respectively ([Bartling et al., 2010, 2011](#)). [LOD or LOQ was not reported.]

### 1.3.4 Food and consumer products

There are standard methods to measure the presence of additives in gasoline. Test method ASTM D5845-21 was named as the standard method for the determination of MTBE, ETBE, TAME, DIPE, methanol, ethanol, and *tert*-butanol in gasoline at concentrations above 0.1 mass% (wt%) by infrared spectroscopy ([ASTM International, 2021](#)). ASTM standard

test method D4815-22 is used for the determination of ethers and alcohols in gasolines by GC, at concentrations from 0.20% to 20.0% and 0.20% to 12.0 wt%, respectively ([ASTM International, 2022](#)).

### 1.3.5 Biomonitoring

MTBE and the other additives evaluated in the present volume have been measured in various human biological samples, including urine, blood, and exhaled breath. The major metabolite of MTBE, TBA, measured in exhaled breath, and the urinary biomarker 2-hydroxyisobutyric acid (2-HIBA; also known as 2-hydroxyisobutyrate and alpha-hydroxyisobutyric acid) behave similarly to MTBE in blood and give similarly valuable exposure estimates ([Pleil et al., 2007](#)).

For additional data on metabolites, see Section 4.1.

[Pleil et al. \(2007\)](#) performed a series of controlled human MTBE exposure tests and applied first-order kinetic calculations to estimate the ability of spot measurements of MTBE and TBA to predict various scenarios of previous exposure to MTBE. They determined that measurement of these biomarkers in both blood and breath samples could reliably reconstruct recent exposure via inhalation. They also determined that the urinary metabolite, 2-HIBA, could serve as a biomarker of recent exposure to MTBE.

#### (a) Blood

GC-MS is the main technique used to detect MTBE and TBA in blood ([Prah et al., 2004](#); [Silva et al., 2008](#); [Zhang et al., 2015a](#)).

[Bonin et al. \(1995\)](#) developed an isotope-dilution method using purge-and-trap GC-MS for measuring MTBE and TBA in whole blood in humans. The minimum LODs obtained (in 5–10 mL of sample) were 0.01 µg/L for MTBE and 0.06 µg/L for TBA ([Bonin et al., 1995](#)).

[Silva et al. \(2008\)](#) developed a method using headspace solid-phase microextraction (HS-SPME) and GC-MS to measure MTBE, ETBE, DIPE, and TAME in blood, with an LOD of 0.6 ng/L.

[Zhang et al. \(2015a\)](#) used HS-SPME and GC-MS to measure MTBE and TBA in serum samples and obtained good analytical performance in terms of sensitivity, with LODs in serum (1 mL) of 0.03 µg/L for MTBE and 0.05 µg/L for TBA ([Zhang et al., 2015a](#)).

[Prah et al. \(2004\)](#) used a purge-and-trap technique followed by GC-MS to measure MTBE and TBA in blood. The purge-and-trap technique is highly suitable for trace-level analysis of VOCs in aqueous samples, particularly when high sensitivity is essential. Using this method, the minimum detection levels of MTBE and TBA were 0.05 and 0.06 µg/L, respectively.

#### (b) Urine

In a study in gasoline pump repairers and inspectors, [Vainiotalo et al. \(2006\)](#) used urine samples to assess exposure to MTBE and TAME. The following compounds were measured by SPME GC-MS: MTBE and its metabolite TBA, and TAME and its metabolite *tert*-amyl alcohol (TAA). The LOQs were [0.44 µg/L] for MTBE and [0.51 µg/L] for TAME. [Fustinoni et al. \(2010\)](#) also analysed urine samples to measure MTBE and ETBE. In this study, HS-SPME followed by GC-MS analysis was used, with LOQs of 10 ng/L for MTBE and 15 ng/L for ETBE.

#### (c) Exhaled breath

In addition to samples of blood and urine, samples of exhaled breath have also been analysed to measure MTBE exposure. Analyses of the samples collected in canisters were performed via GC-MS using protocols derived from US EPA method TO-14 ([Prah et al., 2004](#)).

## 1.4 Occurrence and exposure

### 1.4.1 Environmental occurrence

The extent of the presence of MTBE in the environment at the end of the 20th century was described in *IARC Monographs Volume 73* ([IARC, 1999a](#)). The present section highlights data made available after the previous evaluation had been completed. [The Working Group noted that the amount of MTBE in gasoline in the USA has been declining since the year 2000, and therefore studies from that period might not reflect more recent environmental occurrence in the USA and some other parts of the world.]

Of the 524 toxic chemicals reported to the US EPA Toxic Release Inventory in 2022, MTBE ranked number 128 in total releases (with rank 1 being the highest). In the USA, 131 facilities reported releasing a total of [99.97 tonnes] of MTBE on-site; [97.70 tonnes] in air, [1.86 tonnes] in water, and [0.44 tonnes] on land. An additional [44.77 tonnes] were disposed of off-site ([US EPA, 2024](#)). Comparable data on environmental releases from other countries were not available.

Measurements of MTBE in various matrices have been reported in many environmental studies. An overview of selected studies representing different settings and countries is presented below and in [Table 1.2](#).

#### (a) Air

MTBE is degraded in the atmosphere by hydroxyl radicals and has an estimated half-life of 3–7 days ([Concawe, 2012](#)).

In the USA, data from ambient air monitoring locations across the country from 2000 to 2021 demonstrated a sharp decline in overall average MTBE air levels beginning in 2005, consistent with the phasing out of MTBE use in automotive gasoline. The median value of daily mean MTBE concentrations was [1.8 µg/m<sup>3</sup>] in 2000 and below the LOD ([0.03 µg/m<sup>3</sup>]) in 2021

([ATSDR, 2023](#)). Higher air levels were reported in areas near MTBE contamination sites. For example, in a study of VOCs in two communities in New Jersey, USA, in 2005, the highest ambient air levels of MTBE, up to 159 µg/m<sup>3</sup>, were measured near a facility where cars were scrapped. The authors speculated that MTBE was evaporating from the gas tanks in the cars ([Zhu et al., 2008](#)).

In service stations at toll roads in New Jersey and Pennsylvania, USA, in 1996–1997 (a period when MTBE was used in winter oxygenated fuels and reformulated gasoline), MTBE levels were measured while gasoline was being pumped. MTBE concentrations inside the vehicle (mean, [1.08 mg/m<sup>3</sup>]) were higher than those outside by the pump (mean, [0.10 mg/m<sup>3</sup>]), and concentrations inside the vehicle were higher when the windows were open during refuelling ([Vayghani and Weisel, 1999](#)).

In Taoyuan, Taiwan, China, air samples collected at stations without vapour recovery systems revealed MTBE concentrations of up to [1.01 mg/m<sup>3</sup>], roughly 20 times as high as those at stations with vapour recovery systems ([Wu et al., 2006](#)).

In Helsinki, Finland, mean concentrations of MTBE in ambient air obtained via stationary sampling at two stations with 95-RON gasoline and vapour recovery systems were 0.16 and 0.07 mg/m<sup>3</sup>, respectively ([Vainiotalo et al., 1999](#)).

In Taegu, Republic of Korea, indoor and outdoor air samples were collected in 2000 in two residential areas, one within 50 m of a service station on a major road, and the other at least 100 m from a service station or major road. Homes that were closer to the service stations had higher indoor and outdoor concentrations of MTBE, and levels were higher in the summer months. Indoor and outdoor measurements of MTBE were not significantly different ([Jo and Oh, 2001](#)).

**Table 1.2 Overview of measurements of MTBE in air and water**

| Sample type                     | Location and collection date                       | No. of samples                                                                                                           | Concentration                                                                                                                                                                              |                                                                                                                                                                                            | Analytical method (detection limit)                             | Comments                                                                                                       | Reference                                  |
|---------------------------------|----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|--------------------------------------------|
|                                 |                                                    |                                                                                                                          | Mean (range)                                                                                                                                                                               | Median (IQR)                                                                                                                                                                               |                                                                 |                                                                                                                |                                            |
| Ambient air                     | Camden, New Jersey, USA, 2005                      | 42 (total) in two communities; means are from the community with air toxic hot spots                                     | July: 5.1 <sup>a</sup> (3.0–29) µg/m <sup>3</sup><br>August: 10 <sup>a</sup> (4.9–159) µg/m <sup>3</sup><br>December: 1.2 <sup>a</sup> (0.49–5.7) µg/m <sup>3</sup>                        |                                                                                                                                                                                            | Passive organic vapour monitors (0.28 µg/m <sup>3</sup> )       | Values not significantly different between the two communities.                                                | <a href="#">Zhu et al. (2008)</a>          |
| Ambient air at service stations | Taoyuan City, Taiwan, China, June 2002             | Four gas stations (16 samples each)                                                                                      | 419 (52–1010) µg/m <sup>3</sup>                                                                                                                                                            |                                                                                                                                                                                            | 6 L canisters, 30 seconds, GC-MS                                |                                                                                                                | <a href="#">Wu et al. (2006)</a>           |
| Ambient air at service stations | New Jersey and Pennsylvania, USA, 1996–1997        | 46 in cabin, 36 at pump                                                                                                  | 1.1 (< 0.02 to 4.7) mg/m <sup>3</sup><br>Pump: 0.1 (< 0.02 to 0.6) mg/m <sup>3</sup>                                                                                                       | In cabin: 0.36 mg/m <sup>3</sup><br>Pump: 0.08 mg/m <sup>3</sup>                                                                                                                           | Thermal desorption coupled with GC-MS                           |                                                                                                                | <a href="#">Vayghani and Weisel (1999)</a> |
| Ambient air at service stations | Helsinki, Finland, August 1996                     | Pump islands at two stations, sampled for 4 days                                                                         | Station A: 0.16 (SD, 0.08) mg/m <sup>3</sup><br>Station B: 0.069 (SD, 0.031) mg/m <sup>3</sup>                                                                                             |                                                                                                                                                                                            | Methods described in published article (0.4 µg/m <sup>3</sup> ) |                                                                                                                | <a href="#">Vainiotalo et al. (1999)</a>   |
| Ambient air at residences       | Taegu, Republic of Korea, 2000 (winter and summer) | Indoor area A, <i>n</i> = 98; indoor area B, <i>n</i> = 97; outdoor area A, <i>n</i> = 97; outdoor area B, <i>n</i> = 93 | Indoor A: [9.4 (2.2–2.7) µg/m <sup>3</sup> ]<br>Indoor B: [6.9 (1.4–18) µg/m <sup>3</sup> ]<br>Outdoor A: [10 (2.2–24) µg/m <sup>3</sup> ]<br>Outdoor B: [7.2 (1.1–18) µg/m <sup>3</sup> ] | Indoor A: [8.7 (5.8–12.3) µg/m <sup>3</sup> ]<br>Indoor B: [6.5 (4.7–8.3) µg/m <sup>3</sup> ]<br>Outdoor A: [9.4 (6.5–12) µg/m <sup>3</sup> ]<br>Outdoor B: [6.9 (4–9) µg/m <sup>3</sup> ] | Thermal desorption system coupled to GC-FID (5 ng)              | Area A: within 50 m of a gas station on a major road. Area B: at least 100 m from a gas station or major road. | <a href="#">Jo and Oh (2001)</a>           |
| Ambient air                     | Nanning, China, 2012                               | 52 samples from six zones                                                                                                | 1.91 (0.18–6.73) µg/m <sup>3</sup>                                                                                                                                                         | 1.47 µg/m <sup>3</sup>                                                                                                                                                                     | GC-MS (0.1 µg/m <sup>3</sup> )                                  |                                                                                                                | <a href="#">Zhang et al. (2015b)</a>       |
| Groundwater                     | USA, 1992–2002                                     | 3964 wells                                                                                                               |                                                                                                                                                                                            | Major aquifer, 0.19 µg/L; agricultural land use, 0.35 µg/L; urban land use, 0.4 µg/L                                                                                                       | Purge-and-trap, GC-MS (0.01–1.66 µg/L; median, 0.17 µg/L)       |                                                                                                                | <a href="#">Moran et al. (2005)</a>        |

**Table 1.2 (continued)**

| Sample type                 | Location and collection date                              | No. of samples                          | Concentration                                                                                                 |                                                                        | Analytical method (detection limit)                                  | Comments                                                                | Reference                                 |
|-----------------------------|-----------------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|----------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------|
|                             |                                                           |                                         | Mean (range)                                                                                                  | Median (IQR)                                                           |                                                                      |                                                                         |                                           |
| Groundwater                 | Los Angeles, California, USA, 2000–2001                   | 718 leaking underground fuel tank sites | 900 <sup>a</sup> (0.46–16 000 000) µg/L                                                                       | 1220 µg/L                                                              | US EPA method 8260B, GC-MS (2 µg/L)                                  | The tested sites were near community drinking-water sources.            | <a href="#">Shih et al. (2004)</a>        |
| Groundwater                 | Germany, 1999–2003                                        | 74 nonurban sites; 67 urban sites       |                                                                                                               | Nonurban, 0.18 µg/L (max., 2.2 µg/L); urban, 0.06 µg/L (max., 48 µg/L) | HS-SPME, GC-MS (0.01 µg/L)                                           | Measures at seven contaminated sites ranged from < 0.01 to 43 000 µg/L. | <a href="#">Kolb and Püttmann (2006)</a>  |
| Drinking-water              | Kingdom of the Netherlands, 2001                          | 45                                      |                                                                                                               | 0.02 µg/L (max., 2.9 µg/L)                                             | Purge-and-trap, TDS-GC-MS (0.01 µg/L)                                | Samples were taken at water treatment plants.                           | <a href="#">Morgenstern et al. (2003)</a> |
| Public drinking-water       | California, 1998–2002                                     | 11 132                                  | 6.0 µg/L                                                                                                      | 3.0 µg/L                                                               |                                                                      | Analytical method, detection limit and ranges not stated.               | <a href="#">Williams et al. (2004)</a>    |
| Resident tap drinking-water | Nanning, China, 2012                                      | 24                                      | 0.13 (0.04–0.33) µg/L                                                                                         | 0.12 µg/L                                                              | Purge-and-trap, GC-MS (0.04 µg/L)                                    |                                                                         | <a href="#">Zhang et al. (2015b)</a>      |
| Water, recreational boating | Southern California, 1999                                 | Six marinas                             | Santa Monica Bay: 3.5 (SD, 3.3) µg/L<br>Mission Bay: 20.1 (SD, 8.3) µg/L<br>San Diego Bay: 2.9 (SD, 0.2) µg/L |                                                                        | US EPA method 8260B, purge-and-trap, GC-MS (0.5 µg/L)                | Receiving water samples collected at the surface and near the bottom.   | <a href="#">Brown et al. (2001)</a>       |
| Water, recreational boating | Lake Texoma, Oklahoma/Texas, USA, June 1999–November 2000 | Five marinas, three locations at each   | Boat dock: 2.9 (SD, 2.5) µg/L<br>Gas station: 2.6 (SD, 2.0) µg/L<br>Entrance: 1.8 (SD, 0.8) µg/L              |                                                                        | US EPA methods 502.2 and 503.1, purge-and-trap GC procedure (1 µg/L) | Samples at 1-foot [0.30 m] depth.                                       | <a href="#">An et al. (2002)</a>          |

GC, gas chromatography; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; HS, headspace; IQR, interquartile range; max., maximum; MTBE, methyl *tert*-butyl ether; SD, standard deviation; SPME, solid-phase microextraction; TDS-GC-MS, thermal desorption-gas chromatography-mass spectrometry; USA, United States of America; US EPA, United States Environmental Protection Agency.

<sup>a</sup> Geometric mean.



In Nanning, China, MTBE was measured in 52 samples of ambient air collected in 2012 across six zones; concentrations ranged from 0.18 to 6.73  $\mu\text{g}/\text{m}^3$  (Zhang et al., 2015b).

[The Working Group noted that ambient air levels of MTBE vary substantially, and the data may not reflect current exposures levels in areas where MTBE is more commonly used today.]

#### (b) Water

##### (i) Groundwater

MTBE is mobile and persistent in groundwater, which makes it a potential groundwater contaminant (IARC, 1999a). Cases of groundwater contamination contributed to the discontinuation of its use in gasoline in many states in the USA in the 2000s (see Section 1.2).

In the USA, federal scientists from the United States Geological Survey reported that MTBE levels in wells were higher in urban areas than in agricultural areas or major aquifers. Of the thousands of samples tested between 1992 and 2002, MTBE levels exceeded the lower limit of the US EPA Drinking-water Advisory (20  $\mu\text{g}/\text{L}$ ) in 0.3% of samples, nearly all of which were collected in urban areas. MTBE was more frequently detected in groundwater in areas with high MTBE use in gasoline (Moran et al., 2005).

In a study of groundwater contamination at leaking underground fuel tanks in Los Angeles county, California, USA, in 2000–2001, MTBE was detected at 82.7% of sites tested, often at concentrations and plume lengths that indicated the potential for contamination of nearby groundwater wells and community sources of drinking-water (Shih et al., 2004).

In Germany, MTBE was detected in groundwater wells more frequently at urban than non-urban sites (see Table 1.2). The authors concluded that low levels of MTBE (median, 0.18  $\mu\text{g}/\text{L}$  at non-urban sites; 0.06  $\mu\text{g}/\text{L}$  at urban sites) were ubiquitous in shallow groundwater and comparable to MTBE detection frequencies

in groundwater wells in the USA (Kolb and Püttmann, 2006).

In 2012, an industry trade association issued a report that summarized data on the occurrence of MTBE in groundwater in several countries in Europe in 2000–2010. Data from a few countries are presented as examples. In Austria, MTBE concentrations at six contaminated sites had a median value of 369  $\mu\text{g}/\text{L}$  and a maximum value of 1594  $\mu\text{g}/\text{L}$ . At 95 other sites in Austria, the median value was 0.036  $\mu\text{g}/\text{L}$  and the maximum value was 10.6  $\mu\text{g}/\text{L}$ . In Switzerland, MTBE was detected at six out of 129 locations in Basel, with a maximum value of 0.2  $\mu\text{g}/\text{L}$ , and at seven out of 65 locations in Canton St Gallen, with a maximum value of 2.12  $\mu\text{g}/\text{L}$ . In Germany, MTBE was detected in 49–66% of locations in urban areas and in 9–29% of locations in non-urban areas, generally at concentrations below 20  $\mu\text{g}/\text{L}$ . In Bavaria, Germany, MTBE was detected in 75 out of 626 locations (12%), with a median value of 0.1  $\mu\text{g}/\text{L}$  and a maximum value of 1600  $\mu\text{g}/\text{L}$ . In groundwater monitoring data from the Netherlands, MTBE contamination was widespread; at three out of 207 locations (1.4%), MTBE was detected at concentrations above 20  $\mu\text{g}/\text{L}$  (Concawe, 2012).

##### (ii) Drinking-water

In California, USA, the mean concentration of MTBE from water sources in the state drinking-water monitoring database in 1998–2002, before the state ban on MTBE in gasoline, was 6.0  $\mu\text{g}/\text{L}$ . MTBE concentrations exceeded the California maximum contaminant level of 13  $\mu\text{g}/\text{L}$  in 9.2% of the drinking-water sources (Williams et al., 2004).

In the Netherlands in 2001, the concentration of MTBE in drinking-water exceeded the reporting level of 0.01  $\mu\text{g}/\text{L}$  in 35 of 45 samples collected at treatment plants. The median value for all samples was 0.02  $\mu\text{g}/\text{L}$ . High concentrations of 2.9  $\mu\text{g}/\text{L}$  detected in samples collected at one location were attributed by the authors

to a historical spill at a gas station nearby ([Morgenstern et al., 2003](#)). Water quality samples collected at locations along the Rhine River between 2000 and 2018 detected MTBE in 88 out of 1627 samples (5.4%). The authors created an “effort index” to characterize difficulty of removal from drinking-water; the effort index for MTBE was 82, the highest of all the 18 micro-pollutants tested, meaning that MTBE was the most difficult to remove by conventional water treatment methods ([Pronk et al., 2023](#)).

MTBE also has been detected in drinking-water from water systems that installed cross-linked polyethylene (PEX) pipes. In Norway, MTBE was measured at concentrations between 0.2 and 213 µg/L in leakage water from six pipes ([Lund et al., 2011](#)).

In Nanning, China, MTBE levels exceeded the LOD (0.04 µg/L) in all 24 water samples collected from residential taps in 2012. The maximum value was 0.33 µg/L ([Zhang et al., 2015b](#)).

### (iii) *Recreational boating*

In southern California, USA, MTBE was detected in samples of receiving water at all six marinas tested in 1999, with a mean value of 8.8 µg/L and a maximum of 34 µg/L. MTBE levels from surface water samples were about double those from bottom-level samples at two stations. The authors speculated that inefficient two-stroke engines on boats and other watercraft led to MTBE contamination of marine waters ([Brown et al., 2001](#)).

MTBE was measured in water samples at five boating marinas located at a lake in the southwestern USA in 1999–2000. During the winter months, levels fell below the LOD, but during the summer months, MTBE levels tracked powerboat usage and reached levels as high as 10.2 µg/L. MTBE was detected more frequently at the boat dock than at other locations, possibly because of gasoline leaks during engine start-up ([An et al., 2002](#)).

### (c) *Soil*

In England, Wales, and Scotland, soil samples were collected near 631 gasoline stations between 2003 and 2009. MTBE was detected in 31% of samples, at concentrations ranging from 0.1 mg/kg to 73 500 mg/kg; < 10% of the samples revealed contamination levels above 10 mg/kg ([Concawe, 2012](#)). In Sweden, MTBE concentrations measured in soil in 2008 were 22 µg/kg at a background site, 37 µg/kg at an urban site with traffic, 18 µg/kg at a gasoline station, 11 µg/kg near a refinery, and 55 µg/kg at the site of an ETBE manufacturer. In Finland, MTBE measurements reported in 2009 from 25 soil samples indicated that well over half were above 5 mg/kg, nearly 30% were above 50 mg/kg, and the highest value was 1400 mg/kg ([Concawe, 2012](#)).

### (d) *Food and consumer products*

No empirical measurement data were available regarding MTBE in food. Other than for gasoline (described in Section 1.2.2), no empirical measurement data were available for MTBE in consumer products.

## 1.4.2 Occupational exposure

Occupational exposure to MTBE is expected during its production and use as an oxygenated additive in gasoline ([US EPA, 2016](#)). [The Working Group noted that no data were available on exposure during MTBE production, and that since the use of MTBE as an additive to oxygenated gasoline is decreasing in many countries, exposure to MTBE via gasoline is expected to change.]

MTBE is volatile at room temperature, as is gasoline. Consequently, exposure scenarios identified for gasoline, such as manufacturing, blending, distribution, storage, and releases from refuelling activities, will also by analogy pertain to MTBE exposure if gasoline contains this additive (see Section 1.4.2 of the monograph on automotive gasoline in the present volume).

for a description of occupational exposure to gasoline). MTBE exposures vary across occupational groups owing to factors such as job type, tasks, and implemented protective measures. Jobs with potential exposure to MTBE through handling of gasoline include gasoline production (depending on the type of gasoline being produced), transportation and dispensing of gasoline, and automobile maintenance. MTBE evaporates quickly, and the main route of exposure is expected to be inhalation (see Section 4.1). [Table 1.3](#) gives an overview of the available data on occupational exposure data.

The Agency for Toxic Substances and Disease Registry (ATSDR) ([ATSDR, 2023](#)) conducted a review of data on exposure to MTBE that is summarized below. [The Working Group noted that the Agency for Toxic Substances and Disease Registry (ATSDR) review focused on data from the USA, some of which were quite old (from 1992–1995). There were no more recent data available from countries that represent major MTBE users, such as India and Mexico.]

*(a) Occupational exposure via air*

Several studies were available, and a general overview of the results is given in the present section. For detailed results, please see [Table 1.3](#). Workers involved in the blending and formulation of gasoline may be exposed to MTBE, particularly if they are working with fuel additives or in facilities where MTBE is used. Seventy-two sets of data on MTBE were compiled from 14 sources for workplace air concentrations such as manufacturing, blending gasoline, transporting MTBE, distributing and dispensing gasoline, and vehicle repair and related tasks ([Brown, 1997](#)). The data comprised published and unpublished data from 1989 to 1995 and included mainly quantified air concentrations obtained for automobile mechanics and service station attendants. Manufacturing, blending, transportation, and distributing were also mentioned, but did not contribute any quantitative data (as

shown in Table 2 of [Brown, 1997](#)). [The Working Group noted that these values were compiled from 1989 to 1995 and might not be representative of current exposures.] Estimations of geometric mean air concentrations were provided for the following worker populations: manufacturing workers, 0.3 mg/m<sup>3</sup>; blending workers, 1.0 mg/m<sup>3</sup>; transportation workers, 0.87 mg/m<sup>3</sup>; distribution workers, 0.47 mg/m<sup>3</sup>; service station workers, 2.0 mg/m<sup>3</sup>; mechanics, 0.3 mg/m<sup>3</sup>; professional drivers, 0.04 mg/m<sup>3</sup>; and other drivers, 0.02 mg/m<sup>3</sup> ([Brown, 1997](#)).

Estimations of MTBE exposures among manufacturing workers were lower than those measured for MTBE plant operators, for whom personal air concentration averaged < 3.6 mg/m<sup>3</sup> ([Concawe, 1997](#)). Average personal exposures when handling neat MTBE ranged from 2.4 to 45.5 mg/m<sup>3</sup> in 24 measurements performed for different tasks (plant operator, ship handling, hose disconnection and check tank levels). Samples taken over shorter periods showed higher levels for workers involved in tanker loading, particularly top loading and maintenance activities. Under some conditions, exposure concentrations may exceed 100 mg/m<sup>3</sup> ([Concawe, 1997](#)).

*(i) Ship tanker loading and unloading*

MTBE exposures have been associated with specific loading tasks. Ship tanker loading (45.5 mg/m<sup>3</sup>) and hose connections (23.3 mg/m<sup>3</sup>) were tasks (15–40 minutes) associated with high air levels ([Concawe, 1997](#)). Bottom gasoline-loading workers had lower average exposures (20.6 mg/m<sup>3</sup>) than did top-loading workers (85.6 mg/m<sup>3</sup>) in one study ([Concawe, 1997](#)). This was corroborated by another study ([Hakkola and Saarinen, 1996](#)) in which the mean for bottom loaders was 13.0 mg/m<sup>3</sup> (range, 2.8–42 mg/m<sup>3</sup>) and for top loaders was 91 mg/m<sup>3</sup> (range, 20–226 mg/m<sup>3</sup>).

**Table 1.3 Overview of occupational exposure measurements of methyl *tert*-butyl ether**

| Occupational group or job type, and location                                                                                                                                                                                           | Date                                                  | Air sampling and analytical method (detection limit)                  | Geometric mean concentration (range)                                                                                                           |                                                                                                                                                                     | Comments                                                                                                                                                                                    | Reference                                                           |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|-----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
|                                                                                                                                                                                                                                        |                                                       |                                                                       | Air                                                                                                                                            | Blood                                                                                                                                                               |                                                                                                                                                                                             |                                                                     |
| Automobile mechanics or service station attendants<br>Service station attendants<br>Other – spent most of their time around traffic or motor vehicles<br>Referent group: commuted to work in their cars<br>USA (Stamford, Connecticut) | 5–16 April 1993 (~5 mo after oxyfuel programme began) | Personal air sampling and NIOSH method 1615 (LOD, 0.02 mg per sample) | LOD (0.1 mg/m <sup>3</sup> ) to 43 mg/m <sup>3</sup> ( <i>n</i> = 37)                                                                          | 1.73 µg/L (0.17–36.7 µg/L) ( <i>n</i> = 21)<br>15 µg/L (7.6–28.9 µg/L) ( <i>n</i> = 3)<br>0.1 µg/L ( <i>n</i> = 6)<br>0.11 µg/L (< 0.05–2.60 µg/L) ( <i>n</i> = 14) | Cross-sectional study. Participants exposed to gasoline containing 15% MTBE during work and/or travel from work.                                                                            | CDC 1993 HHE Report No. 93-802. <a href="#">White et al. (1995)</a> |
| Occupationally exposed to motor vehicle exhaust or gasoline fumes<br>Alaska, USA                                                                                                                                                       | December 1992<br><br>February 1993                    | Stationary sampling with a canister plus GC analysis                  | 0.37 mg/m <sup>3</sup> (0.02–2.92 mg/m <sup>3</sup> )<br><br>0.13 mg/m <sup>3</sup> (ND to 0.51 mg/m <sup>3</sup> )                            | 1.8 µg/L (0.2–37.0 µg/L) ( <i>n</i> = 18)<br><br>0.24 µg/L (0.05–1.44 µg/L) ( <i>n</i> = 28)                                                                        | December 1992 gasoline contained MTBE (15% by volume) and February 1993 no MTBE in gasoline.                                                                                                | <a href="#">Moolenaar et al. (1994)</a>                             |
| Automobile mechanics<br>Finland                                                                                                                                                                                                        | October 1994<br>August 1995                           | Personal air sampling plus GC analysis (LOQ, 0.05 µg/mL)              | 4.3 mg/m <sup>3</sup> (0.75–21.9 mg/m <sup>3</sup> ) ( <i>n</i> = 15)<br>6.4 mg/m <sup>3</sup> (0.57–33.7 mg/m <sup>3</sup> ) ( <i>n</i> = 20) | 143 nmol/L (61–545 nmol/L) ( <i>n</i> = 15)<br>213 nmol/L (18–993 nmol/L) ( <i>n</i> = 20)                                                                          | In October, all the gasolines (95, 98, and 99 RON) contained only MTBE (11.8% v/v) as an oxygenate. In August the 98 and 99 RON gasolines contained 11.4 ± 11.5% MTBE and 0.2 ± 0.25% TAME. | <a href="#">Vainiotalo et al. (1998)</a>                            |
| Ship tanker loading                                                                                                                                                                                                                    |                                                       |                                                                       | 45.5 mg/m <sup>3</sup>                                                                                                                         |                                                                                                                                                                     | Task duration, 15–40 minutes.                                                                                                                                                               | <a href="#">Concawe (1997)</a>                                      |
| Hose disconnection                                                                                                                                                                                                                     |                                                       |                                                                       | 23.3 mg/m <sup>3</sup>                                                                                                                         |                                                                                                                                                                     | Task duration, 15–40 minutes.                                                                                                                                                               | <a href="#">Concawe (1997)</a>                                      |
| Top gasoline loader                                                                                                                                                                                                                    |                                                       |                                                                       | 85.6 mg/m <sup>3</sup>                                                                                                                         |                                                                                                                                                                     |                                                                                                                                                                                             | <a href="#">Concawe (1997)</a>                                      |
| Bottom gasoline loader                                                                                                                                                                                                                 |                                                       |                                                                       | 20.6 mg/m <sup>3</sup>                                                                                                                         |                                                                                                                                                                     |                                                                                                                                                                                             | <a href="#">Concawe (1997)</a>                                      |

**Table 1.3 (continued)**

| Occupational group or job type, and location  | Date | Air sampling and analytical method (detection limit) | Geometric mean concentration (range)                                                                                                                                    |       | Comments                                                                                                                                                                                                                                                                                                                                                      | Reference                                   |
|-----------------------------------------------|------|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
|                                               |      |                                                      | Air                                                                                                                                                                     | Blood |                                                                                                                                                                                                                                                                                                                                                               |                                             |
| Service station attendants                    |      |                                                      | Range of averages from 3.6 to 85.6 mg/m <sup>3</sup> , with shift average exposures of < 1 mg/m <sup>3</sup>                                                            |       | MTBE added as an octane enhancer with levels up to 15% by volume.                                                                                                                                                                                                                                                                                             | <a href="#">Concawe (1997)</a>              |
| Top gasoline loader<br>Finland                |      | Personal air sampling plus GC analysis               | 91 mg/m <sup>3</sup> (20–226 mg/m <sup>3</sup> ) ( <i>n</i> = 4)                                                                                                        |       | Gasoline contains about 10–15% MTBE in liquid phase.                                                                                                                                                                                                                                                                                                          | <a href="#">Hakkola and Saarinen (1996)</a> |
| Bottom gasoline loader                        |      |                                                      | 13.0 mg/m <sup>3</sup> (2.8–42 mg/m <sup>3</sup> ) [36 ppm, 0.78–12 ppm] ( <i>n</i> = 6)                                                                                |       |                                                                                                                                                                                                                                                                                                                                                               | <a href="#">Hakkola and Saarinen (1996)</a> |
| Tank truck drivers<br>Finland                 |      | Personal air sampling plus GC analysis (FID)         | 6.5 mg/m <sup>3</sup> (0.1–28 mg/m <sup>3</sup> ) during loading ( <i>n</i> = 14)<br>15 mg/m <sup>3</sup> (1.1–90 mg/m <sup>3</sup> ) during unloading ( <i>n</i> = 19) |       | The median urinary MTBE concentration was 80 nmol/L (range, 24–234 nmol/L) and the metabolite TBA 388 nmol/L (range, 173–1293 nmol/L) ( <i>n</i> = 11). The time-weighted MTBE air concentrations were highly correlated with urinary MTBE concentrations ( <i>r</i> = 0.84) collected post-shift and TBA ( <i>r</i> = 0.49) collected before the next shift. | <a href="#">Saarinen et al. (1998)</a>      |
| Unloading rail tanker cars                    |      | NIOSH method 1615                                    | 4043 mg/m <sup>3</sup> (3664–6318 mg/m <sup>3</sup> ) ( <i>n</i> = 4)                                                                                                   |       | Monitoring done during winter months from October through January.                                                                                                                                                                                                                                                                                            | <a href="#">Lillquist and Zeigle (1998)</a> |
| Service station attendants<br>Cincinnati, USA |      | NIOSH method 1615                                    | One sample above the LOD (0.58 mg/m <sup>3</sup> ) for MTBE ( <i>n</i> = 32)                                                                                            |       | Cincinnati – small amount of MTBE was used in fuel (0–0.18% v/v).                                                                                                                                                                                                                                                                                             | <a href="#">Hartle (1993)</a>               |

**Table 1.3 (continued)**

| Occupational group or job type, and location                                                         | Date                    | Air sampling and analytical method (detection limit)                                                        | Geometric mean concentration (range)                                                                                                                                                     |                                                             | Comments                                                                                                                         | Reference                              |
|------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
|                                                                                                      |                         |                                                                                                             | Air                                                                                                                                                                                      | Blood                                                       |                                                                                                                                  |                                        |
| Service station attendants<br>Phoenix, USA                                                           |                         | NIOSH method 1615                                                                                           | 1.1 mg/m <sup>3</sup><br>(0.14–14 mg/m <sup>3</sup> ) ( <i>n</i> = 40)                                                                                                                   |                                                             | MTBE content averaged 12.5–13% v/v.                                                                                              | <a href="#">Hartle (1993)</a>          |
| Service station attendants<br>Los Angeles, USA                                                       |                         | NIOSH method 1615                                                                                           | 0.51 mg/m <sup>3</sup><br>(0.07–2.6 mg/m <sup>3</sup> ) ( <i>n</i> = 48)                                                                                                                 |                                                             | Pumps equipped with vapour recovery devices.                                                                                     | <a href="#">Hartle (1993)</a>          |
| Gasoline and exhaust exposed workers                                                                 | 1993                    | NIOSH method 1615                                                                                           | 1.5 mg/m <sup>3</sup><br>(ND to 5.4 mg/m <sup>3</sup> ) ( <i>n</i> = 19)                                                                                                                 | 0.24 <sup>a</sup> µg/L<br>(ND to 1.5 µg/L) ( <i>n</i> = 19) |                                                                                                                                  | <a href="#">Mannino et al. (1995)</a>  |
| Gasoline attendants<br>New Jersey, USA                                                               | 1994                    | NIOSH method 1615                                                                                           | 1.4 mg/m <sup>3</sup> (range,<br>0.29–5.1 mg/m <sup>3</sup> ) ( <i>n</i> = 21)                                                                                                           |                                                             | Elevated peak exposure concentrations (> 50 ppm) were obtained during refuelling. Average refuelling time was about 3–4 minutes. | <a href="#">Cook and Kovein (1997)</a> |
| Petrol station workers<br>NAFLD group<br>Petrol station workers<br>Non-NAFLD group<br>Southern China | April to September 2014 | Personal air sampling done with vapour monitor conducted for three consecutive 8-hour work days.<br>HSGC-MS | Mean (± SD):<br>293 ± 155 µg/m <sup>3</sup> ( <i>n</i> = 11)<br>287 ± 122 µg/m <sup>3</sup> ( <i>n</i> = 60)                                                                             | ND                                                          | Cross-sectional study.                                                                                                           | <a href="#">Yang et al. (2016)</a>     |
| Service station attendants<br><br>Support staff<br>Southern China                                    | August to October 2013  | Personal air sampling with vapour monitor and GC-MS analysis                                                | Mean concentrations per petrol station: 293.5, 346.5, 377.9, 423.9, 394.7, 406.2, 379.9, and 423 µg/m <sup>3</sup><br>62.1, 51.7, 73.9, 80.7, 68.2, 85, 51.6, and 75.8 µg/m <sup>3</sup> |                                                             | Cross-sectional study in eight petrol stations.                                                                                  | <a href="#">Hu et al. (2016)</a>       |



**Table 1.3 (continued)**

| Occupational group or job type, and location                                                                                                                      | Date | Air sampling and analytical method (detection limit) | Geometric mean concentration (range) |                                                                                              | Comments                                                                                                                     | Reference                          |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------------------------------------------------|--------------------------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
|                                                                                                                                                                   |      |                                                      | Air                                  | Blood                                                                                        |                                                                                                                              |                                    |
| Service station attendants, including refuelling workers<br>Support staff (logistics attendants and administrators of the petrol stations)<br>South-eastern China | 2019 |                                                      |                                      | 0.71 µg/L (0.62–0.80 µg/L) ( <i>n</i> = 100)<br>0.19 µg/L (0.18–0.20 µg/L) ( <i>n</i> = 101) | Cross-sectional study. Service station attendants with significant higher values of MTBE metabolites than did support staff. | <a href="#">Guo et al. (2024a)</a> |

CDC, Centers for Disease Control and Prevention; FID, flame ionization detector; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; HHE, health hazards evaluation; HS-GC-MS, headspace gas chromatography-mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; mo, month(s); MTBE, methyl *tert*-butyl ether; ND, not detected; NAFLD, non-alcoholic fatty liver disease; NIOSH, National Institute for Occupational Safety and Health; ppm, parts per million; RON, research octane number; SD, standard deviation; TAME, *tert*-amyl methyl ether; TBA, *tert*-butyl alcohol; USA, United States of America; v/v, volume per volume.

<sup>a</sup> Median.

*(ii) Railroad tanker loading and unloading*

Other short-term tasks associated with greater MTBE exposures were sampling and unloading railroad cars transporting MTBE (geometric means, [22 mg/m<sup>3</sup>] and [12.6 mg/m<sup>3</sup>], respectively) in the USA ([Lillquist and Zeigle, 1998](#)). Railroad car sampling requires filling a glass bottle for laboratory testing. The operators' exposures increased when cleaning of the valve on the railroad car was required. The 5-gallon (18.9 L) bucket used to drain the valve after sample collection was frequently left in the working area, with product vapourizing from it. Time-weighted average (TWA) geometric means were recorded of [4.0 mg/m<sup>3</sup>] for operators unloading MTBE railroad cars and [6.1 mg/m<sup>3</sup>] for laboratory workers testing oxyfuel ([Lillquist and Zeigle, 1998](#)). Leaks from the fitting surfaces contributed to the unloading operators' exposures ([Lillquist and Zeigle, 1998](#)). [The Working Group noted that current unloading and loading technology would be expected to result in much lower exposures because of the use of closed systems.]

*(iii) Road tanker loading and unloading*

Major exposure of road-tanker drivers to gasoline occurs during loading and delivery ([Hakkola and Saarinen, 1996](#)); thus, exposure depends on number of loads per day, content of the loads (gasoline, diesel oil, or kerosene), load size, working habits, vapourization of the oxygenates, leakage, and climate conditions such as temperature and wind speed ([Hakkola and Saarinen, 1996](#); [Saarinen et al., 1998](#)). [The Working Group noted that this statement would also hold for other tanker loading jobs (railroad, ship, etc.).] Road tanker drivers ( $n = 12$ ) in Finland had median MTBE air concentrations of 6.5 mg/m<sup>3</sup> (range, 0.1–28 mg/m<sup>3</sup>) during loading (14 samples) and 15 mg/m<sup>3</sup> (range, 1.1–90 mg/m<sup>3</sup>) during unloading (19 samples) ([Saarinen et al., 1998](#)). These road tanker drivers started their work shifts by loading the truck with gasoline

at the district depot (equipped with vapour recovery systems) and then unloading the gasoline at one or several stations (none had vapour recovery systems). The mean size of the gasoline loads was 13 000 L (range, 1800–36 000 L). The 95-octane gasoline contained on average about 6% of both MTBE and TAME. For drivers, the median urinary concentration was 80 nmol/L (range, 24–234 nmol/L) for MTBE and 388 nmol/L (range, 173–1293 nmol/L) for the metabolite TBA ([Saarinen et al., 1998](#)). The time-weighted air concentrations of MTBE were highly correlated with urinary concentrations of MTBE ( $r = 0.84$ ) collected post-shift and TBA ( $r = 0.49$ ) collected before the next shift ([Saarinen et al., 1998](#)). [The Working Group noted that in many countries several improvements had been made since these studies were published in the late 1990s. Top-loading systems have been replaced by bottom-loading systems. Hoses and couplings, which are prone to leaks, have been replaced by dry break couplings and durable hoses designed to withstand high pressures and resist degradation from gasoline. Manual processes that required workers to handle hoses, valves, and gauges directly (leading to spills, overfills, and accidents) have been replaced by automated systems that reduce the risk of overfill and allow workers to manage the entire process at a distance. Stage I vapour recovery systems capture gasoline vapours released during fuelling operations by returning the vapours displaced from the tank during loading to the storage facility (for details on vapour recovery systems, see Section 1.4.1 of the monograph on automotive gasoline in the present volume). Therefore, transportation workers are likely to have lower vapour exposures at the present time.]

*(iv) Service station attendants*

The primary role of service station attendants is to assist customers with refuelling their vehicles, operating the pumps, checking oil levels, and cleaning windshields. [The Working Group

noted that “gas station attendant”, “petrol station attendant”, “pump attendant”, “pump service attendant”, and “fuelling pump worker” can be used as job titles for the same job in some countries. The job title used in the present monograph is “service station attendant”.] Workers were seldom exposed to only MTBE or MTBE in gasoline but were often also exposed to exhaust fumes. In this occupational setting, average exposure levels to MTBE, reported in various publications, ranged from 0.3 to 85.6 mg/m<sup>3</sup>, with peak exposures of [252 mg/m<sup>3</sup>] also reported ([Table 1.3](#)).

Estimated geometric mean MTBE air concentration for service station attendants was 2 mg/m<sup>3</sup> in a review that included data until 1995 ([Brown, 1997](#)).

MTBE exposure in service station attendants was studied in the USA in the early 1990s ([Hartle, 1993](#)). In Cincinnati, Ohio, where a small amount of MTBE was used in fuel (0–0.18 liquid volume percentage, or % v/v), only in one of 32 personal air samples was MTBE present at above the LOD of [0.58 mg/m<sup>3</sup>]. In Phoenix, Arizona, where the MTBE content averaged 12.5–13% v/v, exposures averaged [1.1 mg/m<sup>3</sup>] (geometric mean), and ranged from [0.14 to 14 mg/m<sup>3</sup>] (all except one of 40 samples contained MTBE at a concentration above the analytical LOD). In Los Angeles, where gasoline pumps were equipped with vapour recovery devices, MTBE exposures averaged [0.5 mg/m<sup>3</sup>], ranging from [0.07] to [2.63 mg/m<sup>3</sup>], and 15 of 48 samples contained MTBE at a concentration above the LOD. Higher MTBE exposures were correlated with working at the service station distributing fuel containing the highest MTBE content (11% v/v). [The Working Group noted that the rise in self-service service stations had reduced the number of attendants in some regions, but that full-service service stations were still prevalent in certain countries.]

The geometric mean TWA of personal MTBE air concentrations among service station attendants ( $n = 21$  samples of personal sampling) in

New Jersey, USA, was [1.4 mg/m<sup>3</sup>] (range, [0.29 to 4.7 mg/m<sup>3</sup>]) in 1994 ([Cook and Kovein, 1997](#)). Using video exposure monitoring, the authors demonstrated that manual refuelling was significantly responsible for exposures to oxygenated fuels, characterized by peak exposures. The authors noted that estimated MTBE peak exposures of 1–2 seconds in duration may be as high as approximately [252 mg/m<sup>3</sup>] ([Cook and Kovein, 1997](#)).

Eighteen workers who were occupationally exposed routinely to motor vehicle exhaust or gasoline fumes in Alaska, USA, were recruited to a study in 1992 investigating before and after MTBE use in gasoline. ([Moolenaar et al., 1994](#)). Eight participants were animal control officers, meter and telephone technicians, and a garbage collector who spent most of their workday in motor vehicles. Ten participants were mechanics and workers at service stations and automobile dealerships. All participants were monitored for MTBE in December 1992 while gasoline contained MTBE (15% by volume) and in February 1993 when this was suspended. MTBE concentrations in the workplace air were measured by an evacuated canister placed in the vicinity of the work area where the worker spent most of the work shift. All workers were heavily exposed to MTBE in workplace air while MTBE was still in use (median 8-hour TWA, 0.37 mg/m<sup>3</sup>; range, 0.02–2.92 mg/m<sup>3</sup>) and much less after the suspension of MTBE use (median, 0.13 mg/m<sup>3</sup>; range, not detected to 0.51 mg/m<sup>3</sup>;  $P$  for difference between both time points, 0.0001) ([Moolenaar et al., 1994](#)). [The Working Group noted that this type of stationary sampling of workplace air using canister will not include the variability a worker experiences during a workday, thus might underestimate MTBE air concentrations. MTBE is rapidly eliminated from the blood; therefore, blood concentrations measured in samples collected after a work shift may underestimate the actual exposure levels.]

A study conducted in southern China evaluated a possible relationship between MTBE exposure and non-alcoholic fatty liver disease (NAFLD) among service station attendants ( $n=71$ ) (Yang et al., 2016). Personal air concentrations were measured, and average exposure concentrations of MTBE were  $292 \pm 155 \mu\text{g}/\text{m}^3$  and  $287 \pm 122 \mu\text{g}/\text{m}^3$  in NAFLD and non-NAFLD groups, respectively (no statistical difference). These occupational exposure concentrations of MTBE among service station attendants were much higher than that of outside air samples (using air sampling canister) collected in the same region, the Pearl River delta, that ranged from 0 to  $1.25 \mu\text{g}/\text{m}^3$  (Wang et al., 2007) [this article being in Chinese, this information was taken from an article by Hu et al., 2016].

Inhalation exposures to MTBE among service station attendants ( $n=97$ ) recruited from August to October 2013 from eight service stations located near the Pearl River in southern China, i.e. service stations labelled A, B, C, D, E, F, G, and H were assessed (Hu et al., 2016). The mean MTBE exposure concentrations of the operating workers at service stations A through H were from 293 to  $423 \mu\text{g}/\text{m}^3$ , while those of the support staff were from 52 to  $85 \mu\text{g}/\text{m}^3$ . The results showed that the exposure levels of MTBE in operating workers were much higher than among support staff ( $P < 0.01$ ).

In summary, MTBE exposure among service station attendants in the USA in the early 1990s varied by location and fuel composition. In Cincinnati (0–0.18% v/v MTBE), only one sample exceeded detection. In Phoenix (12.5–13% v/v), exposures averaged  $1.1 \text{ mg}/\text{m}^3$ . In Los Angeles, with vapour recovery, exposures were lower ( $0.5 \text{ mg}/\text{m}^3$ ). Higher exposures were linked to higher MTBE in fuel (11% v/v). A 1992 study in Alaska monitored 18 workers exposed to gasoline fumes before and after MTBE (15% v/v) was removed from fuel. MTBE air concentrations dropped from  $0.37 \text{ mg}/\text{m}^3$  to  $0.13 \text{ mg}/\text{m}^3$  after its suspension. However, these stationary

air concentrations may have underestimated actual service station attendants' exposures. In a study published in 2016, reported MTBE concentrations ranged from 293 to  $423 \mu\text{g}/\text{m}^3$  (0.08–0.1 ppm) for service station attendants in southern China and from 52 to  $85 \mu\text{g}/\text{m}^3$  (0.01–0.02 ppm) for support staff (Hu et al., 2016).

(v) *Automobile maintenance and car mechanics*

Automobile maintenance and car mechanics have been exposed to gasoline containing MTBE and will also have co-exposures to many other carcinogens (e.g. polyaromatic hydrocarbons, engine exhaust, used engine oil). The estimated geometric mean air concentrations for mechanics was  $0.3 \text{ mg}/\text{m}^3$  in a review of studies published up to 1995 (Brown, 1997). Car mechanics were also included in the study by Moolenaar et al. (1994) (see Section 1.4.2(e)).

In a study conducted in Stamford, Connecticut, USA, 21 MTBE-exposed workers inside car-repair garages (mechanics, supervisors, and service advisors) had personal TWA MTBE air concentrations ranging from below the LOD [ $<0.1 \text{ mg}/\text{m}^3$ ] to  $43 \text{ mg}/\text{m}^3$ , with inhalation exposures varying by location (White et al., 1995). Mechanics working on diesel vehicles had minimal exposures to MTBE (White et al., 1995).

(b) *Biomonitoring of service station attendants and fuel tanker drivers*

In addition to air measurements, workers' exposure to MTBE has been assessed using bio-monitoring. Gasoline-and-exhaust-exposed workers ( $n=19$ ) had personal air MTBE concentrations of  $1.5 \text{ mg}/\text{m}^3$  (range,  $<\text{LOD}$  to  $5.4 \text{ mg}/\text{m}^3$ ), which correlated well with MTBE blood levels (median,  $0.24 \mu\text{g}/\text{L}$ ; range,  $<\text{LOD}$  to  $1.5 \mu\text{g}/\text{L}$ ) (Mannino et al., 1995).

Biomonitoring was carried out in workers who were routinely occupationally exposed to motor vehicle exhaust or gasoline fumes in Alaska,

USA, and who were recruited to a study investigating MTBE blood levels before (1992) and after (1993) the use of MTBE in gasoline was discontinued ([Moolenaar et al., 1994](#)). Median post-shift blood MTBE concentrations were higher (1.8 µg/L; range, 0.2–37.0 µg/L;  $n = 18$  workers) before the use of MTBE was suspended than after (0.24 µg/L; range, 0.05–1.44 µg/L;  $n = 28$  workers) ( $P = 0.0001$ ). Participants without occupational exposure also showed a decline in blood concentrations after MTBE use was discontinued.

A similar study conducted in Stamford, Connecticut, USA, included 30 MTBE-exposed workers, namely, (i) workers inside car-repair garages (mechanics, supervisors, and service advisors) ( $n = 21$ ); (ii) service station attendants ( $n = 3$ ); and (iii) “other” workers (including workers in the city’s traffic and sanitation departments who spent time in traffic) ( $n = 6$ ). The study also included 14 non-occupationally exposed participants (commuters) ([White et al., 1995](#)). The personal TWA MTBE air concentrations are described in Section 1.4.2(f). Blood MTBE levels were highly variable among car-repair workers, with the highest levels exceeding the lowest by 200-fold and generally being higher than those in non-occupationally exposed individuals. Service station attendants who pumped gasoline had blood MTBE levels that were one to two orders of magnitude higher than those of non-occupationally exposed individuals, while other job categories, including workers in the city’s traffic and sanitation department who spent time in traffic, showed levels comparable to this group. Blood levels of TBA, a metabolite of MTBE, were consistently higher than those of MTBE, with similar trends across job groups, i.e. highest levels in those pumping gas and elevated levels in car repair workers compared with commuters. Blood levels of MTBE strongly correlated with those of TBA. MTBE air concentrations for occupationally exposed individuals strongly correlated with blood levels of both MTBE and TBA ([White et al., 1995](#)). [The Working Group

noted that this study reported exposures in the milligrams per cubic metre range, whereas [Moolenaar et al. \(1994\)](#) reported exposures in the micrograms per cubic metre range; this could be explained by the lower MTBE air concentrations in vehicle exhaust owing to incomplete combustion and the air sampling method used (personal air sampling versus stationary sampling), but also to the lack of vapour recovery systems.]

[Vainiotalo et al. \(1998\)](#) reported a strong correlation between personal MTBE air concentrations of fuel tanker drivers during loading procedures and blood MTBE levels approximately 20 minutes after loading; there was no significant correlation between MTBE air and urinary TBA concentrations.

A cross-sectional study in the Anhui Province of south-eastern China was conducted in 2019 among service station attendants, including mainly refuelling workers as the group with high exposure (MTBE occupationally exposed population,  $n = 83$ ) and support staff such as logistics attendants and administrators of the service stations as the group with low exposure (non-MTBE occupationally exposed population,  $n = 118$ ) ([Guo et al., 2024b](#)). The mean blood concentrations of MTBE were 0.19 µg/L (range, 0.18–0.20 µg/L) and 0.71 µg/L (range, 0.62–0.80 µg/L) in the low- and high-exposure groups, respectively ( $P < 0.001$ ). Similarly, levels of the metabolite TBA in the high-exposure group (mean concentration) were also significantly higher than those in the low-exposure group: mean concentrations, 5.34 µg/L (range, 4.34–6.34 µg/L) and 3.47 µg/L (range, 2.93–4.02 µg/L), respectively.

[The Working Group noted that, overall, the highest measured exposures occurred in tanker loading or unloading (especially top loading) and were on the order of tens of milligrams per cubic metre. However, since the implementation of vapour recovery systems in some countries and technological improvements for ship, rail, and road tanker cars, these exposures are very



low. Service station attendants and mechanics have moderate exposures in the range of 0 to 10 mg/m<sup>3</sup>, but service station attendants can also experience very high peak exposures (peak exposure over 1–2 seconds, 265 mg/m<sup>3</sup>) several times per day, depending on the number of customers. Fairly recent biomonitoring data have shown that blood MTBE levels are higher among service station attendants (0.71 µg/L) than among service station support staff (see [Table 1.3](#).)

### 1.4.3 Exposure of the general population

Exposure of the general population to MTBE can occur via inhalation of MTBE vapour, ingestion of drinking-water contaminated with MTBE, and skin absorption from direct contact with MTBE in contaminated soils, water, or automotive gasoline that contains MTBE. The unpleasant odour of MTBE can be detected at very low concentrations in water (15–680 µg/L), and absorption from skin is lower than from inhalation or ingestion ([ATSDR, 2023](#)). The dominant route of exposure to MTBE for the general population is via inhalation of MTBE vapour, particularly near sources of MTBE-blended gasoline and point sources of MTBE contamination. Measures of MTBE in the environment vary greatly and are described in Section 1.4.1(a).

#### (a) Air monitoring

At two service stations in Finland in August 1996, the adjusted geometric mean exposure to MTBE among customers while refuelling (based on 167 individual personal breathing-zone air samples collected using charcoal tubes) was 3.0 mg/m<sup>3</sup> overall, with a 1-minute average of 3.3 mg/m<sup>3</sup>. The 95-RON gasoline also contained TAME, and both service stations had vapour recovery systems ([Vainiotalo et al., 1999](#)).

During the 1999–2000 survey cycle, a subsample of participants in the population-based NHANES survey wore passive VOC-exposure monitors for 48–72 hours. MTBE levels exceeded

the LOD (0.68 µg/m<sup>3</sup>) in 28% of the measurements from 641 study participants; the six highest measurements ranged from 98 to 182 µg/m<sup>3</sup> ([Jia et al., 2008](#)).

In Milan, Italy, personal air exposure to MTBE and other VOCs was measured among schoolchildren aged 7–11 years during spring 2018 ( $n=82$ ) and winter 2019 ( $n=89$ ). Median exposure values were more than twice as high in the winter (1.1 µg/m<sup>3</sup>) as in the summer (0.5 µg/m<sup>3</sup>) ([Boniardi et al., 2023](#)).

#### (b) Biomonitoring

In the USA, the population-based National Health and Nutrition Examination Survey (NHANES) included direct measures of blood MTBE levels for 16 937 survey participants aged ≥ 12 years from 2001 to 2012. This study period spanned the time during and after the use of MTBE in gasoline in the USA. During 2001–2002, when MTBE was still in use in much of the country, 93.9% of survey participants had MTBE blood levels that exceeded the LOD (1.4 ng/L); the median value was 25.8 (interquartile range, 6.08–68.1) ng/L. For the period 2007–2012, after the cessation of use of MTBE in 2006, median MTBE levels were below the LOD of 1.4 ng/L ([Silva et al., 2019](#)).

In Mexico City, Mexico, blood levels of MTBE and other VOCs were measured in blood taken from a convenience sample of 82 office workers in 1998, immediately after their morning commute to work. About half of the participants arrived in personal vehicles, and the majority of others relied on buses. The median blood MTBE concentration was 0.24 µg/L, and values ranged from 0.037 to 92 µg/L ([Lemire et al., 2004](#)).

In central Italy, urinary levels of MTBE and three other gasoline oxygenates (ETBE, TAME, and DIPE) were measured in a sample of Caucasian [White] schoolchildren aged 5–11 years. Samples were collected during summer 2017 ( $n=313$ ) and winter 2018 ( $n=263$ ) in two areas with population and traffic density



**Table 1.4 Regulatory limits and guideline values for workplace exposure to methyl *tert*-butyl ether in various countries**

| Country, location               | 8-hour TWA                                   | STEL                             | Reference                                                            |
|---------------------------------|----------------------------------------------|----------------------------------|----------------------------------------------------------------------|
| Australia                       | 25 ppm (92 mg/m <sup>3</sup> )               |                                  | <a href="#">IFA (2024)</a>                                           |
| Belgium                         | 40 ppm (146 mg/m <sup>3</sup> )              |                                  | <a href="#">IFA (2024)</a>                                           |
| Canada – Ontario                | 40 ppm [144 mg/m <sup>3</sup> ]              |                                  | <a href="#">Government of Ontario (2024)</a>                         |
| China – China Health Commission | 180 mg/m <sup>3</sup>                        | 270 mg/m <sup>3</sup>            | <a href="#">Wu et al. (2019)</a>                                     |
| Denmark                         | 40 ppm (144 mg/m <sup>3</sup> )              |                                  | <a href="#">IFA (2024)</a>                                           |
| European Union                  | 50 ppm (183.5 mg/m <sup>3</sup> )            | 100 ppm (367 mg/m <sup>3</sup> ) | <a href="#">EU-OSHA (2023)</a>                                       |
| New Zealand                     | 25 ppm (92 mg/m <sup>3</sup> )               |                                  | <a href="#">IFA (2024)</a>                                           |
| Singapore                       | 40 ppm (144 mg/m <sup>3</sup> )              |                                  | <a href="#">IFA (2024)</a>                                           |
| Sweden                          | 30 ppm (110 mg/m <sup>3</sup> )              |                                  | <a href="#">IFA (2024)</a>                                           |
| USA – ACGIH                     | 50 ppm [180 mg/m <sup>3</sup> ]              |                                  | <a href="#">ACGIH (2024)</a>                                         |
| USA – California OSHA           | 40 ppm (144 mg/m <sup>3</sup> ) <sup>a</sup> |                                  | <a href="#">California Department of Industrial Relations (2024)</a> |

ACGIH, American Conference of Governmental Industrial Hygienists; OSHA, Occupational Safety and Health Administration; ppm, parts per million; STEL, short-term exposure limit; TWA, time-weighted average; USA, United States of America.

<sup>a</sup> Permissible exposure limit (PEL).

characterized as low (rural) or medium (urban). Mean concentrations of urinary MTBE were significantly higher in the summer (69.9 ng/L) than in the winter (53.3 ng/L). Multiple linear regression analyses showed summer urinary MTBE levels were higher among children who had spent an hour or more in vehicles during the sampling day. Urinary MTBE levels did not vary by area of residence, an unexpected finding that the authors attributed to the fact that differences between ambient pollution levels in the different study areas were not large enough to significantly influence biomarker levels ([Antonucci et al., 2021](#)).

## 1.5 Exposure limits, regulations, and guidelines

An overview of occupational exposure limits is presented in [Table 1.4](#). The European Union established an exposure limit (50.0 ppm, 183.5 mg/m<sup>3</sup>) with a short-term exposure limit (STEL) (100 ppm, 367.0 mg/m<sup>3</sup>) for its member states in 2009 ([EU-OSHA, 2023](#)). China, the Republic of Korea, and the United Kingdom have

similar exposure limit values for MTBE ([Wu et al., 2019](#); [IFA, 2024](#)).

Several countries have established lower TWA exposure limit values. In Belgium, Canada (Ontario and Quebec provinces), Denmark, Singapore, and the state of California, USA, the TWA is 40 ppm [144 mg/m<sup>3</sup>]; in Sweden, the TWA is 30 ppm [110 mg/m<sup>3</sup>]; and in Australia and New Zealand, the TWA is 25 ppm [92 mg/m<sup>3</sup>] ([California Department of Industrial Relations, 2024](#); [Government of Ontario, 2024](#); [IFA, 2024](#)). In 2002, the American Conference of Governmental Industrial Hygienists (ACGIH) adopted its current recommended 8-hour TWA occupational exposure limit for MTBE of 50 ppm [180.0 mg/m<sup>3</sup>] and no short-term exposure limit (STEL), on the basis of a chamber study of 10 persons noting no symptoms at this level, animal evidence, and the “preferred value approach”. Since 1995, ACGIH has classified MTBE as an A3 animal carcinogen with “unknown relevance to humans” ([ACGIH, 2024](#)).

**Table 1.5 Regulatory limits and guideline values for environmental exposure to methyl *tert*-butyl ether in various countries**

| Regulatory limit or guideline value        | Country, location | Description or applicability | Value and units                   | Comments                                                                                                   | Reference                                                             |
|--------------------------------------------|-------------------|------------------------------|-----------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|
| <i>Environmental drinking-water limits</i> |                   |                              |                                   |                                                                                                            |                                                                       |
| Government of Canada                       | Canada            | AO                           | 15 µg/L                           | This AO is the odour threshold of MTBE.                                                                    | <a href="#">Health Canada (2006)</a>                                  |
| Danish Ministry of the Environment         | Denmark           |                              | 5 µg/L                            |                                                                                                            | <a href="#">Kjølholt et al. (2014)</a>                                |
| US EPA                                     | USA               | Advisory                     | 20–40 µg/L or below               | To avert unpleasant taste and odour effects.                                                               | <a href="#">US EPA (1997)</a>                                         |
| State Water Resources Control Board        | USA, California   | MCL<br>SMCL                  | 13 µg/L<br>5 µg/L                 | MCL corresponds to de minimis cancer risk based on animal studies; SMCL based on taste and odour concerns. | <a href="#">California State Water Resources Control Board (2024)</a> |
| <i>Ambient air limit</i>                   |                   |                              |                                   |                                                                                                            |                                                                       |
| US EPA                                     | USA               | RfC                          | 3 mg/m <sup>3</sup><br>(0.83 ppm) | A daily exposure without appreciable risk of deleterious effects during a lifetime.                        | <a href="#">US EPA (1993a)</a>                                        |

AO, aesthetic objective; MCL, maximum contaminant level; MTBE, methyl *tert*-butyl ether; ppm, parts per million; RfC, reference concentration; SMCL, secondary maximum contaminant level; USA, United States of America; US EPA, United States Environmental Protection Agency.

In the USA, 25 states issued regulations that provided partial or complete bans on the use of MTBE in gasoline between 2000 and 2009 after the contamination of water resources with MTBE ([Stout and Healey, 2024](#)). As described in Section 1.4.1, these actions and federal policy changes led to the voluntary cessation of the use of MTBE and other oxygenates except ethanol in gasoline in the USA after 2005 ([Di Girolamo et al., 2021](#); [Stout and Healey, 2024](#)). See also Section 1.5 of the monograph on automotive gasoline in the present volume for regulations related to gasoline and its composition.

In 1993, the US EPA issued an inhalation reference concentration of 3 mg/m<sup>3</sup> for chronic exposure to MTBE, on the basis of health outcomes other than cancer, and no oral reference dose ([US EPA, 1993a](#)).

Drinking-water limits for MTBE have been established and are typically between 5 and 40 µg/L. An overview of environmental and other regulations and guidelines is reported in [Table 1.5](#).

## 2. Cancer in Humans

One pilot case–control study by [Rodriguez et al. \(2024\)](#) on urinary MTBE in cases of pancreatic ductal adenocarcinoma and controls was considered to be uninformative because of the pilot nature of the study, the small number of cases and controls, and the lack of formal comparison of odds ratios. Therefore, there were no epidemiological studies on the association between exposure to MTBE and cancer in humans that were considered to be informative.

### 3. Cancer in Experimental Animals

See [Table 3.1](#).

#### 3.1 Mouse

##### 3.1.1 Inhalation (whole-body)

In a study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female CD-1 mice (age, 7–8 weeks) were exposed to MTBE vapour (purity, >99%) at concentrations of 0, 400, 3000, or 8000 ppm [0, 1430, 10700, and 28600 mg/m<sup>3</sup>] in air for 6 hours per day, 5 days per week, for 18 months, after which the experiment was terminated ([Bird et al., 1997](#); also reported by [Mennear, 1997](#)). The estimated daily doses were 340, 2550, and 6810 mg/kg bw at the respective exposure levels. Mice in the group at the highest concentration exhibited reduced body-weight gain in both males and females. Survival time decreased in males from 510 days in the control group to 438 days in the group at the highest concentration, and in females from 519 days to 489 days. At study termination, survival was 34/50, 39/50, 33/50, and 26/50 in males, and 37/50, 41/50, 39/50, and 34/50 in females, for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively. All animals underwent complete necropsy. The liver, spleen, and submandibular lymph nodes in males, as well as the liver, uterus, and stomach in females, were also histologically examined in all groups.

In females, there was a statistically significant increase ( $P < 0.05$ , [ $P = 0.0277$ ], two-tailed Fisher exact test; [ $P = 0.0139$ , one-tailed Fisher exact test]) in the incidence of hepatocellular adenoma in the group at the highest concentration – 2/50 (4%), 1/50 (2%), 2/50 (4%), and 10/50 (20%) for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively – and the incidence at the highest concentration exceeded the upper bound of the range (range, 0–4.0%) observed in

historical controls from this laboratory. The incidence of hepatocellular adenoma or carcinoma (combined) – 2/50 (4%), 2/50 (4%), 2/50 (4%), and 11/50 (22%) for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively – was significantly increased in the group at the highest concentration ( $P = 0.0073$ , one-tailed Fisher exact test]). The incidence of hepatocellular carcinoma was 0/50, 1/50, 0/50, and 1/50 in the groups at 0 (control), 400, 3000, and 8000 ppm, respectively.

In males, there were no significant increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatocellular adenoma or carcinoma combined. However, the incidence of hepatocellular carcinoma showed a tendency to increase in the group at the highest concentration – 2/49, 4/50, 3/50, and 8/49 for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively. A statistical re-analysis after adjustment for survival concluded that there was a statistically significant increase ( $P < 0.05$ , one-tailed Fisher exact test) in the incidence of hepatocellular carcinoma in male mice at the highest concentration – 2/42, 4/45, 3/41, and 8/34, for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively ([CalEPA OEHHA, 1999](#)). The same analysis concluded that, although not statistically significant, the incidence of the combination of hepatocellular adenoma and carcinoma in male mice – 12/47 (26%), 12/47 (26%), 12/46 (26%), and 16/37 (43%) for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively – was above the historical control data (33%) in the group at the highest concentration group (43%).

Regarding non-neoplastic lesions, there was no significant increase in incidence in either males or females. [The Working Group noted that this was a well-described study that complied with GLP, and it was strengthened by the use of multiple concentrations, males and females (with respective control groups), an adequate duration of exposure and observation, and an adequate number of animals per group.]

**Table 3.1 Studies of carcinogenicity in experimental animals exposed to methyl *tert*-butyl ether**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                       | Tumour incidence                                                                                                                                                                                                                                                              | Significance                                                                                                                                        | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Mouse, CD-1 (M)<br>7–8 wk<br>18 mo<br><a href="#">Bird et al. (1997)</a> | Inhalation (whole-body)<br>MTBE, > 99%<br>Air<br>0, 400, 3000, 8000 ppm<br>(approximately 0, 340, 2550, and 6810 mg/kg bw)<br>6 h/day, 5 days/week for 18 mo<br>50, 50, 50, 50<br>34, 39, 33, 26 | <i>Liver</i><br>Hepatocellular adenoma<br>11/49, 11/50, 9/50, 12/49<br>Hepatocellular carcinoma<br>2/49, 4/50, 3/50, 8/49<br>Hepatocellular adenoma or carcinoma (combined)<br>12/49 (24%), 12/50 (24%),<br>12/50 (24%), 16/49 (43%)                                          | NS<br>NS ( $P = 0.0456$ )<br>NS                                                                                                                     | <i>Principal strengths:</i> Well-conducted long-term study that complied with GLP, multiple concentrations used, male and female animals used, adequate duration of exposure and observation, adequate number of animals per group.<br><br><i>Other comments:</i> A statistical re-analysis after adjustment for survival concluded that there was a statistically significant increase ( $*P < 0.05$ , Fisher exact test) in the incidence of hepatocellular carcinoma (2/42, 4/45, 3/41, 8/34*) in male mice at the highest concentration ( <a href="#">CalEPA OEHHA, 1999</a> ). The same analysis concluded that, although not statistically significant, the incidence of the combination of hepatocellular adenoma and carcinoma in male mice (12/47, 12/47, 12/46, 16/37 for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively) was above the value for historical control data (33%) in the group at the highest concentration (43%). |
| Full carcinogenicity<br>Mouse, CD-1 (F)<br>7–8 wk<br>18 mo<br><a href="#">Bird et al. (1997)</a> | Inhalation (whole-body)<br>MTBE, > 99%<br>Air<br>0, 400, 3000, 8000 ppm<br>(approximately 0, 340, 2550, and 6810 mg/kg bw)<br>6 h/day, 5 days/week for 18 mo<br>50, 50, 50, 50<br>37, 41, 39, 34 | <i>Liver</i><br>Hepatocellular adenoma<br>2/50 (4%), 1/50 (2%),<br>2/50 (4%), 10/50* (20%)<br>Hepatocellular carcinoma<br>0/50 (0%), 1/50 (2%),<br>0/50 (0%), 1/50 (2%)<br>Hepatocellular adenoma or carcinoma (combined)<br>2/50 (4%), 2/50 (4%),<br>2/50 (4%), 11/50 (22%)* | $*P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0139$ , one-tailed Fisher exact test]<br>NS<br>[* $P = 0.0073$ , one-tailed Fisher exact test] | <i>Principal strengths:</i> Well-conducted long-term study that complied with GLP, multiple concentrations used, male and female animals used, an adequate number of animals per group, adequate duration of exposure and observation.<br><br><i>Other comments:</i> Hepatocellular adenoma: historical range was 0–4% for this strain and this laboratory.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                    | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                            | Tumour incidence                                                                                                                                                                         | Significance                                                                                        | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Initiation–<br>promotion (tested<br>as promoter)<br>Mouse, B6C3F <sub>1</sub><br>(F)<br>12 days<br>32 wk<br><a href="#">Moser et al.<br/>(1996a)</a> | Inhalation (whole-body<br>exposure)<br>MTBE, > 99.95%<br>Air<br>Saline, DEN, saline + MTBE,<br>DEN + MTBE<br>DEM: 5 mg/kg, 7.1 mg/kg bw;<br>MTBE: 8000 ppm; 6 h/day,<br>5 days/week for 32 wk<br>12, 12, 12, 12<br>NR | <i>Liver</i><br>No significant increase in the incidence of tumours                                                                                                                      |                                                                                                     | <i>Principal strengths:</i> Well-conducted study,<br>adequate duration of exposure and observation.<br><br><i>Principal limitations:</i> One concentration, small<br>number of animals per group, one sex, number<br>of animals evaluated for histopathology not<br>reported, details on incidence of liver tumours<br>were not available.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Full<br>carcinogenicity<br>Rat, SD (M)<br>8 wk<br>104 wk<br><a href="#">Belpoggi et al.<br/>(1995)</a>                                               | Oral administration (gavage)<br>MTBE, > 99%<br>Olive oil<br>0, 250, 1000 (mg/kg bw)<br>Once daily; 4 days/week for<br>104 wk<br>60, 60, 60<br>26, 25, 32                                                              | <i>Testis</i><br>Leydig cell tumour (interstitial cell adenoma)<br>2/26, 2/25, 11/32*<br><br><i>Haemolymphoreticular system</i><br>Lymphoma or leukaemia (combined)<br>10/59, 9/59, 7/58 | * $P < 0.05$ , prevalence<br>analysis; [ $P = 0.0153$ , one-<br>tailed Fisher exact test]<br><br>NS | <i>Principal strengths:</i> Well-conducted long-term<br>GLP study, adequate number of animals per<br>group, long-term study, male and female animals<br>used.<br><br><i>Principal limitations:</i> Unusual observation<br>period (lifetime) and the lack of mortality-<br>adjusted tumour incidence analysis, historical<br>controls were undocumented.<br><br><i>Other comments:</i> The number of rats used<br>for tumour incidence analysis was based on<br>the number of rats alive at age 96 wk, when<br>the first Leydig cell tumour was observed.<br><a href="#">Belpoggi et al. (1998)</a> conducted a pathological<br>characterization: Leydig cell tumours were<br>characterized as interstitial cell adenomas;<br>the haemolymphoreticular neoplasia was of<br>lymphatic origin and included lymphoblastic<br>lymphoma, lymphoblastic leukaemia, and<br>lymphoimmunoblastic lymphoma. |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                  | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                               | Tumour incidence                                                                             | Significance                                                                                                                             | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, Sprague-<br>Dawley (F)<br>8 wk<br>104 wk<br><a href="#">Belpoggi et al. (1995)</a> | Oral administration (gavage)<br>MTBE, > 99%<br>Olive oil<br>0, 250, 1000 (mg/kg bw)<br>Once daily, 4 days/week for<br>104 wk<br>60, 60, 60<br>58, 51, 47 | <i>Haemolymphoreticular system</i><br>Lymphoma or leukaemia (combined)<br>2/58, 6/51, 12/47* | [ $P = 0.004$ , Cochran–<br>Armitage trend test]<br>* $P < 0.01$ , log-ranked test;<br>[ $P = 0.0011$ , one-tailed Fisher<br>exact test] | <i>Principal strengths:</i> Well-conducted long-term<br>GLP study, adequate number of animals per<br>group, male and female animals used.<br><br><i>Principal limitations:</i> Unusual observation<br>period (lifetime) and the lack of mortality-<br>adjusted tumour incidence analysis, leukaemia<br>or lymphoma might be mistakenly attributed to<br>treatment-related effects because of significant<br>confounding from respiratory infections,<br>historical controls were undocumented.<br><br><i>Other comments:</i> The number of rats used<br>for tumour incidence analysis was based on<br>the number of rats alive at age 56 wk, when<br>the first leukaemia was observed. <a href="#">Belpoggi<br/>et al. (1998)</a> conducted a pathological<br>characterization: Leydig cell tumours were<br>characterized as interstitial cell adenomas;<br>the haemolymphoreticular neoplasia was of<br>lymphatic origin and included lymphoblastic<br>lymphoma, lymphoblastic leukaemia, and<br>lymphoimmunoblastic lymphoma. |



**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                             | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                | Tumour incidence                                                             | Significance                                                                            | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Rat, Wistar (Han, GALAS) (M)<br>6–8 wk<br>24 mo<br><a href="#">Dodd et al. (2013)</a> | Drinking-water<br>MTBE, > 99.99%<br>Reverse osmosis-purified water<br>0, 0.5, 3, 7.5 mg/mL for 104 wk<br>50, 50, 50, 50<br>40, 38, 39, 38 | <i>Brain</i><br>Astrocytoma<br>1/50 (2%), 1/50 (2%),<br>1/50 (2%), 4/50 (8%) | $P = 0.032$ , Cochran–Armitage trend test (one-tailed); $P = 0.037$ , poly-3 trend test | <i>Principal strengths:</i> Well-conducted GLP study, adequate number of animals per group, males and female animals, multiple doses, the duration of exposure and observation was adequate.<br><br><i>Other comments:</i> Incidence in historical controls: brain astrocytoma, 13/3696 (0.35%); range, 0–3%.<br><br>The Working Group considered brain astrocytoma to be a rare tumour. Microscopic examination of all tissues was conducted for males and females in the control group and group at the highest dose, as well for as for any animal that was moribund (and euthanized). Microscopic examination of the kidneys and brain, but no other tissues, was conducted in animals in the groups at 0.5 and 3 mg/mL. |
| Full carcinogenicity<br>Rat, Wistar (Han, GALAS) (F)<br>6–8 wk<br>24 mo<br><a href="#">Dodd et al. (2013)</a> | Drinking-water<br>MTBE, > 99.99%<br>Reverse osmosis-purified water<br>0, 0.5, 3, 15 mg/mL for 104 wk<br>50, 50, 50, 50<br>42, 32, 38, 36  | <i>Brain</i><br>Astrocytoma<br>0/50, 0/50, 0/50, 1/50 (2%)                   | NS                                                                                      | <i>Principal strengths:</i> Well-conducted GLP study, adequate number of animals per group, males and female animals, multiple doses, the duration of exposure and observation was adequate.<br><br><i>Other comments:</i> Incidence in historical controls: brain astrocytoma, 7/3669 (0.19%); range, 0–2%. Microscopic examination of all tissues was conducted for males and females in the control group and group at the highest dose, as well for as for any animal that was moribund (and euthanized). Microscopic examination of the kidneys and brain, but no other tissues, was conducted in animals in the groups at 0.5 and 3 mg/mL.                                                                             |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                      | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                              | Tumour incidence                                                                                                                                                                                  | Significance                                                                                                       | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|--------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Rat, F344 CDF (M)<br>7–8wk<br>82–104 wk<br><a href="#">Bird et al. (1997)</a>  | Inhalation (whole-body)<br>MTBE, > 99%<br>Air<br>0, 400, 3000, 8000 ppm<br>(approximately 0, 223, 1670, and 4450 mg/kg bw) 6 h/day,<br>5 days/week for 82 or 104 wk<br>50, 50, 50, 50<br>NR             | <i>Kidney</i><br>Renal tubular adenoma<br>1/50, 0/50, 5/50, 3/50<br>Renal tubular carcinoma<br>0/50, 0/50, 3/50, 0/50<br>Renal tubular adenoma or carcinoma (combined)<br>1/50, 0/50, 8/50*, 3/50 | NS<br>NS<br>* <i>P</i> < 0.05, two-tailed Fisher exact test<br>[* <i>P</i> = 0.0309, one-tailed Fisher exact test] | <i>Principal strengths:</i> Well-described GLP study, adequate number of animals per group, multiple concentrations used, male and female animals used, adequate duration of exposure and observation.<br><br><i>Principal limitations:</i> Lack of mortality-adjusted tumour incidence analysis, incomplete histopathological examination for animals at the lowest and intermediate dose.<br><br><i>Other comments:</i> The Working Group considered renal tubular tumours to be rare. Males at 3000 and 8000 ppm were euthanized at week 82 and week 97, respectively, because of excess mortality from severe progressive nephrosis. The other groups were euthanized at 24 mo. Early mortality in the group at 8000 ppm may have influenced liver tumour incidence ( <a href="#">Bird et al., 1997</a> ). |
| Full carcinogenicity<br>Rat, F344 CDF (F)<br>7–8 wk<br>82–104 wk<br><a href="#">Bird et al. (1997)</a> | Inhalation (whole-body)<br>MTBE, > 99%<br>Air<br>0, 400, 3000, 8000 ppm<br>(approximately 0, 223, 1670, and 4450 mg/kg bw) 6 h/day,<br>5 days/week for 82 or 104 wk<br>50, 50, 50, 50<br>30, 27, 24, 25 | <i>Kidney</i><br>No significant increase in the incidence of tumours                                                                                                                              |                                                                                                                    | <i>Principal strengths:</i> Well-described GLP study, adequate number of animals per group, multiple concentrations used, male and female animals used, adequate duration of exposure and observation.<br><br><i>Principal limitations:</i> Lack of mortality-adjusted tumour incidence analysis, incomplete histopathological examination for animals at the lowest and intermediate dose.                                                                                                                                                                                                                                                                                                                                                                                                                    |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                   | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals             | Tumour incidence                                                                               | Significance                                                                                                                                                                            | Comments                                                                                                                                                                                               |
|---------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Co-carcinogenicity<br>Rat, F344 (CDF<br>F344 CrIBR) (M)<br>7–8 wk<br>104 wk<br><a href="#">Benson et al. (2011)</a> | Inhalation (whole-body exposure)                                                                                       | <i>Kidney</i><br>Renal tubule adenoma<br>0/50, 0/50, 6/50*, 2/50                               | $*P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0133$ , one-tailed Fisher exact test]                                                                                              | <i>Principal strengths:</i> Well-conducted GLP study, adequate number of animals per group, male and female animals used, multiple concentrations used, adequate duration of exposure and observation. |
|                                                                                                                     | Air                                                                                                                    | Renal tubule carcinoma<br>0/50, 1/50, 0/50, 1/50                                               | NS                                                                                                                                                                                      | <i>Principal limitations:</i> MTBE was co-administered with gasoline (GMVC), making it difficult to evaluate the carcinogenic potential of MTBE itself based on this study alone.                      |
|                                                                                                                     | GMVC (0.4 g/m <sup>3</sup> ) (low),<br>GMVC (2 g/m <sup>3</sup> ) (intermediate),<br>GMVC (4 g/m <sup>3</sup> ) (high) | Renal tubule adenoma or carcinoma (combined)<br>0/50, 1/50, 6/50*, 3/50                        | $*P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0133$ , one-tailed Fisher exact test]                                                                                              |                                                                                                                                                                                                        |
|                                                                                                                     | GMVC (gasoline containing MTBE at 0, 0.4, 2, or 4 g/m <sup>3</sup> , respectively), 6 h/day, 5 days/week for 104 wk    | <i>Testis</i><br>Interstitial cell adenoma (Leydig cell tumour)<br>43/50, 47/50, 48/50, 50/50* | $*P < 0.05$ , two-tailed Fisher exact test [ $P = 0.0062$ , one-tailed Fisher exact test]                                                                                               | <i>Other comments:</i> The Working Group considered renal tubular tumours to be rare.                                                                                                                  |
|                                                                                                                     | NR                                                                                                                     | <i>Spleen</i><br>Mononuclear cell leukaemia<br>27/50, 31/39*, 31/38**, 32/50                   | $*P < 0.05$ , two-tailed Fisher exact test [ $P = 0.0105$ , one-tailed Fisher exact test]<br>$**P < 0.05$ , two-tailed Fisher exact test [ $P = 0.0060$ , one-tailed Fisher exact test] |                                                                                                                                                                                                        |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                   | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                                                                                                      | Tumour incidence                                                  | Significance                                                                                                                                                                              | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|---------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Co-carcinogenicity<br>Rat, F344 (CDF<br>F344 CrIBR) (F)<br>7–8 wk<br>104 wk<br><a href="#">Benson et al. (2011)</a> | Inhalation (whole-body exposure)<br>GMVC vapour condensate (15% by volume); purity, NA<br>Air<br>GVC (control),<br>GMVC (0.4 g/m <sup>3</sup> ) (low),<br>GMVC (2 g/m <sup>3</sup> ) (intermediate),<br>GMVC (4 g/m <sup>3</sup> ) (high)<br>GMVC (containing MTBE at 0, 0.4, 2, or 4 g/m <sup>3</sup> , respectively), 6 h/day, 5 days/week for 104 wk<br>50, 50, 50, 50<br>NR | <i>Mammary gland</i><br>Fibroadenoma<br>0/49, 3/20*, 2/22, 5/47** | * $P < 0.05$ , two-tailed Fisher exact test [ $P = 0.0218$ , one-tailed Fisher exact test]<br>** $P < 0.05$ , two-tailed Fisher exact test [ $P = 0.0251$ , one-tailed Fisher exact test] | <i>Principal strengths:</i> Well-conducted GLP study, male and female animals used, multiple concentrations used, adequate number of animals per group, adequate duration of exposure and observation.<br><br><i>Principal limitations:</i> MTBE was co-administered with gasoline, making it difficult to evaluate the carcinogenic potential of MTBE itself based on this study alone.<br><br><i>Other comments:</i> It was unclear why the number of rats examined in the groups at the lowest and intermediate dose was about half that in the control group and at the highest dose, which may have caused an apparent but not true increase in incidence in these groups. |

bw, body weight; DEN, *N*-nitrosodiethylamine; F, female(s); F344, Fischer 344; GLP, Good Laboratory Practice; GMVC, vapour from gasoline plus methyl *tert*-butyl ether (15% by volume) vapour condensate; GVC, vapour from unleaded gasoline; h, hour(s); M, male(s); mo, month(s); MTBE, methyl *tert*-butyl ether; NA, not applicable; NR, not reported; NS, not significant; ppm, parts per million; SD, Sprague-Dawley; wk, week(s).

### 3.1.2 Initiation–promotion studies (inhalation)

In an initiation–promotion model of liver carcinogenesis, groups of 12 female B6C3F<sub>1</sub> mice (age, 12 days) received a single intraperitoneal injection of *N*-nitrosodiethylamine (DEN) at 5 mg/kg bw or saline. Beginning at age 8 weeks, the mice were exposed by inhalation (whole-body) to MTBE (purity, >99.95%) at 0 or 8000 ppm (28 800 mg/m<sup>3</sup>) in air for 6 hours per day, 5 days per week, and were euthanized after 32 weeks. The mean exposure concentration ( $\pm$  standard deviation, SD) was measured as 7919  $\pm$  198 ppm (99.0% of target concentration). At study termination, survival was 23/24 (96%) for controls and 21/24 (88%) for MTBE-exposed mice. MTBE exposure significantly decreased body weight in the controls and in the mice exposed to DEN only (DEN-initiated) ([Moser et al., 1996a](#)). [The 24 control mice included 12 mice in the air (control) group and 12 in the group exposed to DEN only, while the 24 MTBE-exposed mice included 12 mice in the group exposed to MTBE only and 12 in the group exposed to DEN plus MTBE. The number of surviving animals in each of the four respective groups was not reported.] The liver was isolated and microscopically examined for all animals.

Exposure to MTBE for 32 weeks after initiation with DEN did not increase the number of hepatocellular adenomas or carcinomas compared with the group treated with DEN only. No hepatocellular tumours were observed in the group exposed to MTBE only.

Regarding preneoplastic lesions, MTBE exposure did not significantly alter the number of hepatic foci when comparing the group exposed to DEN plus MTBE with the group exposed to DEN only.

[The Working Group noted that this was a well-conducted study, strengthened by the use of an adequate duration of exposure and observation. However, it was limited by the use of one

concentration only, one sex, and a small number of animals per group. In addition, the number of animals evaluated for histopathology and the incidence of liver tumours were not reported.]

## 3.2 Rat

### 3.2.1 Oral administration (gavage)

In a well-conducted study of chronic toxicity and carcinogenicity that complied with GLP ([Belpoggi et al., 1995](#); also reported by [Belpoggi et al., 1997](#)), groups of 60 male and 60 female Sprague-Dawley rats (age, 8 weeks) were treated by gavage with MTBE (purity, >99%) at doses of 0, 250, or 1000 mg/kg bw in 1 mL of olive oil. The dosing schedule was 4 days per week, with administration once daily on two consecutive days, followed by a day of rest, then another 2 days of dosing with 2 days of rest. This regimen continued for 104 weeks, and the rats were observed until death. All rats underwent complete necropsy, and all organs and tissues were examined microscopically. At study termination, survival was 26/60, 25/60, and 32/60 in males, and 58/60, 51/60, and 47/60 in females, for the groups at 0 (control), 250, and 1000 mg/kg bw, respectively. In males, survival was comparable among the treated groups and the control group at up to 88 weeks, except that a higher survival rate was observed in the group at the highest concentration beyond 80 weeks. However, in females, survival decreased in a dose-dependent manner after 24 weeks [statistical significance not reported; mean survival times not provided]. No difference in water or food consumption, or in mean body weights, was observed between treated and control animals of either sex.

In males, there was a statistically significant increase ( $P < 0.05$ , prevalence analysis; [ $P = 0.0153$ , one-tailed Fisher exact test]) in the incidence of interstitial cell adenoma of the testis [calculated based on the number of surviving rats at age 96 weeks, when the first interstitial

cell adenomas in the testis were observed] in the group at 1000 mg/kg bw, the incidence being 2/26 (8%), 2/25 (8%), and 11/32 (34%) for the groups at 0 (control), 250, and 1000 mg/kg bw, respectively. In addition, the overall incidence of interstitial cell adenoma in the testis [calculated based on the number of rats at the start] was significantly increased in the group at 1000 mg/kg bw [ $P=0.0079$ , one-tailed Fisher exact test], the incidence being 2/60 (3%), 2/60 (3%), and 11/60 (18%) for the groups at 0 (control), 250, and 1000 mg/kg bw, respectively. A histological re-analysis with the aim of pathological characterization of these testicular tumours and lymphomas or leukaemias, and their precursors, in these Sprague-Dawley rats, was conducted by [Belpoggi et al. \(1998\)](#). In that study, interstitial cell tumours in rats were confirmed to be interstitial cell adenomas. [The Working Group noted that the group sizes reflected the number of rats alive at 96 weeks, when the first interstitial cell tumour was observed. However, since the incidence of interstitial cell adenoma typically increases with age and the survival rate at the highest dose was higher than that in the control group and at 250 mg/kg bw, this observed significance may be misleading.] In females, there was a significant positive trend [ $P=0.004$ , Cochran–Armitage trend test] in the incidence of lymphoma or leukaemia (combined), with the incidence – 2/58 (3%), 6/51 (12%), and 12/47 (26%) for the groups at 0 (control), 250, and 1000 mg/kg bw, respectively [calculated based on the number of surviving rats at age 56 weeks, when the first leukaemia was observed] – being significantly increased in the group at 1000 mg/kg bw compared with the control group ( $P<0.01$ , log-ranked test; [ $P=0.0011$ , one-tailed Fisher exact test]). In addition, the overall incidence of lymphoma or leukaemia (combined) was significantly increased [ $P=0.0042$ , one-tailed Fisher exact test] in the group at 1000 mg/kg bw – 2/60 (3%), 6/60 (10%), and 12/60 (20%) for the groups at 0 (control), 250, and 1000 mg/kg bw,

respectively [calculated based on the number of rats at the start]. [The authors reported that the incidence in the group at 1000 mg/kg bw exceeded the historical control incidence rates for lymphoma and leukaemia (combined) ( $<10\%$ ) from this laboratory; however, information on historical controls was not documented.] The haemolymphoreticular neoplasia was of lymphatic origin and included lymphoblastic lymphoma, lymphoblastic leukaemia, and lymphoimmunoblastic lymphoma ([Belpoggi et al., 1998](#)). [The Working Group noted that the group sizes refer to the number of rats alive at 56 weeks, when the first leukaemia was observed. The Working Group also noted that the United States National Toxicology Program (NTP) has concluded that the leukaemias/lymphomas identified in these studies were probably misinterpreted as being treatment-related, primarily owing to extensive confounding by respiratory infections ([Gift et al., 2013](#)).] There was an increased incidence of uterine sarcoma (8.3%) in the group at 250 mg/kg bw compared with the control group (0%); however, the incidence was 0% in the group at 1000 mg/kg bw.

[The Working Group noted that this was a long-term study that complied with GLP and was strengthened by the use of an adequate number of animals per group, both males and females, two doses, and an adequate duration of exposure. However, it was limited by the study observation period (lifetime) that increased uncertainty in interpreting the incidence of tumours, the lack of mortality-adjusted tumour incidence analysis, and the historical controls that were mentioned but not documented. In addition, after the histopathological re-analysis by the NTP, haemolymphoreticular neoplasms (lymphoma/leukaemia) were considered to have been misinterpreted and were not taken into consideration by the Working Group for the present assessment of carcinogenicity.]



### 3.2.2 Oral administration (drinking-water)

In a study of chronic toxicity and carcinogenicity that complied with GLP, groups of 50 male and 50 female Wistar rats (Han, GALAS) (age, 6–8 weeks), were treated with drinking-water (reverse osmosis-purified water) containing MTBE (purity, > 99%) at concentrations of 0, 0.5, 3, or 7.5 mg/mL for males, and 0, 0.5, 3, or 15 mg/mL for females, for 104 weeks ([Dodd et al., 2013](#)). The combined mean ( $\pm$  SD) daily doses of MTBE for males during the 2 years of exposure were  $25 \pm 11$  mg/kg,  $140 \pm 63$  mg/kg, and  $330 \pm 139$  mg/kg bw per day for the groups at 0.5, 3, and 7.5 mg/mL, respectively. For females, the mean ( $\pm$  SD) daily doses of MTBE were  $49 \pm 14$  mg/kg,  $232 \pm 66$  mg/kg, and  $1042 \pm 280$  mg/kg bw per day for the groups at 0.5, 3, and 15 mg/mL, respectively. At study termination, survival was 40/50, 38/50, 39/50, and 38/50 in males for the groups at 0 (control), 0.5, 3, and 7.5 mg/mL, respectively, and 42/50, 32/50, 38/50, and 36/50 in females for the groups at 0 (control), 0.5, 3, and 15 mg/mL, respectively. No differences in survival or body weight were observed between the treated and control groups. All animals underwent a complete necropsy and more than 60 tissues were collected per animal. Kidney and brain were evaluated in all animals in the study, whereas other tissues were assessed only for animals in the control group and at the highest dose.

In males, there was a significant positive trend ( $P=0.032$ , Cochran–Armitage trend test;  $P=0.037$ , poly-3 trend test adjusted for survival differences) in the incidence of brain astrocytoma – 1/50 (2%), 1/50 (2%), 1/50 (2%), and 4/50 (8%) for the groups at 0 (control), 0.5, 3, and 7.5 mg/mL, respectively. No significant increase in the incidence of brain astrocytoma was observed in any treated group. The incidence of astrocytoma was within the range for historical controls for Wistar rats at 0.5 and 3 mg/mL and exceeded the upper bound of this range for the group at 7.5 mg/mL

(incidence in historical controls, 13/3696 (0.35%); range, 0–3%) from the Harlan Laboratory. [The Working Group considered brain astrocytoma to be a rare tumour. The Working Group also noted that MTBE is reported to be distributed in the brain.]

In female rats, the incidence of brain astrocytoma was 0/50 (0%), 0/50 (0%), 0/50 (0%), and 1/50 (2%) in the groups at 0 (control), 0.5, 3, and 7.5 mg/mL, respectively, indicating no significant increase in the incidence of brain astrocytoma. The incidence of brain astrocytoma was within the historical control range for Wistar rats (7/3669 (0.19%); range of 0–2%) from the Harlan Laboratory.

The incidence of neoplastic lesions of the kidney did not exceed one in males and females in either the control or MTBE-exposed groups. No evidence of MTBE-induced tumorigenicity was observed in other organs.

[The Working Group noted that this was a well-conducted long-term study that complied with GLP, and used an adequate number of animals per group, both males and females, and multiple doses.]

### 3.2.3 Inhalation (whole-body)

In a study of chronic toxicity and carcinogenicity that complied with GLP, groups of 50 male and 50 female Fischer 344 (F344) CDF rats (age, 7–8 weeks) were exposed to MTBE vapour (purity, > 99%) at concentrations of 0, 400, 3000, or 8000 ppm [0, 1430, 10 700, and 28 600 mg/m<sup>3</sup>] in air for 6 hours per day, 5 days per week, for 24 months ([Bird et al., 1997](#); also reported by [Mennear, 1997](#)). The estimated doses were 223, 1670, and 4450 mg/kg bw at the respective exposure levels. Body weights were significantly decreased in both sexes in the group at 8000 ppm throughout the study. Males at the intermediate and highest doses were euthanized at week 97 and week 82, respectively, because of excessive morbidity from severe progressive nephrosis.

Animals in the remaining groups (0 and 400 ppm) were euthanized at 24 months. [The number of animals per group that survived to study termination was not reported.] The mean survival times for males in the control group and at the lowest dose were 632 and 617 days, respectively. For females in the groups at 0, 400, 3000, or 8000 ppm, the survival times were 697, 683, 697, and 676 days, respectively. All animals underwent complete necropsy. The liver, kidneys, and testes of males, as well as the liver of females, were evaluated for all dose groups, and other tissues were examined only for animals in the control group and the group at 8000 ppm.

In males, there was a statistically significant increase ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0309$ , one-tailed Fisher exact test]) in the incidence of renal tubular cell adenoma or carcinoma (combined) in the group at 3000 ppm, with incidence being 1/50 (2%), 0/50, 8/50 (16%), and 3/50 (6%) for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively. The incidence of renal tubular cell adenoma – 1/50 (2%), 0/50, 5/50 (10%), and 3/50 (6%) for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively – and of renal tubular cell carcinoma – 0/50, 0/50, 3/50 (6%), and 0/50 for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively – was slightly but not significantly increased. [The Working Group considered renal tubular tumours to be rare.]

The incidence of interstitial cell adenoma in the testis – 32/50 (64%), 35/50 (70%), 41/50 (82%), and 47/50 (94%) for the groups at 0 (control), 400, 3000, and 8000 ppm, respectively – was significantly increased in the groups at 3000 ppm ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0352$ , one-tailed Fisher exact test]) and at 8000 ppm ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0002$ , one-tailed Fisher exact test]) compared with the control group. [The Working Group noted that the incidence of interstitial cell adenoma of the testis fell within the range of spontaneous

incidence previously observed in rats of the same strain ([Mennear, 1997](#)). The Working Group also noted the unusually low incidence of interstitial adenoma of the testis in control rats compared with the incidence in historical controls in the same laboratory, and that mortality-adjusted analysis was not performed.] A statistical re-analysis of the incidence of renal tubule tumours and of interstitial cell tumours, after adjustment for survival, confirmed the original statistical analysis ([CalEPA OEHHA, 1999](#)).

In females, one renal tubular cell adenoma was observed in one animal at the intermediate concentration; no treatment-related increases in tumour incidence were observed.

Regarding non-neoplastic lesions, the authors reported an exposure-related increase in the incidence and severity of renal changes associated with chronic progressive nephropathy (CPN) in males across all MTBE-exposed groups, and to a lesser extent in females in the groups at 3000 and 8000 ppm. Furthermore, nephropathy was identified as the most common cause of death and morbidity in male rats in the groups at 3000 and 8000 ppm.

[The Working Group noted that this was a well-described study that complied with GLP, and was strengthened by the use of multiple concentrations, both males and females (with respective control groups), an adequate duration of exposure and observation, and an adequate number of animals per group. However, it was limited by the lack of mortality-adjusted tumour incidence analysis, and an incomplete histopathological examination for animals exposed at the lowest and intermediate doses.]

In a study of subchronic toxicity ([Sarhan et al., 2019](#)), groups of 15 adult male Wistar albino rats [age, not reported] were exposed by inhalation to MTBE vapour (purity, commercial grade) at a concentration of 60 µL/3 minutes per day for 3, 6, or 12 months. [No details on how the exposure was conducted were provided.] The control group were exposed to non-MTBE

vapour for 12 months. After the exposure period, five rats were selected randomly, euthanized, dissected, and stained with haematoxylin and eosin to monitor histopathological changes.

After 12 months of exposure to MTBE, there were noticeable changes in the accretion of tracheal adenomas in the tracheal lamina propria and alterations in lung adenomas at the interalveolar septa of air alveoli and perivascular tissues, in comparison with the trachea and lungs of the control rats. Microscopic investigations revealed an accumulation of tracheal adenomas in the tracheal tissue of the group exposed to MTBE for 6 months. No significant changes were observed at the 3-month interim. [There were no original data on weight, survival, or tumour incidence.]

Regarding non-neoplastic lesions, exposure to MTBE for 3–12 months induced hyperplasia and polyp formation in the trachea. After 12 months, squamous metaplasia and inflammatory infiltration in the lamina propria were observed.

[The Working Group noted that no incidence rates for the reported tumours were provided. Moreover, the lesions identified as “tracheal adenoma in the tracheal lamina propria” (Fig. 2 of the publication) and “lung adenoma” (Fig. 4 of the publication) did not appear to meet standard diagnostic criteria for tumours and therefore should not be classified as such. The study lacked details on how the exposure of 60  $\mu\text{L}$ /3 minutes per day was conducted. Only one sex was used, the number of animals per group was small, and the duration of exposure per day was short. Therefore, the Working Group considered that this study was uninformative for the evaluation of the carcinogenicity of MTBE in experimental animals.]

### 3.2.4 Administration with known carcinogens or other agents (inhalation)

In a well-conducted study that complied with GLP and compared the potential carcinogenicity of vapour from unleaded gasoline (GVC) and gasoline containing MTBE (GMVC), groups of 50 male and 50 female F344 rats (age, 7–8 weeks) were exposed to GVC or GMVC at total vapour concentrations of 0, 2, 10, or 20  $\text{g}/\text{m}^3$ . [The GMVC used in the study was well characterized and included 21.3% added MTBE and 1.5% benzene. The exposure concentrations of MTBE were 0, 0.4, 2, and 4  $\text{g}/\text{m}^3$  for the groups at vapour concentrations of 0, 2, 10, or 20  $\text{g}/\text{m}^3$ , respectively.] The rats were exposed for 6 hours per day (plus 14 minutes, the time for the vapour concentration to reach 90% of equilibrium), 5 days per week, for 104 weeks, for both GVC and GMVC ([Benson et al., 2011](#)). [The concurrent experiment with GVC is reviewed in the monograph on automotive gasoline in the present volume.] The authors reported no significant effects on survival. Body weights of male and female rats exposed to GMVC at the highest concentration (20  $\text{g}/\text{m}^3$ ) remained below those for the controls for most of the study. Body weights of males and females at 10  $\text{g}/\text{m}^3$  were below those for the controls for some or most of the study. All animals underwent complete necropsy. All tissues of rats in the control group and the group at the highest concentration were evaluated histologically. Additionally, tissues from the respiratory tract, testis, and kidneys were examined histologically for male rats in the groups at 2 and 10  $\text{g}/\text{m}^3$ .

In males, the incidence of renal tubular adenoma was 0/50 (0%), 0/50 (0%), 6/50 (12%), and 2/50 (4%), the incidence of renal tubular carcinoma was 0/50 (0%), 1/50 (2%), 0/50 (0%), and 1/50 (2%), and the incidence of renal tubular adenoma or carcinoma (combined) was 0/50 (0%), 1/50 (2%), 6/50 (12%), and 3/50 (6%) for the groups at 0 (control), 2, 10, and 20  $\text{g}/\text{m}^3$ , respectively. Both the incidence of renal tubular

adenoma and the incidence of renal tubular adenoma or carcinoma (combined) were significantly increased ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0133$ , one-tailed Fisher exact test]) in the group exposed to GMVC at  $10 \text{ g/m}^3$ , compared with the controls. [The Working Group noted that similar increases were observed in the concurrent experiment with GVC at  $10 \text{ g/m}^3$  and that there were no significant differences between the GMVC and GVC groups at  $10 \text{ g/m}^3$ .]

The incidence of interstitial cell adenoma of the testis – 43/50 (86%), 47/50 (94%), 48/50 (96%), and 50/50 (100%) for the groups at 0 (control), 2, 10, and  $20 \text{ g/m}^3$ , respectively – was significantly increased ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0062$ , one-tailed Fisher exact test]) in the group exposed to GMVC at  $20 \text{ g/m}^3$ . The background incidence of testicular interstitial cell adenoma was 48/50 in the GVC controls and 43/50 in GMVC controls. Exposure to GVC did not significantly increase the incidence of this tumour. [The lower incidence for controls in the GMVC study compared with the GVC study probably contributed to the statistical significance observed in the group exposed to GMVC at  $10 \text{ g/m}^3$ , because the incidence in rats exposed to either GMVC or GVC was similar, ranging from 94% to 100%.] [The Working Group noted that in comparison with the sham control group, the incidence of interstitial cell tumours in all treated groups was not significantly different. Overall, the Working Group concluded that the interstitial cell tumours were not treatment-related, as this is a very common background pathology of F344 rats.] The incidence of mono-nuclear cell leukaemia of the spleen – 27/50 (54%), 31/39 (79%), 31/38 (81%), and 32/50 (64%) for the groups at 0 (control), 2, 10, and  $20 \text{ g/m}^3$ , respectively – was significantly increased in the groups at  $2 \text{ g/m}^3$  ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0105$ , one-tailed Fisher exact test]), and at  $10 \text{ g/m}^3$  ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0060$ , one-tailed Fisher exact test]). [The Working Group noted that, compared with

the sham control group, the incidence of mono-nuclear cell leukaemia in all treated groups was not significantly different. The Working Group also noted this is a very common background pathology of F344 rats.]

In females, there was a significant increase in the incidence of fibroadenoma of the mammary gland – 0/49 (0%), 3/20 (15%), 2/22 (9%), and 5/47 (11%) for the groups at 0 (control), 2, 10, and  $20 \text{ g/m}^3$ , respectively – in the groups at  $2 \text{ g/m}^3$  ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0218$ , one-tailed Fisher exact test]) and at  $20 \text{ g/m}^3$  ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0251$ , one-tailed Fisher exact test]) compared with the controls. [The Working Group noted that the incidence in the control group was unusually low at 0%. Furthermore, the number of rats examined in the groups at 2 and  $10 \text{ g/m}^3$  was approximately half that in the control group and the group at  $20 \text{ g/m}^3$ , possibly because the mammary glands were not examined in all rats in the groups at the low and intermediate concentrations. Instead, they were examined only in animals that were dead, moribund, or had gross lesions at the final euthanasia. These factors may have contributed to an apparent, but not actual, increase in incidence in the treatment groups.]

Regarding non-neoplastic lesions, CPN was observed in nearly all males and females in all groups, with a slightly but not significantly higher severity in GMVC-exposed groups compared with controls. In males, the average severity was 1.9 in the control group and 2.6 and 2.7, respectively, in the groups at 10 and  $20 \text{ g/m}^3$ . In females, an average severity of 1.0 was observed in the controls, and 1.5 in the group at  $20 \text{ g/m}^3$ . There were exposure-related increases (significantly increased in females) in the incidence of respiratory epithelial degeneration in the nose at turbinate level 2 in males and females. In males at turbinate level 2, the incidence of respiratory degeneration was 0/50, 1/50, 2/50, and 3/50 in the groups at 0 (control), 2, 10, and  $20 \text{ g/m}^3$ ,



respectively. In females at turbinate level 2, the incidence of respiratory epithelial degeneration was 0/50, 5/50, 7/50, and 6/50 in the groups at 0 (control), 2, 10, and 20 g/m<sup>3</sup>, respectively. There was an exposure-related increase in the incidence of olfactory epithelial degeneration at levels 2 and 3 in male (significantly increased) and female rats. At turbinate level 2, the incidence was 0/50, 5/50, 7/50, and 6/50 in males and 4/50, 5/50, 5/50, and 10/50 in females in the groups at 0, 2, 10, and 20 g/m<sup>3</sup>, respectively. At turbinate level 3, the incidence was 2/50, 1/50, 7/50, and 7/50 in males and 4/50, 2/50, 10/50, and 8/50 in females in the groups at 0, 2, 10, and 20 g/m<sup>3</sup>, respectively.

[The Working Group noted that this was a well-conducted study that complied with GLP, and it was strengthened by the use of an adequate duration of exposure and observation, an adequate number of animals per group, both males and females, and multiple concentrations. The most significant limitation of the study was that MTBE was co-administered with gasoline, making it difficult to evaluate the carcinogenic potential of MTBE itself on the basis of this study alone.]

### 3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of MTBE has been assessed in four well-conducted GLP studies in male and female Wistar rats (Han, GALAS) treated by oral administration (drinking-water) ([Dodd et al., 2013](#)), in male and female Sprague-Dawley rats treated by oral administration (gavage) ([Belpoggi et al., 1995](#); also reported by [Belpoggi et al., 1997](#)) [histological re-analysis by [Belpoggi et al. \(1998\)](#)]; in male and female CD-1 mice exposed by inhalation (whole-body) ([Bird et al., 1997](#); also reported by [Mennear, 1997](#)); and in male and female F344 CDF rats exposed by inhalation (whole-body) ([Bird et al., 1997](#); also

reported by [Mennear, 1997](#)). In addition, the co-carcinogenicity of MTBE has been assessed in a well-conducted GLP study in male and female F344 (CDF F344 CrIBR) rats exposed by inhalation (whole-body) ([Benson et al., 2011](#)). The carcinogenicity of MTBE was also evaluated in studies that did not comply with GLP. Specifically, MTBE was tested and by inhalation in a study of subchronic (12-month) toxicity in male Wistar albino rats ([Sarhan et al., 2019](#)). In addition, an initiation–promotion study was conducted in female B6C3F<sub>1</sub> mice exposed by inhalation (whole-body) ([Moser et al., 1996a](#)).

In the well-conducted GLP study in male and female Wistar rats (Han, GALAS) treated with MTBE by oral administration (drinking-water) ([Dodd et al., 2013](#)), there was a significant positive trend in the incidence of brain astrocytoma in males. [The Working Group considered brain astrocytoma to be a rare tumour. The Working Group also noted that MTBE is reported to be distributed to the brain.]

In the well-conducted GLP carcinogenicity study in male and female Sprague-Dawley rats treated with MTBE by oral administration (gavage) ([Belpoggi et al., 1995](#); also reported by [Belpoggi et al., 1997](#)), there was a statistically significant increase in the incidence of interstitial cell adenoma of the testis in males. In addition, the overall incidence of testicular interstitial cell adenoma [calculated based on the number of rats at the start] was significantly increased. The histological re-analysis by [Belpoggi et al. \(1998\)](#) confirmed the interstitial cell adenomas, and the lymphoid tumours as lymphoblastic lymphomas, lymphoblastic leukaemias and lymphoimmunoblastic lymphomas. In females, there was a significant positive trend in the incidence of lymphoma or leukaemia (combined), with the adjusted incidence significantly increasing. [The Working Group noted that an NTP Pathology Working Group had concluded that the leukaemias/lymphomas identified in these studies were probably misinterpreted as being treatment-

related, primarily because of extensive confounding by respiratory infections ([Gift et al., 2013](#)). The Working Group agreed with the conclusion of the NTP Pathology Working Group review. The Working Group also noted that the study observation period (lifetime) increased uncertainty in interpreting the incidence of tumours. In addition, the Working Group noted that group sizes reflected the number of rats alive at 96 weeks, when the first interstitial cell tumour was observed. However, since the incidence of interstitial cell adenoma typically increases with age, and the survival rate in the group at the highest dose was higher than that in the control group or the group at the lowest dose, this observed significance may be misleading.]

In the well-conducted GLP study in male and female CD-1 mice exposed to MTBE by inhalation (whole-body) ([Bird et al., 1997](#); also reported by [Mennear, 1997](#)), there was a significant increase in the incidence of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined) in females.

In the well-conducted GLP study in male and female F344 CDF rats exposed to MTBE by inhalation (whole-body) ([Bird et al., 1997](#); also reported by [Mennear, 1997](#)), there was a significant increase in the incidence of renal tubular cell adenoma or carcinoma (combined) in males. [The Working Group considered renal tubular tumours to be rare.] There was a significant increase in the incidence of interstitial cell adenoma in the testis.

In the well-conducted co-carcinogenicity GLP study in male and female F344 (CDF F344 Cr1BR) rats exposed to MTBE by inhalation (whole-body) ([Benson et al., 2011](#)), there was a significant increase in the incidence of renal tubular adenoma and of renal tubular adenoma or carcinoma (combined) in males. [The Working Group considered renal tubular tumours to be rare.] There was a significant increase in the incidence of interstitial cell

adenoma in the testis, and mononuclear cell leukaemia in the spleen.

In the initiation–promotion study in female B6C3F<sub>1</sub> mice exposed to MTBE by inhalation (whole-body) ([Moser et al., 1996a](#)), there was no increase in the incidence of hepatic tumours.

The study in male Wistar albino rats exposed to MTBE by inhalation ([Sarhan et al., 2019](#)) was considered to be uninformative for the evaluation of the carcinogenicity of MTBE in experimental animals.

## 4. Mechanistic Evidence

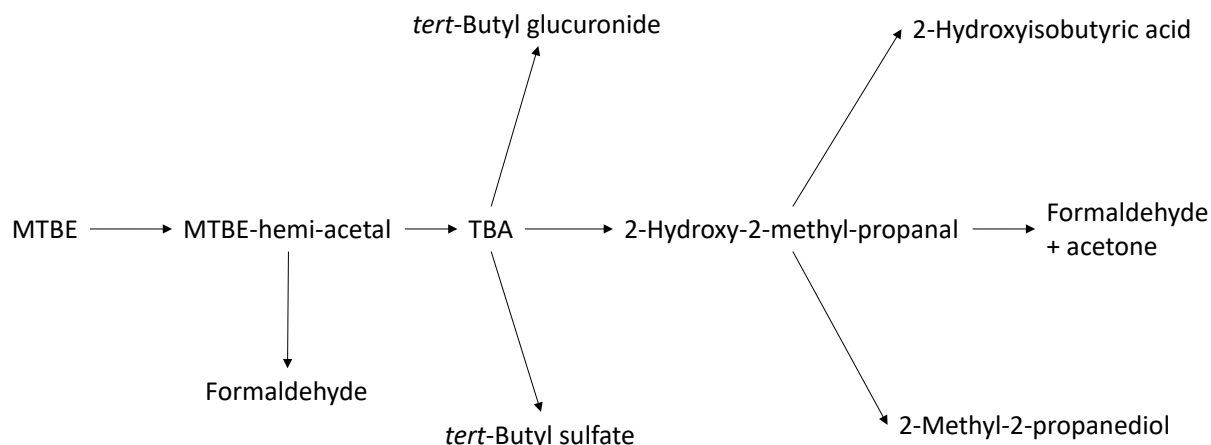
### 4.1 Absorption, distribution, metabolism, and excretion

[The Working Group noted that the structure and physical properties of MTBE are similar to those of the other ethers that are automotive gasoline additives being evaluated in this volume, namely ETBE and TAME; hence, absorption via inhalation, skin, and ingestion as well as distribution in the body are expected to be common to the three agents. In addition, the first and central step in metabolism ([Fig. 4.1](#)), oxidative demethylation (deethylation in the case of ETBE) leading to the formation of TBA (TAA in the case of TAME) and formaldehyde (acetaldehyde in the case of ETBE) is qualitatively similar for the three ethers. The subsequent metabolism of TBA has been described in slightly different ways by different researchers, as detailed in the monograph on TBA in the present volume. Overall, a fraction of TBA is conjugated, and another fraction is further metabolized to 2-HIBA, MPD, formaldehyde, and acetone (see Section 1).]

#### 4.1.1 Exposed humans

Studies addressing the toxicokinetics of MTBE in humans were also described in *IARC Monographs Volume 73* ([IARC, 1999a](#)).



**Fig. 4.1 Metabolism of methyl *tert*-butyl ether**

MTBE, methyl *tert*-butyl ether; TBA, *tert*-butyl alcohol.  
 The intermediary metabolites MTBE-hemiacetal and 2-hydroxy-2-methylpropanal are postulated.  
 Adapted from the scheme for ethyl *tert*-butyl ether in [US EPA \(2021\)](#).

#### (a) Occupational exposure

[Saarinen et al. \(1998\)](#) measured MTBE and TAME in the breathing zone, and MTBE, TBA, TAME, and TAA in urine from 11 road-tanker drivers in Finland. The drivers were exposed to gasoline vapours for short periods (mean  $\pm$  SD,  $21 \pm 14$  minutes) three times during a work shift. The mean concentration of MTBE was  $8.1 \text{ mg/m}^3$  (2.2 ppm) in the air in the breathing zone, and the total uptake during the shift was calculated to be  $106 \text{ }\mu\text{mol}$ . Mean concentrations in urine post-shift and next morning were 113 and 18 nM, respectively. A strong correlation ( $r=0.84$ ) was found between MTBE exposure and post-shift urinary excretion.

#### (b) Inhalation exposure, controlled studies

See [Table 4.1](#).

[Cain et al. \(1996\)](#), [Buckley et al. \(1997\)](#), [Bernauer et al. \(1998\)](#), [Nihlén et al. \(1998a, b\)](#), and [Lee et al. \(2001\)](#) reported on toxicokinetic data in healthy volunteers exposed to MTBE by inhalation. Most of these studies were also described in *IARC Monographs Volume 73* ([IARC, 1999a](#)).

[Cain et al. \(1996\)](#) exposed four healthy volunteers (two men and two women), presumably at rest, to MTBE at a concentration of 1.7 ppm ( $6 \text{ mg/m}^3$ ) for 1 hour in an exposure chamber, followed by exposure to clean air for 1.5 hours. Blood was sampled from the antecubital vein and analysed for MTBE by GC-MS. The concentration of MTBE in venous blood increased during the exposure and reached  $17 \text{ }\mu\text{g/L}$  after 1 hour, and then declined, with an initial half-life of approximately 40 minutes.

[Buckley et al. \(1997\)](#) exposed two healthy sedentary volunteers (one man and one woman) to MTBE at a concentration of  $4.04 \text{ mg/m}^3$  (1.12 ppm) and  $4.88 \text{ mg/m}^3$  (1.35 ppm), respectively, for 1 hour in an exposure chamber, followed by exposure to clean air for 7 hours. Alveolar breath, venous blood, and urine were sampled and analysed for MTBE by GC-MS. Post-exposure decreases in MTBE in alveolar breath and blood were analysed with a three-compartment toxicokinetic model and gave residence times of 2–3 minutes, 15–50 minutes, and 3–13 hours, and 5 minutes, 60 minutes, and 32 hours, respectively. The relative respiratory

**Table 4.1 Comparison of toxicokinetic data obtained from controlled inhalation exposure of healthy volunteers to methyl *tert*-butyl ether (rounded values)**

| No. of participants | Exposure level       | Exposure duration | Relative respiratory uptake | Concentration in blood at end of exposure |                       | Post-exposure half-life of TBA in blood   | Post-exposure excretion in urine (% of absorbed dose) |              | Reference                                |
|---------------------|----------------------|-------------------|-----------------------------|-------------------------------------------|-----------------------|-------------------------------------------|-------------------------------------------------------|--------------|------------------------------------------|
|                     |                      |                   |                             | MTBE                                      | TBA                   |                                           | MTBE                                                  | TBA          |                                          |
| Men, 2;<br>women, 2 | 1.7 ppm              | 1 h               | NR                          | 17 µg/L                                   | NR                    | NR                                        | NR                                                    | NR           | <a href="#">Cain et al. (1996)</a>       |
| Men, 1;<br>women, 1 | 1.12 ppm<br>1.35 ppm | 1 h               | 54%<br>40%                  | 8.2 µg/L<br>14.1 µg/L                     | 6.3 µg/L<br>6.6 µg/L  | 2–3 h<br>2–5 days                         | < 1%<br>< 1%                                          | < 1%<br>< 1% | <a href="#">Buckley et al. (1997)</a>    |
| Men, 10             | 5 ppm <sup>a</sup>   | 2 h               | 47%                         | 1.4 µM                                    | NR                    | NR                                        | 0.09%                                                 | 0.5%         | <a href="#">Nihlén et al. (1998a)</a>    |
|                     | 25 ppm <sup>a</sup>  |                   | 42%                         | 6.5 µM                                    | ~5 µM <sup>b</sup>    | 10.0 h                                    | 0.07%                                                 | 0.7%         |                                          |
|                     | 50 ppm <sup>a</sup>  |                   | 49%                         | 13 µM                                     | ~10 µM <sup>b</sup>   | 10.0 h                                    | 0.09%                                                 | 0.6%         |                                          |
| Men, 3;<br>women, 3 | 4.5 ppm<br>39 ppm    | 4 h               | NR                          | 1.9 µM<br>6.7 µM                          | 2.6 µM<br>21.8 µM     | 6.5 h<br>5.3 h                            | NR<br>0.14%                                           | NR<br>2.1%   | <a href="#">Amberg et al. (1999)</a>     |
|                     |                      |                   |                             |                                           |                       |                                           |                                                       |              |                                          |
| Men, 3;<br>women, 3 | 1.6 ppm              | 15 min            | 69%<br>63%                  | 4–10 µg/L                                 | 2.2 µg/L <sup>c</sup> | 8.0 h <sup>d</sup><br>10.5 h <sup>e</sup> | 0.7%<br>1.2%                                          | 1.3%<br>2.4% | <a href="#">Lee et al. (2001)</a>        |
| Men, 14             | 3 ppm                | 1 h               | NR                          | 0.28 µM <sup>f</sup>                      | 0.19 µM <sup>f</sup>  | NR                                        | NR                                                    | NR           | <a href="#">Prah et al. (2004)</a>       |
| Men, 4              | 25 ppm               | 4 h               | 43%                         | 11 µM                                     | 7 µM <sup>g</sup>     | NR                                        | 0.3%                                                  | 1.0%         | <a href="#">Vainiotalo et al. (2007)</a> |
|                     | 75 ppm               |                   | 42%                         | 28.8 µM                                   | 20 µM <sup>g</sup>    |                                           | 0.2%                                                  | 1.3%         |                                          |

h, hour(s); min, minute(s); MTBE, methyl *tert*-butyl ether; NR, not reported; ppm, parts per million; TBA, *tert*-butyl alcohol.

<sup>a</sup> Light physical exercise (50 W) during the exposure.

<sup>b</sup> Estimated from Fig. 1b in [Nihlén et al. \(1998a\)](#).

<sup>c</sup> Estimated from Fig. 3 in [Lee et al. \(2001\)](#).

<sup>d</sup> One man.

<sup>e</sup> One woman.

<sup>f</sup> Maximum value reached.

<sup>g</sup> Estimated from Fig. 2 in [Vainiotalo et al. \(2007\)](#).

uptake was estimated to be 54% in the man and 40% in the woman (reported by the authors as fractions exhaled at equilibrium of 0.46 and 0.60, respectively). The decrease in the metabolite TBA in blood was slower than that of MTBE, with a residence time of 3–4 hours in the man and 3.5–7 days in the woman (half-lives, 2–3 hours and 2–5 days, respectively). The concentrations of MTBE and TBA in the urine were similar to those in blood and ranged from 0.37 to 15 µg/L and 2 to 15 µg/L, respectively. Urinary elimination accounted for a very small fraction of total MTBE elimination (< 1%).

[Nihlén et al. \(1998a\)](#) exposed 10 healthy male volunteers to MTBE at a concentration of 5, 25, and 50 ppm for 2 hours during light physical exercise (50 W) in an exposure chamber. MTBE and its metabolite, TBA, were monitored in exhaled air, arterialized capillary blood, and urine, which were sampled for up to 3 days after exposure, and analysed by headspace-gas chromatography (HS-GC). The relative respiratory uptake of MTBE was 42–49%, and the post-exposure respiratory exhalation was 32–47% of the inhaled dose. Metabolic clearance from the blood was reported to be 0.34–0.52 L/hour per kg bw. The concentration of MTBE in the blood was described by four exponential phases, with average half-lives of 1 minute, 10 minutes, 1.5 hours, and 19 hours. The post-exposure decrease in MTBE in the urine was described by two half-lives of 20 minutes and 3 hours. The average post-exposure half-lives of TBA in the blood and urine were 10 and 8.2 hours, respectively. The urinary excretion of MTBE and TBA was < 1% of the absorbed dose. The kinetics of MTBE and TBA were linear up to the highest exposure level of 50 ppm.

[Nihlén et al. \(1999\)](#) exposed four male volunteers to <sup>13</sup>C-labelled MTBE at a concentration of 50 ppm for 2 hours during light physical exercise (50 W), via face mask inhalation. Urinary metabolites were studied by nuclear magnetic resonance (NMR) spectroscopy. NMR signals

were not detected for labelled MTBE, TBA, or possible MTBE-derived conjugates. The NMR analysis showed the presence of α-hydroxyisobutyric acid (2-hydroxyisobutyrate, 2-HIBA) and 2-methyl-1,2-propanediol (MPD) that were quantified in two volunteers. The total excretion of these metabolites in urine collected up to 20 hours post-exposure was 11% (2-HIBA) and 1% MPD in one subject and 3% (2-HIBA) and 1% MPD in the other, expressed as a percentage of the MTBE uptake. In addition, blood, exhaled air, and urine were analysed for MTBE, TBA, and acetone by GC. The GC analyses showed a relative uptake of MTBE of 39%, post-exposure exhalation of MTBE of 17%, and low recovery of TBA in the urine of below 1%. The same volunteers had previously been exposed by inhalation (whole-body) to unlabelled MTBE in an earlier study ([Nihlén et al., 1998b](#)), and the toxicokinetics of MTBE and TBA in this face mask exposure did not differ from those in the previous whole-body chamber exposure.

[Amberg et al. \(1999\)](#) exposed healthy volunteers (three men and three women) to MTBE at 4 and 40 ppm for 4 hours in an exposure chamber. The exposures resulted in blood concentrations of MTBE of 1.9 and 6.7 µM, and of TBA of 2.6 and 21.8 µM, respectively. MTBE was rapidly cleared from the blood with an average half-life of 2.6 hours after exposure at 40 ppm, whereas the average half-life for TBA was 5.3 hours. In non-exposed volunteers, TBA was found in the blood and urine; MPD and 2-HIBA were also present in the urine, but at concentrations that were significantly increased after exposure. 2-HIBA was the major metabolite in the urine, with TBA and MPD being minor ones. All the urinary metabolites were rapidly eliminated, with half-lives in the urine of between 9.7 and 17.0 hours. Between 35% and 69% of the retained MTBE was recovered as metabolites in the urine.

[Lee et al. \(2001\)](#) exposed six healthy volunteers (three men and three women) to MTBE (15 vol% in gasoline) at 6 mg/m<sup>3</sup> (1.7 ppm) twice

for 15 minutes. On average, 65.8% of the inhaled MTBE was retained, of which 69.4% was expired within 8 hours after exposure. Three elimination phases were identified for MTBE in breath, with average half-lives of 2.0 minutes, 26 minutes, and 5.0 hours, respectively. A strong positive correlation between concentrations of MTBE in blood and breath was found, with a mean blood:breath ratio of 23.5. TBA concentrations in the blood peaked at around 2–4 hours after exposure. The half-lives of TBA in the blood and urine were 8.0 hours and 0.5 hour, respectively, in men and 10.5 and 10.3, respectively, in women. On average, 1.0% of the inhaled dose was excreted in the urine as MTBE, and 1.9% was excreted as unconjugated TBA within 24 hours. Notably, in all the volunteers MTBE and TBA were also detected in blood sampled before exposure.

[Prah and colleagues \(2004\)](#) exposed 14 healthy male volunteers to MTBE at 3.0 ppm (10.8 mg/m<sup>3</sup>) by inhalation (mouth only) from a scuba tank for 1 hour. Concentrations of MTBE and TBA in the blood increased during exposure and after 60 minutes reached 0.28 µM and 0.19 µM, respectively. Three elimination phases were identified for MTBE in blood, with average half-lives of 1.9 minutes, 1.0 hours, and 5.2 hours ([Prah et al., 2004](#)).

[Vainiotalo et al. \(2007\)](#) exposed four healthy male volunteers to MTBE at 25 and 75 ppm in an exposure chamber for 4 hours. The average relative respiratory uptake of MTBE was 43%. The inhaled doses were 1.6 mmol at 25 ppm and 4.9 mmol at 75 ppm; 55% and 48%, respectively of the inhaled dose was exhaled, and 43% and 42% was retained in the body. Concentrations of MTBE in the blood and breath peaked at the end of exposure, reaching 11.0 µM and 28.8 µM in the blood for exposures at 25 ppm and 75 ppm, respectively. The corresponding concentrations in exhaled air were 0.4 µM and 1.2 µM. Two elimination phases were described for MTBE in the blood and exhaled air, the mean half-lives in the blood being 1.7 hours and 3.8 hours. The decline

in concentrations of MTBE in exhaled air paralleled that in the blood. The amounts of MTBE excreted in the urine up to 48 hours after exposure were 0.3% of the absorbed dose at 25 ppm and 0.2% at 75 ppm. The corresponding values for TBA were 1.0% and 1.3%. The mean half-lives of MTBE in urine (determined graphically) were 3.8 hours at 25 ppm and 4.4 hours at 75 ppm. No half-life was reported for TBA in the blood and urine, as the post-exposure decline, according to the authors, seemed to follow zero-order rather than first-order kinetics. [The Working Group noted that this conclusion seemed to be drawn from the data on TBA in the urine ([Vainiotalo et al., 2007](#), Fig. 2) but was not supported by the data on TBA in the blood ([Vainiotalo et al., 2007](#); Fig. 1), which rather indicated first-order kinetics. Moreover, other controlled exposure studies suggested first-order toxicokinetics, including a first-order decrease in blood TBA concentrations ([Nihlén et al., 1998b](#); [Amberg et al., 1999](#); [Lee et al., 2001](#)).]

[In [Table 4.1](#), the Working Group reported some key toxicokinetic data provided by the available studies on controlled inhalation exposures to MTBE. Although the studies differed in exposure level, exposure duration, end-points studied, sampling design, and toxicokinetic analysis, the Working Group noted that the results were consistent. The studies reported similar and high respiratory uptake (around 40%), high post-exposure respiratory excretion of MTBE, similar decreases in the blood and breath, and similar, low excretion of MTBE and TBA in the urine. The differences in respiratory uptake were likely explained by differences in exposure duration and physical workload. Differences between the studies in the reported half-lives (not shown in [Table 4.1](#)) could be explained by differences in exposure duration, sampling design, and toxicokinetic methodology (graphical inspection, statistical approach). Pre-exposure (background) levels of MTBE and metabolites might also have contributed to some

of the differences. Within studies with a given exposure duration, peak concentrations and areas under the curve (AUCs) for both MTBE and TBA were proportional to the exposure level, suggesting first-order toxicokinetics up to the highest tested level of 75 ppm. The Working Group also noted that there was no indication of sex-specific differences in the disposition of MTBE and its metabolite; however, the studies that included both sexes were conducted in a limited number of study participants.]

(c) *Dermal exposure, controlled studies*

In a study by [Prah et al. \(2002, 2004\)](#), 14 healthy male volunteers were dermally exposed to MTBE by having the forearm immersed for 1 hour in a tank containing MTBE at 52 mg/L (average) in water. The study participants wore a charcoal mask to avoid inhalation of MTBE vapour. The mean skin surface area exposed was 1511 cm<sup>2</sup>, and the tank temperature was 39 °C. Concentrations of MTBE and TBA in the blood increased during exposure and reached 0.05 µM and 0.06 µM, respectively, at 60 minutes. Three elimination phases were identified for MTBE in the blood, with average half-lives of 5.5 minutes, 2.0 hours, and 6.7 hours. The absorbed amount of MTBE was estimated to be 2.2 mg. Skin permeability ( $K_p$ ) was 0.028 cm/hour. Similar  $K_p$  values were calculated for rat skin permeability (0.11–0.28 cm/hour at 4 mg/mL, 0.23–0.58 cm/hour; [Johanson and Rauma, 2008](#)), based on data reported by [Miller et al. \(1997\)](#) [The Working Group considered that these  $K_p$  values corresponded to “very high” permeability ([Johanson and Rauma, 2008](#)).]

(d) *Oral exposure, controlled studies*

[Amberg et al. \(2001\)](#) studied the disposition of ingested MTBE in six healthy volunteers (three men and three women). The volunteers were exposed at an interval of 4 weeks to <sup>13</sup>C-labelled MTBE at doses of 5 mg (57 µmol) and 15 mg (170 µmol) dissolved in 100 mL of drinking-

water. The average maximum MTBE level in blood was 0.10 µM after the lower dose, and 0.69 µM after the higher dose. Three elimination half-lives were found, with average values of 0.7 hours, 1.2 hours, and 3.7 hours for the higher dose and 0.8 hours, 1.8 hours, and 8.1 hours for the lower dose. The maximum concentrations in the blood and the elimination half-life of TBA were, respectively, 0.45 µM and 8.1 hours after the lower dose of MTBE, and 1.82 µM and 8.5 hours after the higher dose. Metabolites determined in the urine were 2-HIBA (36–40% of the administered dose; half-life 17.4–17.8 hours), MPD (33–40%; 10.7–14.2 hours), TBA (0.9–1.3%; 7.7–8.1 hours) and MTBE. The total recovery in urine was 46–49% of the administered dose. Exhalation of MTBE and TBA was measured in the three men given the higher dose. Recoveries amounted to 26% and 6%, respectively. The decreases in MTBE and TBA concentrations in exhaled air paralleled those in the blood.

[Prah et al. \(2004\)](#) studied the disposition of MTBE in 14 healthy male volunteers after controlled exposures by inhalation, dermal, and oral administration. The inhalation and dermal experiments are described in Sections 4.1.1(b) and (c). In the oral experiments, the study participants received MTBE in a lemon–lime commercial drink, because of the objectionable taste of MTBE. The mean final dose was 11.1 mg/L (2.8 mg). Maximum concentrations of MTBE and TBA in the blood were 0.17 µM and 0.23 µM, respectively. Three elimination phases were identified for MTBE in the blood, with half-lives of 15 minutes, 1.7 hours, and 7 hours ([Prah et al., 2004](#)). The authors noted that the late phase of the post-exposure decline in blood and breath MTBE levels was parallel for the three exposure routes; however, the higher AUC ratio of blood TBA:MTBE in the oral experiments indicated greater biotransformation to TBA, implying significant first-pass metabolism. [The Working Group noted that, although the slower elimination of TBA (compared with MTBE) made it a



better biomarker of MTBE exposure, its relevance might vary according to exposure route because of first-pass metabolism.] The authors also noted that most of the study participants had a baseline blood concentration of TBA of 1–3 µg/L ([Prah et al., 2004](#)).

#### 4.1.2 Experimental systems

The toxicokinetics of MTBE in experimental systems were described in *IARC Monographs Volume 73* ([IARC, 1999a](#)). More recent studies are described in the following sections, along with some studies that were not included in the previous monograph.

##### (a) Non-human mammalian systems in vivo

In vivo experiments in rats showed that MTBE can be readily absorbed via inhalation and dermal administration, and completely absorbed via oral administration ([IARC, 1999a](#)). The toxicokinetics were essentially similar for all three exposure routes, and no difference was observed between the sexes. No metabolic saturation was reported after oral or dermal doses of up to 400 mg/kg bw; however, inhalation exposure to MTBE at up to 8000 ppm for 6 hours resulted in saturation, in that a larger fraction of the absorbed dose was excreted as unchanged MTBE via the breath, and smaller fractions were excreted as metabolites via the urine, compared with exposure at 400 ppm. Only a small fraction (<2%) was excreted via the faeces. MTBE was also reported to be oxidatively demethylated to TBA, which is then either conjugated with sulfate or glucuronide, or further metabolized to 2-HIBA and MPD, the last two being the major metabolites excreted in the urine, followed by TBA–glucuronide and TBA–sulfate. Only minute amounts of MTBE and TBA were excreted in the urine. Repeated exposure to MTBE at up to 300 ppm for 6 hours per day for up to 15 weeks showed no change in blood MTBE levels over

weeks, suggesting no or limited bioaccumulation or induction of metabolism ([IARC, 1999a](#)).

##### (i) Exposure by inhalation

[Amberg et al. \(1999\)](#) exposed 10 F344 NH rats (five males and five females) to MTBE at 4.5 ppm and 39 ppm in an inhalation chamber for 4 hours. The estimated absorption was 3.8 µmol at 4.5 ppm, and 33 µmol at 39 ppm. The concentration of MTBE in the blood, measured at 4 hours, was 2.3 µM at 4.5 ppm and 5.9 µM at 39 ppm, whereas that of TBA was 2.9 µM and 36.7 µM, respectively.

The MTBE half-life in blood was 0.5 hours for the exposure at 39 ppm (the half-life for TBA, in blood was not determined). After exposure to MTBE at 39 ppm, the average quantity of metabolites excreted in the urine, and the corresponding average half-lives, were: TBA, 1.2 µmol (3.6% of the absorbed dose), 4.5 hours; MPD, 2.8 µmol (8.5%), 3.4 hours; and 2-HIBA, 11.1 µmol (33.6%), 4.4 hours. [The Working Group noted that considerable amounts of metabolites were found in the non-exposed control rats. MTBE levels in the urine were close to the LOD in both exposed and control rats.]

[Benson et al. \(2003\)](#) exposed 43 F344 male rats to <sup>14</sup>C-labelled MTBE at 4, 40, or 400 ppm, and to the light fraction of unleaded gasoline (LFG) at 20 and 200 ppm (containing <sup>14</sup>C-labelled MTBE at 4 and 40 ppm, respectively), via the nose only, for 4 hours. The rats were also exposed to LFG at 20 and 200 ppm for 4 hours per day, for 7 days. Analysis of the radioactivity showed that MTBE equivalents (measured as <sup>14</sup>C activity) rapidly distributed to all tissues examined, the largest fraction being distributed to the liver. With the increase in the exposure from 4 ppm to 40 ppm, and up to 400 ppm, the fraction of MTBE equivalents was reduced in all tissues examined (lung, liver, kidney, heart, brain, and testis) both after 4 hours and after 72 hours. Furthermore, the concentration of MTBE equivalents in the blood increased more than proportionally with



the exposure level, and the elimination half-life increased; the effects were small at 40 ppm and marked at 400 ppm, compared with 4 ppm.

Both acute and repeated exposure to LFG at 200 ppm, when compared with exposure to MTBE alone at 40 ppm, caused a decrease in the half-life of elimination from the blood, with a reduction in the fraction of MTBE equivalents disposed in the tissues. [The Working Group considered that this result suggested that the components of LFG inhibited the metabolism of MTBE. In contrast, repeated co-exposure to LFG caused a decrease in the body burden of MTBE compared with acute co-exposure, suggesting that the components of LFG caused induction of the metabolism of MTBE.]

#### (ii) Oral exposure

[Prescott-Mathews et al. \(1999\)](#) studied the interaction between orally administered MTBE and  $\alpha_{2u}$ -globulin in male and female F344 rats. Four males and four females were treated by gavage with  $^{14}\text{C}$ -labelled MTBE (dissolved in 10% Emulphor) at a dose of 750 mg/kg bw or with vehicle only for 4 days. The total radioactivity recovered was similar in kidney samples from males and females. Gel filtration with G-25 showed that a slightly higher fraction of the radioactivity coeluted with the protein fraction in male rats than in female rats. However, gel filtration with G-75 and anion exchange chromatography did not demonstrate any coelution of radioactivity with the low-molecular-weight protein fraction or the  $\alpha_{2u}$ -globulin fraction, respectively, in kidney samples from either males or females. The authors reported that these findings indirectly supported the hypothesis that  $\alpha_{2u}$ -globulin-mediated mechanism is a cause of MTBE-induced protein droplet nephropathy in male rats.

Male Sprague-Dawley rats were exposed orally to MTBE at a dose of 0, 250, 500, 1000, or 1500 mg/kg bw, in corn oil, daily for 15 or 28 days ([Williams and Borghoff, 2000](#)). Total

hepatic microsomal cytochrome P450 (CYP) concentration increased by approximately 33% in the group at the highest dose, at 15 days. Regarding specific CYP activities, increases of 0.7- to 5.5-fold were observed for several CYPs (2B1/2, 1A1/2, 2A1, 3A1/2, and C2E1) and for UDP-glucuronosyltransferase (UGT). MTBE also induced its own metabolism, which increased by onefold in the group at the highest dose, at 28 days. The results indicated that MTBE induces selected enzymes involved in testosterone metabolism. The authors suggested that the decrease in serum testosterone observed in a previous study ([Williams et al., 2000](#)) after exposure to MTBE may have been the result of enhanced testosterone metabolism and subsequent clearance.

[Elovaara et al. \(2007\)](#) investigated the effects of orally administered MTBE on triglyceridaemia, liver toxicity, and the induction of CYP2E1, CYP2B1, and phase II enzymes in female Wistar rats ( $n = 4$  per test group). Enzyme induction was studied by enzyme activity and immune-system inhibition assays. Exposure to MTBE (dose range, 10–20 mmol/day per kg bw, for 3 days) resulted in hepatomegaly and induction of CYP activity. MTBE caused an increase of 0.6-fold in CYP2E1 activity and an increase of up to 20-fold in CYP2B1 activity in MTBE-treated rats. Slight or no modifying effects were observed on the activities of the phase II enzymes NADPH:quinone oxidoreductase (NQO1), glutathione *S*-transferase (GST), and UDP-glucuronosyltransferase (UGT). MTBE alone did not induce hypertriglyceridaemia. There was no marked potentiation of drug-/chemical-induced acute liver damage. [The Working Group noted that the study compared the effects of MTBE on lipid metabolism with those exerted by TAME, another oxygenated additive. MTBE differed from TAME in the level of induction of specific CYP activities and of hypertriglyceridaemia (see Section 4.1.2 of the monograph on TAME in the present volume).]

**Table 4.2 In vitro partition coefficients for MTBE and TBA (rounded average values)**

| In vitro partition coefficient |           |            |         |                   |                   | Reference                                |
|--------------------------------|-----------|------------|---------|-------------------|-------------------|------------------------------------------|
| Measured                       |           |            |         | Calculated        |                   |                                          |
| Blood:air                      | Water:air | Saline:air | Oil:air | Oil:blood         | Fat:blood         |                                          |
| MTBE                           |           |            |         |                   |                   |                                          |
| 17.7 <sup>a</sup>              | NR        | 15.2       | 120     | 6.8 <sup>a</sup>  | 5 <sup>a</sup>    | <a href="#">Nihlén et al. (1995)</a>     |
| 11.5 <sup>b</sup>              | NR        | 11.9       | 125     | NR                | NR                | <a href="#">Borghoff et al. (1996)</a>   |
| 14.2 <sup>b</sup>              | 15.7      | NR         | 115     | NR                | NR                | <a href="#">Kaneko et al. (2000)</a>     |
| 20 <sup>a</sup>                | 23        | 23         | 280     | 14 <sup>a</sup>   | NR                | <a href="#">Vainiotalo et al. (2007)</a> |
| TBA                            |           |            |         |                   |                   |                                          |
| 462 <sup>a</sup>               | NR        | 603        | 168     | 0.36 <sup>a</sup> | 0.65 <sup>a</sup> | <a href="#">Nihlén et al. (1995)</a>     |
| 531 <sup>b</sup>               | 638       | NR         | 198     | NR                | NR                | <a href="#">Kaneko et al. (2000)</a>     |
| 1620 <sup>a</sup>              | 1770      | NR         | 1580    | 0.98 <sup>a</sup> | NR                | <a href="#">Vainiotalo et al. (2007)</a> |

MTBE, methyl *tert*-butyl ether; NR, not reported; TBA, *tert*-butyl alcohol.

<sup>a</sup> Pooled human blood.

<sup>b</sup> Rat blood.

(b) *Non-human mammalian systems in vitro*

(i) *Tissue partitioning*

See [Table 4.2](#).

Using HS-GC, [Nihlén et al. \(1995\)](#) determined in vitro partition coefficients ( $K_{pc}$ ) in blood:air, physiological saline:air, and olive oil:air for MTBE, the ethers ETBE and TAME, and for the metabolite TBA. The analysis was performed using pooled human blood. In addition, tissue:blood partition coefficients were calculated for brain, fat, kidney, liver, lung, and muscle in humans. No significant sex difference was seen with respect to the blood:air partition coefficient.

[Borghoff et al. \(1996\)](#) determined tissue:air partition coefficients as a basis for physiologically based pharmacokinetic modelling (PBPK) (see Section 4.1.2(c)) using blood, liver, kidney, muscle, and adipose tissue from male F344 rats, as well as physiological saline and olive oil.

[Kaneko et al. \(2000\)](#) determined liquid:air partition coefficients for the same three ethers and for the main metabolites (TBA and TAA). The liquids and rat tissues assessed in the study included water, olive oil, blood, muscle, brain, kidney, liver, testis, and fat. The values were

similar to those observed by [Nihlén et al. \(1995\)](#) for MTBE and TBA.

[Licata et al. \(2001\)](#) used tissues sampled from accident victims to determine kidney:air and fat:air partition coefficients using the headspace equilibration technique by [Gargas et al. \(1989\)](#): the mean values obtained were 12.8 (three male and three female donors) and 84.8 (three male donors) respectively.

[Vainiotalo et al. \(2007\)](#) determined liquid:air and tissue:air partition coefficients for MTBE, TBA, TAME, and TAA by HS-GC according to the method of Sato and Nakajima. The liquids tested were water, physiological saline, pooled human blood, and olive oil. The coefficients for MTBE and TBA were higher or much higher than values reported in the previous two studies.

Partition coefficient values reported by [Nihlén et al. \(1995\)](#), [Borghoff et al. \(1996\)](#), and [Kaneko et al. \(2000\)](#) showed good agreement, whereas those reported by [Vainiotalo et al. \(2007\)](#) were higher or far higher.

[Poet and Borghoff \(1997\)](#) used a vial equilibration technique to study uptake of MTBE in the male rat kidney and to evaluate interaction with the male rat-specific protein,  $\alpha_{2u}$ -globulin.

MTBE partitioning and binding studies were carried out with freshly prepared, untreated, and heated (60°C) kidney homogenates from male and female rats. The tissue:air partition coefficients were markedly higher in untreated male kidney than in female untreated kidney (66 versus 12), and they were higher in heated male kidney than in heated female kidney homogenates (36 versus 12) (see also Section 4.3).

## (ii) Metabolism

As described previously, experiments with microsomal preparations have shown that oxidative demethylation of MTBE to TBA results in the formation of formaldehyde ([IARC, 1999a](#)).

[Hong et al. \(1999\)](#) used liver microsomes prepared from female 2E1 knockout mice (2E1<sup>-/-</sup>) (age, 7 weeks) to assess the contribution of CYP2E1 to the metabolism of MTBE, ETBE, and TAME. Lack of CYP2E1 activity was confirmed with the *N*-nitrosodimethylamine (NDMA) demethylation assay. The levels of ether-metabolizing activity did not differ significantly from those in age- and strain-matched 2E1<sup>+/+</sup> mice. [The Working Group noted that the results showed that CYP2E1 plays a negligible role in the metabolism of MTBE, ETBE, and TAME in the mouse liver.]

[Le Gal et al. \(2001\)](#) studied the metabolism of MTBE and the other gasoline oxygenate ethers, ETBE and TAME, in microsomes prepared from 12 human liver samples. The data were fitted to a two-site model, yielding apparent  $K_m$  values of 0.25 and 2.9 mM, and  $V_{max}$  values of 0.81 and 3.5 nmol/minute per mg protein. [The Working Group noted that the kinetic data, correlation studies, chemical inhibition, and metabolism by heterologously expressed human CYPs supported the assertion that the major enzyme involved in MTBE metabolism is CYP2A6, with a minor contribution from CYP3A4 at low substrate concentrations.] In a later study using 14 human liver samples, the same group found

24-fold variation in CYP2A6 activity in the O-demethylation of MTBE ([Le Gal et al., 2003](#)).

[Shamsipur et al. \(2012\)](#) investigated the metabolism of MTBE by commercial human CYP2A6 using HS-GC-MS analysis. The  $K_m$  was 3.13 mM,  $V_{max}$  was 0.137 mM minute<sup>-1</sup>, and  $V_{max}/K_m$  was 0.044 minute<sup>-1</sup>, as calculated with a Lineweaver–Burk equation. [The Working Group noted that  $V_{max}$  and  $V_{max}/K_m$  were difficult to compare in this study and in the study by [Le Gal et al. \(2001\)](#) because the amount of enzyme was not reported.]

## (c) Toxicokinetics and physiologically based pharmacokinetic models

[Borghoff et al. \(1996\)](#) developed a PBPK model to address the toxicokinetics of MTBE and TBA in male F344 rats. The model consisted of six compartments for MTBE (lung, rapidly perfused, slowly perfused, fat, kidney and liver) and six (same as for MTBE) or two (total body water and liver) for TBA. Values from the literature were used for physiological parameters, and the authors measured tissue partition coefficients (see Section 4.1.2(b)) in vitro by a vial equilibration technique and metabolic rates by closed chamber gas uptake studies in vivo in male rats (see Section 4.1.2(a)). The metabolism of MTBE was described by two saturable pathways. The model could predict MTBE concentrations in air in the gas uptake experiments (100–2000 ppm initial concentration) and MTBE in the blood of rats exposed by inhalation (400–8000 ppm, 6 hours), intravenously (40 mg/kg), and orally (40 or 400 mg/kg). The two-compartment model could predict TBA concentrations in the blood during and after a 6-hour exposure at 400 ppm, but underpredicted concentrations after exposure at 8000 ppm. The authors concluded that the toxicokinetics of TBA were more complex than those of MTBE, and that this part of the model needed refinement.

[Rao and Ginsberg \(1997\)](#) used a rat and human PBPK model for MTBE and TBA to assess acute effects on the central nervous system (CNS) during bathing/showering in MTBE-contaminated water. The model had eight compartments for MTBE (lungs, same rapidly perfused tissues, slowly perfused tissues, fat, kidney, skin, brain, and liver) and eight for TBA (same as MTBE). Model parameters were collected from the literature. The skin permeability of MTBE was calculated from the log  $K_{ow}$  (octanol:water partition coefficient) and molecular weight. The model could accurately predict rat data on MTBE in the blood (400 and 8000 ppm for 6 hours) and human data on TBA in the blood (25 and 50 ppm MTBE for 2 hours) but overpredicted human data on MTBE in the blood (1.7 ppm for 1 hour, 5 and 50 ppm for 2 hours). The rat PBPK analysis suggested that the acute CNS toxicity seen after MTBE exposure was mainly caused by MTBE itself, since the internal dose of TBA was below that needed for CNS effects. By integrating inhalation and dermal exposure, the PBPK model further suggested that bathing (adults and children aged 3–10 years, for 10 minutes) or showering (adults, age 10 years, 15 minutes) in water containing MTBE at 1 mg/L would produce brain concentrations approximately 1000 times as low as the no-observed-adverse-effect level (NOAEL) for acute CNS effects in animals and twofold below the brain concentration achieved during exposure to MTBE at 2 ppm, the acute minimum risk level developed by the ATSDR.

[Licata et al. \(2001\)](#) used the [Borghoff et al. \(1996\)](#) PBPK model to investigate the influence of variability in MTBE metabolism on MTBE blood levels in humans. Following Michaelis–Menten kinetics, the model had six compartments and two saturable metabolic pathways in the liver. Physiological parameters were taken from the literature. Partition coefficients were from [Nihlén et al. \(1995\)](#), except for those in human kidney and adipose tissue, which were

determined within the study (see Section 4.1.2(b)). The four metabolic parameters ( $V_{max1}$ ,  $K_{m1}$ ,  $V_{max2}$ ,  $K_{m2}$ ) were obtained by extrapolating the in vitro constants reported by [Poet and Borghoff \(1998\)](#), who used liver microsomes prepared from five men and five women. The metabolic parameters were further refined by simultaneous fitting to three toxicokinetic studies in humans exposed by inhalation ([Cain et al., 1996](#); [Amberg et al., 1999](#)) using maximum likelihood techniques. Extrapolated to a hypothetical human weighing 70 kg, the mean in vitro constants were as follows:  $V_{max1}$ , 34 (range, 4.5–118; maximum likelihood estimate, 35)  $\mu\text{mol/kg per hour}$ ;  $V_{max2}$ , 6.2 (2.7–10.2; 8.6)  $\mu\text{mol/kg per hour}$ ;  $K_{m1}$ , 62 (48–94; 61)  $\mu\text{M}$ ; and  $K_{m2}$ , 3.8 (2.0–5.2; 5.0). Thus, the parameters varied by between 2-fold ( $K_{m1}$ ) and 25-fold ( $V_{max1}$ ) among the 10 liver samples. The authors concluded that the PBPK model could accurately predict the venous blood levels of MTBE in the experimental data for exposure at 1.7 ppm and 40 ppm but markedly underpredicted the data for 4 ppm. [The Working Group noted that it was unclear how optimization would have improved the  $V_{max}$  estimates, since the exposure levels in the human studies (1.7, 4, and 40 ppm) were far below metabolic saturation. Furthermore, the Working Group noted that a problem with the comparisons between the modelled and experimental data was that mixed venous blood was used in the model whereas antecubital venous blood was used in the experiments. Thus, under resting conditions, blood flow in the arm is low, and the MTBE level in antecubital venous blood would reflect that in the muscles rather than in mixed venous blood.] Moreover, the blood levels measured in the experiments varied more, by approximately twofold, than was predicted by the PBPK model using the ranges for metabolic parameters obtained in vitro with the 10 samples of human liver. [The Working Group noted that in the simulation of variability the authors used only minimum and maximum values of  $V_{max1}$ , keeping all other model parameters fixed,



including mean values for the other three metabolic parameters. This may severely underestimate the “true” variability. Another explanation that was not addressed by the authors was that variability in MTBE levels in antecubital blood may be caused by changes in physical workload and resulting variability in blood flow.] The variability in blood levels of MTBE in the study by Nihlén et al. in 10 male volunteers exposed to MTBE at 25 and 50 ppm for 2 hours during exercise (50 W) and with sampling of arterialized capillary blood was about  $\pm 10\%$ , expressed as 95% confidence intervals (Nihlén et al., 1998b). This variability was more similar to that simulated by Licata et al. (2001).

Dennison et al. (2003, 2004a, b) developed a PBPK model for the disposition of gasoline vapour. These studies are described in Section 4.1.2(c) of the monograph on automotive gasoline in the present volume.

Kim et al. (2007) developed a multi-route human PBPK model for MTBE and TBA to account for exposure by inhalation and dermal and oral administration. The model had nine systemic compartments for MTBE (lungs, skin, fat, kidney, stomach, intestine, liver, rapidly perfused tissue, and slowly perfused tissue) and three for TBA (central blood, other tissues, and liver) linked via metabolism in the liver. A major difference from previous models was that three rate constants were introduced to improve the toxicokinetic description after oral intake: uptake from the stomach to the liver, transport from the stomach to the intestine, and uptake from the intestine to the liver. Physiological parameters were obtained from the literature. [The Working Group noted that the complexity of the model, limiting the interpretation of the results, was difficult to evaluate because: (i) nearly all chemical-dependent parameters were fitted using the same experiments that were then used for comparing predictions with experimental data; (ii) it was unclear why these parameters were fitted at a time when *in vitro* parameters

had already been published, in particular, partition coefficients (see Section 4.1.2a); and (iii) the goodness of fit was difficult to assess because the time scales (*x* axes) in the graphs comparing observed and predicted values were logarithmic.]

Zhang et al. (2006) used a three-dimensional (3D) computational fluid (CFD) and particle dynamics model to describe the transport and uptake of MTBE and ethanol vapour in the upper airways in humans. The model consisted of an oral airway (oral cavity, pharynx, larynx, and trachea) and the first four generations of the tracheobronchial tree. The total deposited fractions at the five levels were 22%, 12%, and 7% for MTBE and 68%, 52%, and 39% for ethanol at air flow rates of 15, 30, and 60 L/min, respectively. Thus, it was reported that MTBE penetrated deeper into the lower airways than did ethanol. Of the total deposited fraction, 65–70% was deposited in the oral airway for both vapours.

Pleil et al. (2007) used a simple inhalation toxicokinetic model with two compartments for MTBE (and one for TBA or 2-HIBA) to examine how spot measurements of the two chemicals could be expected to predict different hypothetical scenarios of previous exposures. The model assumed inhalation only and first-order (non-saturated) metabolism. Rate constants for MTBE (and TBA) were obtained by best fit to previously published experimental data on inhalation in humans from 14 men exposed to MTBE at 3 ppm for 1 hour (Prah et al., 2004) (see Section 4.1.1). Similarly, rate constants for 2-HIBA were obtained by fitting to the data reported by Amberg et al. (1999, 2001). Not surprisingly, the authors found that blood and breath biomarker measurements gave similar results and that the biological damping effect (longer half-life) of TBA and 2-HIBA gave more accurate estimates of previous exposures. The relationships between exposure to MTBE by inhalation and levels of biomarkers (MTBE and TBA) in breath and blood and 2-HIBA in urine were investigated using four exposure scenarios,

all with a duration of 120 hours: intermittent constant 1 hour every 8 hours, occupational random 8 hours per day for 5 days, environmental random intermittent, and environmental random intermittent smoothed. The calculations gave the following relations: mean MTBE exposure = (MTBE in breath)  $\times$  4.11 = (TBA in breath)  $\times$  25.8 = (MTBE in blood)  $\times$  0.243 = (TBA in blood)  $\times$  0.0355 = (2-HIBA in urine)  $\times$  0.162. The authors concluded that, as an exposure marker, the assessment of MTBE levels in blood or breath was no more relevant than was the measurement of air samples. On the other hand, TBA or 2-HIBA levels in blood were relevant; because of their longer half-lives, it was possible to back-calculate the average exposure to MTBE to within  $\pm$  20%.

[Leavens and Borghoff \(2009\)](#) expanded their previous rat PBPK model ([Borghoff et al., 1996](#); [Rao and Ginsberg, 1997](#)) to include binding of MTBE to the male rat-specific protein  $\alpha_{2u}$ -globulin in the kidney (see Section 4.3). Metabolism of MTBE was assumed to occur only in the liver via two saturable pathways. TBA metabolism was assumed to occur only in the liver via one saturable, low-affinity pathway and to be induced by repeated exposure. The binding of MTBE and TBA to  $\alpha_{2u}$ -globulin was modelled as saturable and competitive and was assumed to affect only the rate of hydrolysis of  $\alpha_{2u}$ -globulin. The eight parameters related to  $\alpha_{2u}$ -globulin (initial concentration and hydrolysis of free and bound  $\alpha_{2u}$ -globulin, binding and unbinding of MTBE and TBA) were obtained by best fit to existing toxicokinetic data in rats, as were two parameters describing the induction of TBA metabolism. The model could accurately predict the time courses of MTBE and TBA observed for blood, brain, kidney, and liver in male and female rats exposed at 100–3000 ppm MTBE for 6 hours (data from the study by [Prescott-Mathews et al., 1997](#)). The model developed described the levels of MTBE and TBA in males and females, the role of MTBE and TBA binding to  $\alpha_{2u}$ -globulin in

male rats, and the observed changes in blood and tissue concentrations after repeated exposure to TBA. The predicted binding affinity of MTBE to  $\alpha_{2u}$ -globulin was greater than that of TBA, and the hydrolysis rate of chemically bound  $\alpha_{2u}$ -globulin was lower than that of unbound protein. The model predicted that increases in total kidney  $\alpha_{2u}$ -globulin after 10 days of inhalation exposure to MTBE at 400, 1500, and 3000 ppm were 9%, 17%, and 27%, respectively. The predicted increases were slightly lower than the experimentally measured values of 10–13%, 19–25%, and 31–55% ([Prescott-Mathews et al., 1997](#)).

[Borghoff et al. \(2010\)](#) expanded the rat PBPK model developed by [Leavens and Borghoff \(2009\)](#) by incorporating four additional experimental studies with a focus on toxicokinetic data on MTBE and TBA in the kidney. Male and female F344, Wistar Han, and Sprague-Dawley rats were exposed by gavage to MTBE at a dose of 250 or 1000 mg/kg in 2% Emulphor or olive oil. Optimization in the PBPK model yielded oral absorption rate constants ( $K_a$ ) of 0.59 (males) and 0.72 hour<sup>-1</sup> (females) for Emulphor. The corresponding  $K_a$  values for olive oil were 0.51 and 0.64 hour<sup>-1</sup>, respectively. In a palatability study, male and female F344 rats were exposed to drinking-water containing MTBE at 0, 1.5, 3.0, 7.0, or 15 mg/mL in for 28 days. The concentrations of MTBE in male kidney and in female kidney were overpredicted by the PBPK model, compared with the experimental data, whereas TBA in female kidney showed good agreement. In a second palatability study in male and female Wistar Han rats, the model predicted far higher tissue levels of MTBE at all doses in male than in female kidney. The paper also mentions 14-day and 13-week toxicity studies in Wistar Han rats. [The Working Group noted that it was not clear if and how these data were incorporated in the model.] The model predicted that approximately 80% of the MTBE administered dose was exhaled after exposure to MTBE at 7.5



or 15 mg/mL in drinking-water, an oral dose of 250 mg/kg, and inhalation at 400 ppm. At higher doses (8000 ppm for inhalation exposure and 1000 mg/kg by oral gavage), the percentage exhaled increased to 88–93%. Lower exposure concentrations (i.e. drinking-water, 0.5 mg/mL) administered at a slower rate tended to result in a higher percentage of the MTBE dose being metabolized when compared with higher concentrations administered at a higher rate (1000 mg/kg). [The Working Group noted that this was caused by the increased influence of metabolic saturation at the higher doses.]

### Synopsis

[The additional human toxicokinetics studies published in the last 25 years confirm and strengthen the information reported in the previous monograph (Volume 73; [IARC, 1999a](#)). MTBE is readily absorbed via all relevant routes of exposure (inhalation, oral, and dermal) and rapidly cleared from the blood in several phases, with half-lives ranging from minutes to several hours; this reflects distribution to other tissues, exhalation, and metabolism. MTBE is oxidatively demethylated to TBA, which is either conjugated or further metabolized; 2-HIBA and MPD are the major metabolites of TBA. The conjugates and metabolites are excreted in the urine. A major portion of the absorbed dose is also excreted as unchanged MTBE via exhaled breath. Only a small fraction is excreted in the urine as MTBE or TBA. Formaldehyde is formed in two steps, in the demethylation of MTBE to TBA and the breakdown of TBA. Most if not all metabolism of MTBE occurs in the liver. Physical properties, PBPK modelling (see Section 4.1.2(c)), as well as scattered experimental data suggest that MTBE and its metabolites are all distributed throughout the body. The kinetics appear to be linear (i.e. internal concentrations and metabolic rates proportional to dose) up to 75 ppm, the highest exposure level tested.

Tissue distribution of MTBE metabolites (measured as  $^{14}\text{C}$ ) has been demonstrated in the lung, liver, kidney, heart, brain, and testis of rats after exposure by inhalation or oral gavage. Disproportional increases in levels of MTBE equivalents in the blood, and increased elimination half-lives indicated metabolic saturation that becomes marked at 400 ppm. Acute co-exposure to LFG (light fraction of gasoline plus MTBE) caused a disproportional increase in MTBE body burden, suggesting competitive inhibition of MTBE metabolism. Repeated co-exposure to LFG, on the other hand, caused decreased body burdens of MTBE compared with acute co-exposure, suggesting induction of MTBE metabolism. Several groups have studied water:air, olive oil:air, and tissue:air partition coefficients for MTBE (and TBA) using vial equilibration techniques. The tissues include blood, muscle, brain, kidney, liver, testis, and fat. One group studied the uptake of MTBE in the kidney and binding to male rat  $\alpha_{2u}$ -globulin.

In vivo and in vitro studies with liver microsomes showed that MTBE induces the activation of several CYP enzymes and enzymes involved in testosterone metabolism, and it also induces its own metabolism. Experiments with microsomes prepared from human liver yielded  $V_{\max}$  and apparent  $K_m$  values for MTBE. Additional in vitro experiments showed that CYP2A6 is the major enzyme involved in the metabolism of MTBE, with a minor contribution from CYP3A4 and a negligible role of CYP2E1 (at least in mice), and that CYP2A6-mediated demethylation of MTBE varies widely in human liver samples.

The reported data on partitioning, uptake, and metabolism have been used in PBPK modelling for MTBE. PBPK models have been developed that can adequately describe the toxicokinetics of MTBE and TBA after intravenous, oral, dermal, and inhalation exposure in rats. One group included binding of MTBE to the male rat-specific protein  $\alpha_{2u}$ -globulin in the kidney in their model. Human models have been

introduced to describe the experimental data on inhalation in humans and to characterize exposure during bathing and showering with MTBE-contaminated water.

A two-compartment model was used to calculate the relations between MTBE in inhaled air and biomarker levels (MTBE and TBA in blood and exhaled breath, 2-HIBA in urine).]

## 4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens ([Smith et al., 2016](#)) encompassed by the agent MTBE. Evidence was available for the key characteristics “is electrophilic or can be metabolically activated to an electrophile”, “is genotoxic”, “alters DNA repair and causes genomic instability”, “induces epigenetic alterations”, “induces oxidative stress”, “induces chronic inflammation”, “is immunosuppressive”, “modulates receptor-mediated effects”, and “alters cell proliferation, cell death, or nutrient supply”. There were no data for the key characteristic “causes immortalization”. Where available, the studies in exposed humans used to support the mechanistic evidence were evaluated for the quality of the study design, exposure assessment, and assay accuracy, and precision.

### 4.2.1 *Is electrophilic or can be metabolically activated to an electrophile*

#### (a) *Humans*

No studies were available to the Working Group.

#### (b) *Experimental systems*

##### (i) *Non-human mammalian systems in vivo*

See [Table 4.3](#).

Two studies ([Du et al., 2005](#); [Yuan et al., 2007](#)) indicated formation of MTBE-derived DNA adducts in the liver, lung, and kidney of mice exposed to MTBE at a single dose of 1–2 µg/kg bw. [Du et al. \(2005\)](#) also performed a decay study and observed that levels of MTBE-derived DNA adducts peaked at 6 hours post-administration in the liver and at 12 hours in the lung, then dropped rapidly until 5 days post-administration, and declined thereafter more slowly ([Du et al., 2005](#)).

##### (ii) *Non-human mammalian systems in vitro*

[Casanova and Heck \(1997\)](#) investigated mechanisms of MTBE-induced hepatocarcinogenesis in vitro. They concluded that: (i) metabolism of MTBE to formaldehyde in mouse and rat hepatocytes reaches a maximal rate at low concentrations of airborne MTBE; (ii) the metabolism of MTBE to formaldehyde is slow relative to the rate of oxidation of formaldehyde, which results in only small amounts of covalent binding of formaldehyde to DNA or RNA, but in relatively large amounts of metabolic incorporation of <sup>14</sup>C into RNA, via [<sup>14</sup>C]formate, the oxidation product of [<sup>14</sup>C]formaldehyde; and (iii) there was no obvious species- or sex-specificity with respect to DNA–protein cross-links or RNA–formaldehyde adduct formation from MTBE, when hepatocytes from female CD-1 mice, male B6C3F<sub>1</sub> mice, and male F344 rats were used.

**Table 4.3 End-points relevant to DNA adducts in non-human mammalian systems in vivo exposed to methyl *tert*-butyl ether**

| End-point   | Species, strain (sex) | Tissue | Results <sup>a</sup> | Dose (LED or HID) | Route, duration, dosing regimen                                                                                                                                                                                                                     | Comments                                                                                   | Reference                          |
|-------------|-----------------------|--------|----------------------|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|------------------------------------|
| DNA adducts | Mouse, Kunming (M)    | Kidney | (+)                  | 0.95 µg/kg        | Gavage, single dose<br>Dose-response study:<br>MTBE, 0.95, 5.71, and 75.59 µg/kg bw and mice euthanized at 2, 6 and 12 h post-administration<br>Decay study:<br>MTBE, 1.09 and 6.18 mg/kg bw and mice euthanized at 1, 3, 5, 8, 11, 15, and 21 days | <sup>14</sup> C-labelled MTBE tested; two regimes tested – dose-dependent and decay study. | <a href="#">Du et al. (2005)</a>   |
|             |                       | Liver  | +                    |                   |                                                                                                                                                                                                                                                     |                                                                                            |                                    |
|             |                       | Lung   | +                    |                   |                                                                                                                                                                                                                                                     |                                                                                            |                                    |
| DNA adducts | Mouse, Kunming (M)    | Kidney | +                    | 1.86 µg/kg        | Gavage, 6 h, single dosage                                                                                                                                                                                                                          | <sup>14</sup> C-labelled MTBE tested.                                                      | <a href="#">Yuan et al. (2007)</a> |
|             |                       | Liver  | +                    |                   |                                                                                                                                                                                                                                                     |                                                                                            |                                    |
|             |                       | Lung   | +                    |                   |                                                                                                                                                                                                                                                     |                                                                                            |                                    |

bw, body weight; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; M, male(s); MTBE, methyl *tert*-butyl ether.

<sup>a</sup> +, positive; (+), positive findings in a study of limited quality.

#### 4.2.2 *Is genotoxic*

##### (a) *Humans*

##### (i) *Exposed humans*

One study in exposed humans was available to the Working Group. [Sørensen et al. \(2003\)](#) measured genotoxicity by comet assay and levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in human lymphocytes and urine collected from 40 volunteers living and working in Copenhagen, and they monitored the volunteers for five consecutive days. The volunteers were exposed to MTBE, toluene, and benzene, at median exposure values of 0.86, 18.7, and 2.5 µg/m<sup>3</sup>. No correlations were observed with the genotoxicity end-points measured by comet assay and biomarkers of exposure ([Sørensen et al., 2003](#)). [The Working Group noted that a significant correlation between SPMA excretion and 8-oxodG in lymphocytes was observed; however, a clear correlation with external exposure to MTBE was not studied. The study is also described in Section 4.2.5 and reported in [Table 4.8](#).]

##### (ii) *Human cells*

See [Table 4.4](#).

In human peripheral blood lymphocytes exposed to MTBE at increasing concentrations (0–200 µM), [Chen et al. \(2008\)](#) observed formation of DNA single-strand breaks (SSB) and double-strand breaks (DSBs), as measured by alkaline and neutral comet assay. The DNA breaks were also observed in the presence of the DNA repair enzyme formamidopyrimidine/8-oxoguanine DNA *N*-glycosylase (Fpg), suggesting that MTBE could induce a variety of DNA damage, including oxidative base modifications (see Section 4.2.5). DNA damage and oxidative DNA damage, as measured by alkaline single-cell gel electrophoresis analysis, were also observed by [He et al. \(2021\)](#) in DNA polymerase-β-deficient human bronchial epithelial cells (16HBE) (DNA polymerase-β knockout cells)

that were exposed to MTBE at a concentration of 50 µmol/L for 24 hours (concentrations tested were 5, 50, 100 µmol/L for 12, 24, 36 hours).

##### (b) *Experimental systems*

See [Tables 4.5](#) and [4.6](#).

##### (i) *Non-human mammalian systems in vivo*

Negative findings for various genotoxicity-associated end-points, including sister-chromatid exchange (SCE), micronucleus formation, DNA migration distance, DNA cross-linking, DNA synthesis, and mutation frequency were reported in non-human mammalian systems in vivo ([US EPA, 1989, 1993b](#); [McKee et al., 1997](#); [Kado et al., 1998](#); [Schreiner et al., 2014](#); [Darwish and Mosallam, 2019](#)). However, induction of chromosomal aberrations in rat bone marrow was observed after exposure to a very high cumulative dose of MTBE (11.2 g/kg bw) over 14 and 28 consecutive days ([Darwish and Mosallam, 2019](#)). Also, positive evidence of SCE was observed in lymphocytes from Sprague-Dawley rats exposed to condensates of gasoline, containing MTBE or another additive, TAME, for > 4 weeks ([Schreiner et al., 2014](#)).

[Yang et al. \(2005\)](#) reported genotoxic effects with MTBE. DNA SSBs, measured as “length of DNA migration”, were increased in tissue from the kidney, lung and liver of mice exposed to MTBE through inhalation (range, 0–4968 mg/m<sup>3</sup>) for 20 days ([Yang et al., 2005](#)). Similarly, DNA strand breaks were induced in lymphocytes from rats treated orally with MTBE ([Alishahi et al., 2020](#)).

In two studies in animals, the maximum exposure dose of MTBE was set at 8000 ppm, based on the US EPA recommendations for 50% of the lower explosion limit of MTBE ([US EPA, 1989, 1993b](#)). There was no evidence of chromosomal damage in rats ([US EPA, 1989](#)), and no increase in the frequency of micronucleated polychromatophilic erythrocytes in mice ([US EPA, 1993b](#)), upon exposure to MTBE at 8000 ppm.

**Table 4.4 End-points relevant to genotoxicity in human cells in vitro exposed to methyl *tert*-butyl ether**

| End-point                         | Tissue, cell line                                                      | Results <sup>a</sup> | Concentration (LEC or HIC) | Comments                                                                                                | Reference                          |
|-----------------------------------|------------------------------------------------------------------------|----------------------|----------------------------|---------------------------------------------------------------------------------------------------------|------------------------------------|
| DNA strand breaks (SSBs and DSBs) | Lymphocytes (primary)                                                  | +                    | 50 µM                      | Both comet assays (alkaline comet assay and neutral comet assay) were tested and gave positive results. | <a href="#">Chen et al. (2008)</a> |
| DNA strand breaks                 | Human lung cell line (16HBE) wildtype or transfected with empty vector | –                    | 100 µmol/L                 | DSBs were observed only in DNA pol-β-deficient cells.                                                   | <a href="#">He et al. (2021)</a>   |
|                                   | DNA pol-β-deficient cells (16HBE cells transfected with DNA pol-β)     | +                    | 5 µmol/L                   |                                                                                                         |                                    |

DNA pol-β, DNA polymerase β; DSB, double-strand break; HIC, highest ineffective concentration; LEC, lowest effective concentration; SSB, single-strand break.  
<sup>a</sup> +, positive, –, negative.

**Table 4.5 End-points relevant to genotoxicity in non-human mammalian systems in vivo exposed to methyl *tert*-butyl ether**

| End-point                                  | Species, strain (sex)       | Tissue                       | Results <sup>a</sup> | Dose (LED or HID)                 | Route, duration, dosing regimen                                                                                         | Comments                                                                                                              | Reference                                   |
|--------------------------------------------|-----------------------------|------------------------------|----------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Chromosomal aberrations                    | Rat (M)                     | Bone marrow                  | +                    | 800 mg/kg bw per day (in 14 days) | Oral gavage, 14 and 28 days, daily exposures                                                                            | MTBE in corn oil.                                                                                                     | <a href="#">Darwish and Mosallam (2019)</a> |
| Chromosomal aberrations                    | Rat, F344 (M, F)            | Bone marrow                  | –                    | 8000 ppm                          | Inhalation 6 h/day, for 5 consecutive days. Euthanasia at 24 h and 48 h post-treatment                                  |                                                                                                                       | <a href="#">McKee et al. (1997)</a>         |
| Chromosomal aberrations                    | Rat, Fischer (M, F)         | Bone marrow                  | –                    | 8000 ppm                          | Inhalation, 6 h/day, for 5 consecutive days. Euthanasia at 6 h or 24 h post-exposure                                    |                                                                                                                       | <a href="#">US EPA (1989)</a>               |
| Chromosomal aberrations                    | Rat, Sprague-Dawley         | Bone marrow                  | –                    | 400 mg/kg                         | Oral gavage, daily, for 5 days                                                                                          | Sex was not reported.                                                                                                 | <a href="#">US EPA (1987)</a>               |
| DNA strand breaks                          | Rat, Wistar (M)             | Blood, lymphocytes           | +                    | 10 mg/kg                          | Oral gavage, daily, for 30 consecutive days                                                                             |                                                                                                                       | <a href="#">Alishahi et al. (2020)</a>      |
| DNA strand breaks (DNA migration distance) | Mouse, Kunming (M, F)       | Kidney                       | (+)                  | 108 mg/m <sup>3</sup>             | Inhalation, 4 h/day, for 20 days                                                                                        | The methodology used to measure DNA breaks was not clearly reported.                                                  | <a href="#">Yang et al. (2005)</a>          |
|                                            |                             | Lung                         | (+)                  | 4968 mg/m <sup>3</sup>            |                                                                                                                         |                                                                                                                       |                                             |
|                                            |                             | Liver                        | (+)                  | 1440 mg/m <sup>3</sup>            |                                                                                                                         |                                                                                                                       |                                             |
| DNA cross linking rate                     |                             | Liver                        | (–)                  | 4968 mg/m <sup>3</sup>            |                                                                                                                         |                                                                                                                       |                                             |
| Micronucleus formation                     | Mouse, CD-1 (M, F)          | Bone marrow                  | –                    | 8000 ppm                          | Inhalation, 6 h/day, for 2 consecutive days, euthanasia at 24 h and 48 h post-treatment                                 | Several relevant end-points were tested.                                                                              | <a href="#">McKee et al. (1997)</a>         |
| Micronucleus formation                     | Mouse, Swiss-Webster (M, F) | Bone marrow                  | – (M)<br>– (F)       | 1.75 g/kg                         | Single i.p. dose; 24 h                                                                                                  |                                                                                                                       | <a href="#">Kado et al. (1998)</a>          |
| Micronucleus formation                     | Rat, Sprague-Dawley (M, F)  | Bone marrow erythrocytes     | – (M, F)             | 20 000 mg/m <sup>3</sup>          | Inhalation, 6 h/day, 5 days/week for a total of 20 exposures over 28 days 13-week studies in rats exposed by inhalation | Inclusion of oxygenates in gasoline did not increase the effects of gasoline alone or produce any cytogenetic hazard. | <a href="#">Schreiner et al. (2014)</a>     |
| Sister-chromatid exchange                  |                             | Peripheral blood lymphocytes | + (M)<br>+ (F)       | 2000 mg/m <sup>3</sup>            |                                                                                                                         | Caused by gasoline alone.                                                                                             |                                             |
| Micronucleus formation                     | Mouse, CD-1 (M, F)          | Bone marrow (PCEs)           | –                    | 8000 ppm                          | Inhalation, 6 h/day, for 2 consecutive days, evaluated at 24 h and 48 h post-exposure                                   | Ratio of PCEs:NCEs was not changed at 8000 ppm.                                                                       | <a href="#">US EPA (1993b)</a>              |



**Table 4.5 (continued)**

| End-point     | Species, strain (sex)         | Tissue                                           | Results <sup>a</sup> | Dose (LED or HID) | Route, duration, dosing regimen              | Comments | Reference                                    |
|---------------|-------------------------------|--------------------------------------------------|----------------------|-------------------|----------------------------------------------|----------|----------------------------------------------|
| Gene mutation | Rat, transgenic Big Blue F344 | Nasal epithelium, bone marrow, liver, and kidney | –                    | 3000 ppm          | Inhalation, 6 h/day, for 28 consecutive days |          | <a href="#">Gollapudi and Rushton (2024)</a> |

bw, body weight; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male(s); MTBE, methyl *tert*-butyl ether; NCE, normochromatic erythrocyte; PCE, polychromatic erythrocyte; ppm, parts per million.  
<sup>a</sup> +, positive, –, negative; (+) or (–), positive or negative findings in a study of limited quality.

**Table 4.6 End-points relevant to genotoxicity in experimental systems in vitro exposed to methyl *tert*-butyl ether**

| End-point<br>(assay)                                | Species, strain, cell, tissue                                    | Results <sup>a</sup>                            |                                                              | Concentration<br>(LEC or HIC) | Comments                                                                                                                    | Reference                                       |                                            |
|-----------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------|--------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|--------------------------------------------|
|                                                     |                                                                  | Without<br>metabolic<br>activation              | With<br>metabolic<br>activation                              |                               |                                                                                                                             |                                                 |                                            |
| Non-human mammalian systems                         |                                                                  |                                                 |                                                              |                               |                                                                                                                             |                                                 |                                            |
| DNA strand breaks<br>(comet assay)                  | Rat fibroblasts (Rat-1 diploid<br>immortalized fibroblasts)      | +                                               | NA                                                           | 0.84 mM                       | MTBE altered the cell cycle, causing<br>an accumulation of cells in S-phase.                                                | <a href="#">Sgambato<br/>et al. (2009)</a>      |                                            |
| Gene mutation<br>(gene mutation assay)              | Mouse lymphoma (L5178Y)                                          | NA                                              | +<br>In the<br>presence of<br>FDH+NAD <sup>+</sup><br>and S9 | 1 µL/mL                       | Mutations were suggested to be<br>caused by formaldehyde formed<br>through microsomal metabolism.                           | <a href="#">Mackerer<br/>et al. (1996)</a>      |                                            |
| Micronucleus formation                              | Mouse embryonal fibroblasts<br>(NIH/3T3)                         | –                                               | –                                                            | 20 µL/mL                      |                                                                                                                             | <a href="#">Zhou et al.<br/>(2000)</a>          |                                            |
| Non-mammalian systems                               |                                                                  |                                                 |                                                              |                               |                                                                                                                             |                                                 |                                            |
| Reverse mutation<br>(Ames test,<br>microsuspension) | <i>Salmonella typhimurium</i> TA100                              | –                                               | –                                                            | MTBE,<br>3700 µg/tube         | Also included in Section 4.2.2(c).                                                                                          | <a href="#">Kado et al.<br/>(1998)</a>          |                                            |
|                                                     | <i>Salmonella typhimurium</i> TA1535                             | –                                               | –                                                            |                               |                                                                                                                             |                                                 |                                            |
|                                                     | <i>Salmonella typhimurium</i> TA104                              | –                                               | –                                                            |                               |                                                                                                                             |                                                 |                                            |
|                                                     | <i>Salmonella typhimurium</i> TA98                               | –                                               | –                                                            |                               |                                                                                                                             |                                                 |                                            |
| Reverse mutation<br>(Ames test)                     | <i>Salmonella typhimurium</i> TA97a,<br>TA98, TA100              | –                                               | –                                                            | 2.5 mg/plate                  |                                                                                                                             | <a href="#">Williams-Hill<br/>et al. (1999)</a> |                                            |
|                                                     | <i>Salmonella typhimurium</i> TA102                              | –                                               | +                                                            | 0.75 mg/plate<br>(LEC)        |                                                                                                                             |                                                 |                                            |
|                                                     |                                                                  | <i>Salmonella typhimurium</i> TA100<br>and TA98 | –                                                            | –                             | 100 µg/plate                                                                                                                |                                                 | <a href="#">Zhou et al.<br/>(2000)</a>     |
|                                                     |                                                                  | <i>Salmonella typhimurium</i> TA100<br>and TA98 | –                                                            | –                             | 15% MTBE                                                                                                                    |                                                 | <a href="#">Westphal<br/>et al. (2010)</a> |
|                                                     | <i>Salmonella typhimurium</i> TA102                              | –                                               | –                                                            | 5000 µg/plate                 |                                                                                                                             | <a href="#">McGregor<br/>et al. (2005)</a>      |                                            |
|                                                     | <i>Salmonella typhimurium</i> TA100,<br>TA98, YG1041, and YG1042 | –                                               | –                                                            | 3 mg/plate                    | Toxicity was tested on seeds of<br><i>Lactuca sativa</i> var. Capitata with<br>negative result, EC <sub>50</sub> = 41.8 mg. | <a href="#">Vosahlíková<br/>et al. (2006)</a>   |                                            |

EC<sub>50</sub>, median effective concentration; FDH+NAD<sup>+</sup>, NAD-dependent formate dehydrogenase; HIC, highest ineffective concentration; MTBE, methyl *tert*-butyl ether; LEC, lowest effective concentration; NA, not applicable; S9, 9000 × g supernatant.

<sup>a</sup> +, positive, –, negative.

No chromosomal aberrations were observed in rats orally exposed to MTBE ([US EPA, 1987](#)). Exposure to MTBE, via whole-body inhalation for 28 consecutive days, did not induce any increase in gene mutation in the nasal epithelium, bone marrow, liver, and kidney of transgenic male Big Blue F344 rats ([Gollapudi and Rushton, 2024](#)).

(ii) *Non-human mammalian systems in vitro*

MTBE induced the formation of DNA SSBs in rat fibroblasts [Sgambato et al. \(2009\)](#). [Mackerer et al. \(1996\)](#) observed an increase in the frequency of gene mutations in mouse lymphoma cells. No increase in micronucleus frequency was observed in NIH/3T3 cells ([Zhou et al., 2000](#)).

(iii) *Non-mammalian systems*

An increase in mutation frequency was reported by [Kado et al. \(1998\)](#), as measured performing the Ames microsuspension test in *Salmonella typhimurium* tester strains TA98, TA100, TA104, and TA1535 at concentrations of between 0.03 and 7.4 mg/tube, only at the high concentration tested of 3.7 mg/tube, but not at lower concentrations. [The Working Group noted that more recent studies questioned these findings, since significant cytotoxicity was observed with MTBE at doses higher than 3 mg/tube, and no mutagenicity was observed at lower doses ([Vosahlíková et al., 2006](#)).] MTBE did not induce increases in mutation frequency in *Salmonella typhimurium* strains TA97a, TA98, or T100, although it did induce DNA damage in the TA102 strain in the presence of metabolic activation ([Williams-Hill et al., 1999](#)). No mutagenicity was later observed by [McGregor et al. \(2005\)](#) in the TA102 strain with MTBE at the highest concentration of 5 mg/plate. Also, no mutagenicity was observed in TA98 and TA100 at the highest ineffective concentration (HIC) of MTBE of 100 µg/plate ([Zhou et al., 2000](#)), or with 15% MTBE ([Westphal et al., 2010](#)).

No genotoxicity, as measured by the amount of green fluorescent protein (GFP) fused to the promoter of RAD54 DNA-dependent ATPase, was reported in the yeast *Saccharomyces cerevisiae* treated with MTBE at a dose of 16 mg/L ([Lichtenberg-Fraté et al., 2003](#)).

*Synopsis*

[Overall, the Working Group noted that there was a paucity of data in exposed humans. An increase in 8-OHdG was reported in a single study in exposed humans that had limitations. In human primary lymphocytes, MTBE induced the formation of DNA SSBs and DSBs. Some evidence of DNA damage was also reported in human cell lines. Genotoxicity-associated end-points were observed in experimental systems in vitro with MTBE mainly at the highest concentrations tested, but also in the presence of oxidative stress or metabolic activation. MTBE did not induce genotoxicity in studies in rodents in vivo.]

#### 4.2.3 Alters DNA repair or causes genomic instability

(a) *Humans*

(i) *Exposed humans*

No studies were available to the Working Group.

(ii) *Human cells*

As described in the previous section, [He et al. \(2021\)](#) observed a significant increase in oxidative stress in human bronchial epithelial cells (16HBE) depleted of >80% of the DNA repair enzyme (polymerase β), and exposed also to increasing concentrations of MTBE. This indicated potential roles for oxidative stress in DNA damage (see Section 4.2.5) and for the base excision repair mechanism in the effects of MTBE.

*(b) Experimental systems*

See [Table 4.7](#).

No evidence of unscheduled DNA synthesis (UDS) was observed in the liver of mice exposed to MTBE at 8000 ppm ([US EPA, 1994](#); [McKee et al., 1997](#)). However, increasing concentrations of MTBE (200, 600, and 1000 µg/mL) induced an increase in UDS in rat hepatocytes ([Zhou et al., 2000](#); [Yang et al., 2005](#)) and in rat type II pneumocytes ([Yang et al., 2005](#)).

*Synopsis*

[Overall, the Working Group noted that there were no data available in exposed humans or human primary cells. A single study in human cells in vitro indicated DNA damage after exposure to MTBE. UDS was reported in vitro in rat hepatocytes and pneumocytes; however, these findings were not confirmed in vivo, in the liver in mice.]

*4.2.4 Induces epigenetic alterations**Humans*

[Rota et al. \(2018\)](#) investigated the effect of exposure to MTBE on epigenetic and transcriptional alterations in DNA repetitive elements. Specifically, the DNA methylation of repetitive elements (*LINE-1* and *Alu*) and their transcription, and the methylation of H3K9 histone, were investigated in peripheral blood lymphocytes from 89 service station workers and 90 non-occupationally exposed study participants (the controls) from the area of Milan, Italy. The median work shift exposure to MTBE in air was 408 µg/m<sup>3</sup> for service station workers, and 3.5 µg/m<sup>3</sup> for the controls. Exposure to benzene was 59 µg/m<sup>3</sup> in the workers, and 4 µg/m<sup>3</sup> in the controls. Overall, the duration of exposure was 5 hours per day. Urinary levels of MTBE were significantly higher in workers (median, 232 ng/L) than in controls (median, 58 ng/L). Samples were collected once per week, in the

morning after the work shift. Of the repetitive elements, only L1-Pa5 was hypomethylated in service station workers compared with controls. Methylation of L1-Ta ( $\beta_{90-10} = -0.279$ ;  $P = 0.0298$ ) and Alu-YD6 ( $\beta_{90-10} = -0.257$ ;  $P = 0.0411$ ) was inversely associated with exposure to MTBE. The methylation status of histone H3K9 was not associated with exposure to either MTBE or benzene.

In a small cross-sectional study in Chinese workers, which aimed to investigate MTBE exposure and the development of metabolic-associated fatty liver disease (MAFLD), [Wang et al. \(2025\)](#) observed alterations in specific metabolic end-points. In addition, the authors reported that blood MTBE levels were positively associated with the prevalence of MAFLD. There was an increase in risk (odds ratio) of 1.243 (95% CI, 1.110–1.393;  $P < 0.001$ ) for every 0.1 µg/L increase of blood MTBE concentration. Furthermore, the expression of miRNA-18a-5p, which is involved in lipid metabolism and MAFLD development, was identified in the workers diagnosed with or without MAFLD, showing a significant inverse dose–response relationship between miRNA expression and MAFLD severity and higher levels of MTBE exposure.

The authors suggested that MTBE promoted liver cholesterol synthesis through the miRNA-18a-5p/PXR/SREBP2 pathway, as also assessed in rodents and in HepaRG cells, thereby inducing MAFLD, which is associated with increased risk of various types of cancer.

*Synopsis*

[The Working Group noted that there was a paucity of data.]

*4.2.5 Induces oxidative stress**(a) Humans**(i) Exposed humans*

See [Table 4.8](#).

**Table 4.7 End-points relevant to alterations in DNA repair or genomic instability in experimental systems exposed to methyl tert-butyl ether**

| End-point (assay) | Species, strain (sex) | Tissue or cells     | Results <sup>a</sup> | Dose (LED or HID) | Route, duration, dosing regimen                                         | Comments                 | Reference                           |
|-------------------|-----------------------|---------------------|----------------------|-------------------|-------------------------------------------------------------------------|--------------------------|-------------------------------------|
| <i>In vivo</i>    |                       |                     |                      |                   |                                                                         |                          |                                     |
| UDS (UDS assay)   | Mouse, CD-1 (M, F)    | Liver               | –                    | 8000 ppm          | Inhalation, 6 h/day, for 2 consecutive days, at 400, 3000, and 8000 ppm |                          | <a href="#">McKee et al. (1997)</a> |
| UDS (UDS assay)   | Mouse, CD-1 (M, F)    | Liver               | –                    | 8000 ppm          | Inhalation, 6 h/day, for 2 consecutive days, at 400, 3000, and 8000 ppm |                          | <a href="#">US EPA (1994)</a>       |
| <i>In vitro</i>   |                       |                     |                      |                   |                                                                         |                          |                                     |
| UDS (UDS assay)   | Rat                   | Primary hepatocytes | +                    | 1000 µg/mL        | 200, 600, and 1000 µg/mL                                                |                          | <a href="#">Zhou et al. (2000)</a>  |
| UDS (UDS assay)   | Rat                   | Type II pneumocytes | +                    | 5 mmol/L          | Cell treatment                                                          | Dose-dependent relation. | <a href="#">Yang et al. (2005)</a>  |
|                   | Rat                   | Hepatocytes         | +                    | 10 mmol/L         | Cell treatment                                                          |                          |                                     |

F, female(s); h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; M, male(s); ppm, parts per million; UDS, unscheduled DNA synthesis.

<sup>a</sup> +, positive, –, negative.

**Table 4.8 End-points relevant to oxidative stress in humans exposed to methyl *tert*-butyl ether**

| End-point            | Assay                   | Biological sample type      | Location, study design, study participants                                                                                                             | Duration of exposure                                                                                                                                                                                                            | Response <sup>a</sup> (significance)                              | Covariates controlled                                                       | Comments                                                                                      | Reference                              |
|----------------------|-------------------------|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------|
| 8-oxodGuo            | LC-MS/MS                | Urine                       | Sicily, Italy<br>Cross-sectional<br>155 children:<br><i>n</i> = 55, living far from an oil refinery<br><i>n</i> = 150, living close to an oil refinery | Exposure to MTBE was assessed by urinary MTBE:<br>0.79 mg/L (evening) and 0.82 mg/L (morning) for children living far from the refinery;<br>0.56 mg/L (morning) or 0.59 mg/L (evening) for children near the refinery           | + Correlation, urinary MTBE with 8-oxodGuo in the evening samples | Distance between home and an oil refinery, age, gender, smoking inhabitants | Children, time living in the area (moving; i.e. duration of exposure) was not considered.     | <a href="#">Andreoli et al. (2015)</a> |
| 8-oxodG              | HPLC                    | Blood lymphocytes and urine | Copenhagen, Denmark<br>Cross-sectional                                                                                                                 | Benzene exposure of people living and working in the area: median, 2.53 µg/m <sup>3</sup> ; Q25–Q75, 1.86–3.61 µg/m <sup>3</sup> ; MTBE exposure: median, 0.86; Q25–Q75, 0.43–1.73 µg/m <sup>3</sup> ; personal sampling, 110 h | NR                                                                | Age, body weight                                                            | MTBE exposure compared with benzene exposure, not to biological end-points; no control group. | <a href="#">Sørensen et al. (2003)</a> |
| Oxidative DNA damage | Fpg comet assay<br>Endo | Blood lymphocytes           | 40 participants: outdoor workers, <i>n</i> = 10; indoor workers, <i>n</i> = 30                                                                         |                                                                                                                                                                                                                                 | NR                                                                |                                                                             |                                                                                               |                                        |

Endo, endonuclease III; Fpg, formamidopyrimidine DNA glycosylase; h, hour(s); HPLC, high-pressure liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MTBE, methyl *tert*-butyl ether; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxodGuo, 8-oxo-7,8-dihydro-2'-deoxyguanosine; NR, not reported.

<sup>a</sup> +, positive.



[Andreoli et al. \(2015\)](#) conducted a cross-sectional study in children (aged 5–11 years;  $n=155$ ) living at various distances from the gasoline industry in the area of Milazzo and Nizza in Sicily, Italy; the aim of the study was to evaluate oxidative damage to DNA in children exposed to environmental pollutants and environmental tobacco smoke (ETS). Urine samples were collected twice from each participant (in the evening and following morning). Oxidated products of nucleic acids – 8-oxodG, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and 8-oxoguanine (8-oxoGua) – were measured by LC-MS/MS. Urinary MTBE and benzene, and their metabolites, were considered as biomarkers of exposure to air pollution; cotinine was considered as a biomarker of exposure to ETS. The authors observed a positive correlation between urinary levels of MTBE with 8-oxoGuo ( $r = 0.14$ ;  $P < 0.05$ ) in the evening samples.

A cross-sectional study was conducted by [Sørensen et al. \(2003\)](#) in 40 adult volunteers living and working in the urban area of Copenhagen, Denmark. The aim was to monitor external cumulative exposure to benzene, toluene, and MTBE during a 5-day period and assess oxidative-stress associated end-points. Internal exposure to benzene (measured as S-phenylmercapturic acid, SPMA) was further analysed for genotoxic effects. DNA damage was measured by levels of 8-oxodG in lymphocyte DNA and in the urine and by comet assay with use of formamidopyrimidine/8-oxoguanine DNA *N*-glycosylase (Fpg) and endonuclease III. A significant correlation between SPMA (main benzene metabolite) excretion and 8-oxodG in lymphocytes was found ( $R_s = 0.39$ ) ([Sørensen et al., 2003](#)). [The Working Group noted that a clear correlation with external exposure to MTBE was not studied. Furthermore, there was no control group, and end-points associated with MTBE exposure were not evaluated.]

## (ii) Human cells

See [Table 4.9](#).

[Chen et al. \(2008\)](#) observed increased levels of oxidative damage to DNA in lymphocytes exposed to MTBE, as measured by comet assay plus incubation with Fpg DNA glycosylase.

[Salimi et al. \(2016\)](#) observed an increase in the generation of reactive oxygen species (ROS) and lipid peroxidation, and a decrease in levels of glutathione (GSH) in human blood lymphocytes treated with MTBE at a concentration of 0.1 mM for 30–180 minutes. [He et al. \(2021\)](#) observed alterations in oxidative stress-associated enzymes in human lung-derived cell lines, except when cells were depleted of > 80% of the DNA repair enzyme, polymerase  $\beta$ .

## (b) Experimental systems

### (i) Non-human or non-mammalian systems in vivo

See [Table 4.10](#).

Oxidative stress-associated end-points were mostly evaluated in vivo in various tissues from mice (liver) and rats (liver, testis, lymphocytes) exposed to MTBE for at least 2 weeks consecutively.

[Yang et al. \(2005\)](#), [Li et al. \(2008\)](#), [Saeedi et al. \(2017\)](#), and [Zhu et al. \(2022\)](#) observed an overall increase in oxidative stress, including ROS generation and lipid peroxidation, as measured by an increase in the levels of malondialdehyde (MDA), induction of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), and reduction in GSH and glutathione peroxidase (GPX1). In contrast, [de Peyster et al. \(2008\)](#) did not observe any alterations in levels of 8-OHdG, MDA, or antioxidant capacity in the liver of mice exposed to MTBE at a concentration of 8000 ppb for > 50 days via drinking-water. [The Working Group considered that the chosen time-point of treatment, day 51, represented a rather late time-point for evaluating changes in oxidative stress, thus limiting the relevance of

**Table 4.9 End-points relevant to oxidative stress in human cells in vitro exposed to methyl *tert*-butyl ether**

| End-point            | Assay                      | Tissue, cell line                                               | Results <sup>a</sup> | Concentration (LEC or HIC), duration | Comments                                               | Reference                            |
|----------------------|----------------------------|-----------------------------------------------------------------|----------------------|--------------------------------------|--------------------------------------------------------|--------------------------------------|
| Oxidative DNA damage | Comet assay with Fpg       | Lymphocytes                                                     | ↑                    | 200 µM                               |                                                        | <a href="#">Chen et al. (2008)</a>   |
| ROS                  | DCFH-DA                    | Lymphocytes                                                     | ↑                    | 0.1 mM; 30, 120, and 180 min         |                                                        | <a href="#">Salimi et al. (2016)</a> |
| Lipid peroxidation   | TBARS                      |                                                                 | ↑                    | 0.1 mM, 180 min                      |                                                        |                                      |
| GSH                  | Spectrofluorometric method |                                                                 | ↓                    | 0.1 mM, 180 min                      |                                                        |                                      |
| SOD                  |                            | Human lung cell line 16HBE transfected with empty vector        | ↓                    | 100 µmol/L, 12–36 h                  | Data on MTBE exposure were not detailed appropriately. | <a href="#">He et al. (2021)</a>     |
|                      |                            | Human lung cell line 16HBE                                      | ↓                    | 100 µmol/L, 12–36 h                  |                                                        |                                      |
|                      |                            | DNA pol-β-deficient cells (16HBE pol-β gene knocked-down cells) | ↑                    | 50 µmol/L, 24 h                      |                                                        |                                      |
| CAT, MDA             |                            | Human lung cell line 16HBE                                      | ↓                    | 100 µmol/L, 12–36 h                  |                                                        |                                      |
|                      |                            | 16HBE transfected with empty vector                             | ↓                    | 100 µmol/L, 12–36 h                  |                                                        |                                      |
|                      |                            | DNA pol-β-deficient cells (16HBE pol-β gene knocked-down cells) | ↑                    | 50 µmol/L, 12–36 h                   |                                                        |                                      |

CAT, catalase; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; Fpg, formamidopyrimidine DNA glycosylase; GSH, glutathione; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; MDA, malondialdehyde; min, minute(s); MTBE, methyl *tert*-butyl ether; pol-β, DNA polymerase β; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

<sup>a</sup> ↑, increase; ↓, decrease.

**Table 4.10 End-points relevant to oxidative stress in experimental systems in vivo exposed to methyl tert-butyl ether**

| End-point                          | Assay                                  | Species, strain (sex)       | Tissue, cell    | Results <sup>a</sup>                                                                                                 | Dose (LED or HID)                                                     | Route, duration, dosing regimen           | Comments                                                                                                                                      | Reference                                |
|------------------------------------|----------------------------------------|-----------------------------|-----------------|----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| <i>Non-human mammalian systems</i> |                                        |                             |                 |                                                                                                                      |                                                                       |                                           |                                                                                                                                               |                                          |
| MDA                                | HPLC                                   | Mouse, BALB/c (M), juvenile | Liver           | No change                                                                                                            | 8000 ppb                                                              | 51 days, drinking-water exposed daily     | The time point evaluated was too late; exposures of 7 and 28 days were also investigated, but the oxidative stress response was not measured. | <a href="#">de Peyster et al. (2008)</a> |
| Antioxidant capacity               | Trolox equivalent antioxidant capacity |                             |                 | No change                                                                                                            |                                                                       |                                           |                                                                                                                                               |                                          |
| 8-OHdG                             | HPLC                                   |                             |                 | No change                                                                                                            |                                                                       |                                           |                                                                                                                                               |                                          |
| MDA                                | TBARS                                  | Rat, Sprague-Dawley (M)     | Liver           | ↑                                                                                                                    | 200 µg/L                                                              | 90 days, drinking-water                   |                                                                                                                                               | <a href="#">Saeedi et al. (2017)</a>     |
| GSH                                | DNTB                                   |                             |                 | ↓                                                                                                                    | 40 µg/L                                                               |                                           |                                                                                                                                               |                                          |
| SOD2, SOD1, CAT                    | qPCR (mRNA)                            | Rat, Sprague-Dawley (M)     | Testis          | No change                                                                                                            | 1200 mg/kg                                                            | Oral gavage, daily from age 35 to 56 days |                                                                                                                                               | <a href="#">Zhu et al. (2022)</a>        |
| GPX1                               | qPCR (mRNA) and western blot           |                             |                 | ↓ mRNA<br>↓ Protein                                                                                                  | 1200 mg/kg<br>600 mg/kg                                               |                                           |                                                                                                                                               |                                          |
| LC3B                               | Western blot                           |                             |                 | ↑                                                                                                                    | 600 mg/kg                                                             |                                           |                                                                                                                                               |                                          |
| MDA                                | TBARS                                  | Rat, Sprague-Dawley (M)     | Lymphocytes     | ↑ At the highest dose after 2 wk<br>↑ At the lowest dose after 4 wk                                                  | 1600 mg/kg per day<br>400 mg/kg per day                               | Oral gavage, daily, 2 or 4 wk             | Range of tested doses: 400, 800, 1200 mg/kg per day.                                                                                          | <a href="#">Li et al. (2008)</a>         |
| Total antioxidant ability          | Assay kit                              |                             |                 | ↑ At 2 wk<br>↑ At 4 wk                                                                                               | 400 mg/kg per day<br>1600 mg/kg per day                               |                                           |                                                                                                                                               |                                          |
| SOD <sub>EX</sub> (extracellular)  | PCR (mRNA)                             |                             |                 | ↑ after 2 wk                                                                                                         | 1600 mg/kg per day                                                    |                                           |                                                                                                                                               |                                          |
| OGG1                               | PCR (mRNA)                             |                             |                 | ↓                                                                                                                    | 1600 mg/kg per day                                                    |                                           |                                                                                                                                               |                                          |
| MDA                                | TBARS                                  |                             |                 |                                                                                                                      |                                                                       |                                           |                                                                                                                                               |                                          |
| MDA                                | TBARS                                  | Mouse, Kunming (M, F)       | Lung and Kidney | No change in lung,<br>↑ kidney at 4968 mg/m <sup>3</sup> in both sexes and also at 1440 mg/m <sup>3</sup> in females | 4968 mg/m <sup>3</sup> (HID)<br>1440 and 4968 mg/m <sup>3</sup> (LED) | Inhalation, 4 h/day, 20 days              |                                                                                                                                               | <a href="#">Yang et al. (2005)</a>       |

**Table 4.10 (continued)**

| End-point                    | Assay                                                                                      | Species, strain (sex)                         | Tissue, cell | Results <sup>a</sup> | Dose (LED or HID)          | Route, duration, dosing regimen     | Comments                                                                                                  | Reference                             |
|------------------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------|--------------|----------------------|----------------------------|-------------------------------------|-----------------------------------------------------------------------------------------------------------|---------------------------------------|
| <b>Non-mammalian systems</b> |                                                                                            |                                               |              |                      |                            |                                     |                                                                                                           |                                       |
| TAC                          | FRAP                                                                                       | Rainbow trout                                 | Serum        | No change            | 50 ppm                     | Oral, 28 days                       | Description of study results was incomplete; unclear data labelling. Effects of antioxidants were tested. | <a href="#">Shiry et al. (2023)</a>   |
| MDA                          | MDA activity                                                                               |                                               |              | No change            | 50 ppm (HID); 10 ppm (LED) |                                     |                                                                                                           |                                       |
| SOD                          | RANSOD biochemical kit                                                                     |                                               |              | ↑                    |                            |                                     |                                                                                                           |                                       |
| CAT                          | Degradation of hydrogen peroxide and formation of a stable complex with ammonium molybdate |                                               |              | ↑                    |                            |                                     |                                                                                                           |                                       |
| GPx                          | NADPH                                                                                      | Nile tilapia ( <i>Oreochromis niloticus</i> ) | Hepatocytes  | ↑                    |                            | Oral (water contamination), 27 days |                                                                                                           | <a href="#">Banacee et al. (2023)</a> |
| TAC                          | FRAP reagent                                                                               |                                               |              | ↓                    | 2.5 µL/L                   |                                     |                                                                                                           |                                       |
| MDA                          | TBARS                                                                                      |                                               |              | ↑                    |                            |                                     |                                                                                                           |                                       |
| SOD                          | Xanthine oxidase enzymatic activity                                                        |                                               |              | ↑                    |                            |                                     |                                                                                                           |                                       |
| CAT                          | Hydrogen peroxidase assay                                                                  |                                               |              | ↑                    |                            |                                     |                                                                                                           |                                       |
| Glutathione reductase        | NADPH reduction to NADP+                                                                   |                                               |              | ↓                    |                            |                                     |                                                                                                           |                                       |

CAT, catalase; DNTB, 5,5'-dithiobis-2-nitrobenzoic acid; F, female(s); FRAP, ferric-reducing antioxidant power; GPx, glutathione peroxidase; GSH, glutathione; h, hour(s); HID, highest ineffective dose; HPLC, high-performance liquid chromatography; LC3B, microtubule-associated protein 1 light chain 3 beta; LED, lowest effective dose; M, male(s); MDA, malondialdehyde; mRNA, messenger RNA; NADP+, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; OGG1, 8-oxoguanine DNA glycosidase; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PCR, polymerase chain reaction; ppb, parts per billion; ppm, parts per million; qPCR, quantitative polymerase chain reaction; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; or no change.

the study.] Induction of oxidative stress was also reported in studies conducted in the non-mammalian species rainbow trout ([Shiry et al., 2023](#)) and Nile tilapia ([Banaee et al., 2023](#)).

(ii) *Non-human mammalian systems in vitro*

See [Table 4.11](#).

In all in vitro studies on MTBE-induced oxidative stress in mammalian cells, positive results were reported for cell cultures derived from rats, mice and Chinese hamster.

*Synopsis*

[Overall, the Working Group noted that urinary levels of MTBE significantly correlated with urinary levels of 8-oxodG in children living near a refinery compared with children living far from a refinery, as reported in one study. Exposure to MTBE induced ROS production, an increase in lipid peroxidation, and a decrease in the GSH:GSSG ratio, in one study in human peripheral blood lymphocytes. MTBE induced alterations in various oxidative end-points, including MDA levels, and antioxidant enzyme activities (GSH, GPX1) in vivo and in several in vitro studies.]

#### 4.2.6 *Induces chronic inflammation*

In the only study in experimental systems in vivo that was available to the Working Group, [Saeedi et al. \(2017\)](#) reported increased inflammation, as assessed by histopathology, in the liver of male Sprague-Dawley rats after 90 days of exposure to drinking-water containing MTBE at 200 and 1000 µg/L ([Saeedi et al., 2017](#)). Inflammation of the liver was also accompanied by increased levels of oxidative stress (see Section 4.2.5).

#### 4.2.7 *Is immunosuppressive*

(a) *Humans*

The production of immunoglobulin (Ig) IgG, IgA, IgM, and IgE antibodies against MTBE was measured by ELISA in the serum of 18 men and 6 women (aged 21–58 years) who had worked as service station attendants for at least 2 years, and in 12 controls (8 men and 4 women; aged 24–60 years) who had never been exposed continuously to refuelling. Seven out of 24 exposed participants and one control had significantly elevated titres of IgG and IgM, although IgA and IgE were not affected ([Vojdani et al., 1997a](#)). [The Working Group noted that the authors did not report exposure data and did not control for covariates.]

(b) *Experimental systems*

See [Table 4.12](#).

Exposure to MTBE via inhalation for 4 weeks did not induce immunosuppression in the spleen of female rats. There was no significant difference in the IgM antibody-forming cell response between animals exposed to MTBE and the control group exposed to vehicle only ([White et al., 2014](#)). In contrast, [Zhu et al. \(2022\)](#) observed a decrease in the expression of cell cycle regulator *Ccnd1* mRNA expression in immature Leydig cells of male rats exposed orally to MTBE at a dose of 1200 mg/kg daily for 21 days by gavage, starting postnatal day 35 ([Zhu et al., 2022](#)). [The Working Group considered the observed immunosuppression to be a result of downregulation of the testicular signalling pathway, which was likely to be caused by the very high cumulative dose of MTBE (for 21 days).]

**Table 4.11 End-points relevant to oxidative stress in experimental systems in vitro exposed to methyl *tert*-butyl ether**

| End-point                         | Assay                           | Species, cells                                | Results <sup>a</sup> | Dose (LED or HID) | Time point    | Comments                                                                                                                                                                                             | Reference                              |
|-----------------------------------|---------------------------------|-----------------------------------------------|----------------------|-------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| DNA oxidative damage              | 8-OHdG                          | Rat, diploid immortalized fibroblasts (Rat-1) | ↑                    | 0.84 mM           | 4 h           |                                                                                                                                                                                                      | <a href="#">Sgambato et al. (2009)</a> |
| ROS                               | DCHF-DA                         | Rat, spermatogenic cells                      | ↑                    | 50 μM             | 1 h, 2 h, 6 h |                                                                                                                                                                                                      | <a href="#">Li et al. (2007)</a>       |
| MDA                               | TBARS                           |                                               | ↑                    | 0.5 μM            | 18 h          |                                                                                                                                                                                                      |                                        |
| SOD (cytosolic)                   | Xanthine oxidase method         |                                               | ↓                    | 50 μM             | 3 h, 6 h      |                                                                                                                                                                                                      |                                        |
| SOD <sub>EX</sub> (extracellular) |                                 |                                               | ↑                    | 5 mM              | 6 h, 12 h     |                                                                                                                                                                                                      |                                        |
| SOD <sub>EX</sub> (extracellular) | Corresponding assay kits        | Chinese hamster ovary cells                   | ↑                    | 50 mM             | 12 h          |                                                                                                                                                                                                      | <a href="#">Xie et al. (2017)</a>      |
| CAT                               | TBARS                           |                                               | ↑                    | 25 mM             |               |                                                                                                                                                                                                      |                                        |
| GPx                               |                                 |                                               | ↑                    | 25 mM             |               |                                                                                                                                                                                                      |                                        |
| MDA                               |                                 |                                               | ↑                    | 5 mM              |               |                                                                                                                                                                                                      |                                        |
| ROS                               | DCHF-DA                         | Rat, Sertoli cells                            | ↑                    | 5 μM              | 3 h           |                                                                                                                                                                                                      | <a href="#">Li et al. (2009)</a>       |
| MDA                               | TBARS                           |                                               | ↑                    | 5000 μM           | 24 h          |                                                                                                                                                                                                      |                                        |
| SOD <sub>EX</sub> (extracellular) | Xanthine oxidase method and PCR |                                               | ↑                    | 5000 μM           | 6 h, 12 h     | Wrong concentration listed in the Table of the paper for the xanthine method. Only condition with positive result – tested three different concentrations and four time points (3, 6, 12, and 24 h). |                                        |
| OGG1                              | PCR                             |                                               | ↑                    | 50 μM             | 3 h           |                                                                                                                                                                                                      |                                        |
| ROS                               | DCHF-DA – fluorescent staining  |                                               | ↑                    | 0.125 mmol/L      | 6 h           |                                                                                                                                                                                                      | <a href="#">Ma et al. (2017)</a>       |
| MDA                               | Commercially available kits     |                                               | ↑                    | 0.25 mmol/L       |               |                                                                                                                                                                                                      |                                        |
| SOD                               |                                 |                                               | ↓                    | 0.5 mmol/L        |               |                                                                                                                                                                                                      |                                        |
| GSH or GSSG                       |                                 |                                               | ↓                    | 0.125 mmol/L      |               |                                                                                                                                                                                                      |                                        |
| ROS                               | DCFH-DA                         | Rat, immature Leydig cells                    | ↑                    | 200 mM            | 24 h          |                                                                                                                                                                                                      | <a href="#">Zhu et al. (2022)</a>      |

CAT, catalase; DCHF-DA, 2',7'-dichlorodihydrofluorescein diacetate; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized glutathione; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; OGG1, 8-oxoguanine DNA glycosidase; PCR, polymerase chain reaction; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

<sup>a</sup> ↑, increase; ↓, decrease.



**Table 4.12 End-points relevant to immunosuppression in non-human mammalian systems in vivo exposed to methyl *tert*-butyl ether**

| End-point                          | Species, strain, (sex)  | Tissue, cells         | Results <sup>a</sup> | Dose (LED or HID)        | Route, duration, dosing regimen           | Comments                                                                       | Reference                           |
|------------------------------------|-------------------------|-----------------------|----------------------|--------------------------|-------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------|
| IgM antibody-forming cell response | Rat, Sprague-Dawley (F) | Spleen                | No change            | 20 000 mg/m <sup>3</sup> | Inhalation, 5 days/week for 4 wk          |                                                                                | <a href="#">White et al. (2014)</a> |
| <i>Ccnd1</i> mRNA (qPCR)           | Rat, Sprague-Dawley (M) | Immature Leydig cells | ↓                    | 1200 mg/kg               | Oral gavage, daily from age 35 to 56 days | Immunosuppression was considered to be due to cumulative effects at high dose. | <a href="#">Zhu et al. (2022)</a>   |

F, female(s); HID, highest ineffective dose; Ig, immunoglobulin; LED, lowest effective dose; M, male(s); mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; wk, week(s).

<sup>a</sup> ↓, decrease; or no change.

#### 4.2.8 Modulates receptor-mediated effects

##### (a) Humans or human cells

No study in exposed humans was available to the Working Group. In a single study in the human adrenal gland cell line (H295R) in vitro, MTBE treatment at 0.0001, 0.001, 0.01, 0.1, 1, 10, or 100 µM did not induce steroidogenesis compared with controls, thus there was no alteration in the production of testosterone or estradiol ([de Peyster et al., 2014](#)).

##### (b) Experimental systems

See [Table 4.13](#).

In several studies in vivo ([Moser et al., 1996b](#); [Williams et al., 2000](#); [Williams and Borghoff, 2000](#); [de Peyster et al., 2003](#); [Li et al., 2008](#); [Zhu et al., 2022](#)) in rats and mice, and two experiments in vitro ([de Peyster et al., 2009](#); [Zhu et al., 2022](#)), the authors reported a decrease in the production of testosterone, and an increase in testosterone metabolism, including induction of various CYP enzymes, in the testis, liver, blood serum, or Leydig cells.

#### 4.2.9 Alters cell proliferation, cell death, or nutrient supply

##### (a) Humans

One study in exposed humans was available to the Working Group. The study had a case-control design and assessed apoptosis and cell cycle processing in lymphocytes from individuals in the USA exposed to water (through drinking, bathing, and showering) contaminated with MTBE and benzene for a period of 5–8 years, and compared with non-exposed individuals ([Vojdani et al., 1997b](#)). The 60 exposed participants (38 men and 22 women) had experienced fatigue and other symptoms; no details were given on individual exposure and its duration. The assessment of water contamination revealed MTBE levels ranging between 1 and 76 ppb [µg/L] and benzene levels ranging between 0.2 and 14 ppb [µg/L]. [The Working Group noted that the authors did not specify when or how water contamination was assessed, individual exposure to MTBE and benzene was not evaluated, and there was no discrimination between the effects of these contaminants.] The authors stated that the cases did not show evidence of pre-existing drug abuse, alcoholism, or smoking, or other diseases or complicating conditions. Additionally,

**Table 4.13 End-points relevant to the modulation of receptor-mediated effects in experimental systems in vivo and in vitro exposed to methyl *tert*-butyl ether**

| End-point                                            | Assay                                                | Species, strain (sex)             | Tissue, cells    | Results <sup>a</sup>                             | Dose or concentration (LED or HID, LEC or HIC)             | Route, duration, dosing regimen                                | Comments                                                                                                                               | Reference                                |
|------------------------------------------------------|------------------------------------------------------|-----------------------------------|------------------|--------------------------------------------------|------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| <i>In vivo</i>                                       |                                                      |                                   |                  |                                                  |                                                            |                                                                |                                                                                                                                        |                                          |
| Testosterone levels                                  | RIA, <sup>125</sup> I Coat-a-Count kits              | Rat, Sprague-Dawley (M)           | Blood serum      | ↓                                                | 1500 mg/kg                                                 | Oral gavage, 4–5 h post-exposure, one dose                     | Short exposure period, no repeated exposure, no other time point or dose investigated.                                                 | <a href="#">de Peyster et al. (2003)</a> |
| CYP enzyme induction                                 | Method of <a href="#">Omura and Sato (1967)</a>      |                                   | Liver            | ↑                                                | 1500 mg/kg (days 1–12)<br>750 mg/kg (days 13–27)           | Oral gavage, 14 times, over 27 days (exposure every other day) | Also investigated changes in organ weight upon exposure to MTBE (only significant change in pituitary weight and overall body weight). |                                          |
| Testosterone levels                                  | RIA, <sup>125</sup> I Coat-a-Count kits              |                                   | Blood serum      | ↑ After 28 days<br>No changes after 7 or 14 days | 800 mg/kg (LED after 28 days)                              | Oral gavage, 7, 14, or 28 days (exposure every day)            |                                                                                                                                        |                                          |
| Corticosterone levels                                | RIA, Rat-specific <sup>125</sup> I Coat-a-Count kits |                                   | Blood serum      | ↑                                                | 40 mg/kg (LED after 14 days)<br>800 mg (LED after 28 days) | Oral gavage, 14 days or 28 days (exposure every day)           |                                                                                                                                        |                                          |
| Prolactin and LH levels                              | RIA kits                                             |                                   | Blood serum      | No changes                                       | 800 mg/kg                                                  | Oral gavage, 28 days (exposure every day)                      |                                                                                                                                        |                                          |
| Aromatase activity                                   | Method of <a href="#">Lephart and Simpson (1991)</a> |                                   | Liver and testis | ↓                                                | 1200 mg/kg                                                 | Oral gavage, 14 days (exposure every day)                      |                                                                                                                                        |                                          |
| Hormonal effects on hypothalamic–pituitary functions |                                                      | Rat, Sprague-Dawley (M castrated) | Blood serum      | No changes                                       | 800 mg/kg                                                  | Oral gavage, 5 days, daily                                     |                                                                                                                                        |                                          |

**Table 4.13 (continued)**

| End-point                                   | Assay                                                   | Species, strain (sex)   | Tissue, cells         | Results <sup>a</sup>                                   | Dose or concentration (LED or HID, LEC or HIC)  | Route, duration, dosing regimen                | Comments                     | Reference                                |  |  |  |  |
|---------------------------------------------|---------------------------------------------------------|-------------------------|-----------------------|--------------------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------|------------------------------------------|--|--|--|--|
| Testosterone production                     | EIA kits                                                | Rat, Sprague-Dawley (M) | Blood serum           | ↓                                                      | 800 mg/kg (1st week); 1000 mg/kg (2nd week)     | Oral gavage, 2 wk, daily                       |                              | <a href="#">de Peyster et al. (2014)</a> |  |  |  |  |
| Estradiol                                   |                                                         |                         |                       | No change                                              | 1200 (1st week)/1500 (2nd week) mg/kg estradiol |                                                |                              |                                          |  |  |  |  |
| Testosterone production                     | Chemiluminescence immunoassay, Immulite2000 total T kit | Rat, Sprague-Dawley (M) | Blood serum           | ↓                                                      | 300 mg/kg                                       | Oral gavage, daily from age 35 days to 56 days |                              | <a href="#">Zhu et al. (2022)</a>        |  |  |  |  |
| LH                                          |                                                         |                         |                       | No change                                              | 1200 mg/kg                                      |                                                |                              |                                          |  |  |  |  |
| FSH                                         | Immunohisto-chemical staining                           |                         | Immature Leydig cells | No change                                              | 1200 mg/kg                                      |                                                |                              |                                          |  |  |  |  |
| CYP11A1+ (involved in biosynthetic process) |                                                         |                         |                       | ↓                                                      |                                                 |                                                |                              |                                          |  |  |  |  |
| Testosterone production                     | RIA kits                                                | Rat, Sprague-Dawley (M) | Blood serum           | ↑ At 800 mg/kg and ↓ at 1200 mg/kg with 2 wk treatment | 800 or 1200 mg/kg per day                       | Oral gavage, daily, 2 or 4 wk                  |                              | <a href="#">Li et al. (2008)</a>         |  |  |  |  |
| LH                                          | ↓ At 800 mg/kg with 2 wk treatment                      |                         |                       |                                                        |                                                 |                                                |                              |                                          |  |  |  |  |
| FSH                                         | ↑ After 2 wk treatment                                  |                         |                       | 400 mg/kg per day                                      |                                                 |                                                |                              |                                          |  |  |  |  |
| ABP secretion                               | Agarose gel electrophoresis                             |                         | Testis                | ↑ After 2 wk treatment                                 | 800 mg/kg per day                               |                                                |                              |                                          |  |  |  |  |
|                                             |                                                         |                         |                       | ↓ After 2 wk treatment                                 | 1600 mg/kg per day                              |                                                |                              |                                          |  |  |  |  |
|                                             |                                                         |                         |                       | ↓ After 4 wk treatment                                 | 800 mg/kg per day                               |                                                |                              |                                          |  |  |  |  |
| Testosterone production                     | RIA kits                                                | Rat, Sprague-Dawley (M) | Blood serum           | ↓ After 15 days treatment<br>No changes after 28 days  | 1500 mg/kg per day                              | Oral gavage, daily, 15 or 28 days              | Organ weights were assessed. | <a href="#">Williams et al. (2000)</a>   |  |  |  |  |

**Table 4.13 (continued)**

| End-point                                                                   | Assay | Species,<br>strain (sex)       | Tissue,<br>cells               | Results <sup>a</sup>                                       | Dose or<br>concentration<br>(LED or HID,<br>LEC or HIC) | Route,<br>duration,<br>dosing<br>regimen | Comments | Reference                                                 |
|-----------------------------------------------------------------------------|-------|--------------------------------|--------------------------------|------------------------------------------------------------|---------------------------------------------------------|------------------------------------------|----------|-----------------------------------------------------------|
| Testosterone<br>production                                                  |       |                                | Interstitial<br>fluid          | ↓ After 15 days<br>No changes<br>after 28 days             |                                                         |                                          |          | <a href="#">Williams<br/>et al.<br/>(2000)</a><br>(cont.) |
| LH                                                                          |       |                                | Blood<br>serum                 | ↓ After 28 days<br>and no changes<br>after 15 days         |                                                         |                                          |          |                                                           |
| FSH                                                                         |       |                                |                                | No change                                                  |                                                         |                                          |          |                                                           |
| Prolactin                                                                   |       |                                |                                | ↓ After 15 days,<br>no changes<br>after 28 days            |                                                         |                                          |          |                                                           |
| Testosterone<br>hydroxylase<br>(2α-OHT)<br>activity,<br>CYP2C11             |       | Rat,<br>Sprague-<br>Dawley (M) | Hepatic<br>liver<br>microsomes | No change                                                  | 1500 mg/kg per day                                      | Oral gavage,<br>daily, 15 or<br>28 days  |          | <a href="#">Williams<br/>and<br/>Borghoff<br/>(2000)</a>  |
| Testosterone<br>hydroxylase<br>(2β-OHT;<br>6β-OHT)<br>activity,<br>CYP3A1/2 |       |                                |                                | No change in<br>2β-OHT levels<br>↑ 6β-OHT after<br>28 days |                                                         | Oral gavage,<br>daily, 28 days           |          |                                                           |
| Testosterone<br>hydroxylase<br>(7α-OHT)<br>activity, CYP2A1                 |       |                                |                                | ↑ After 15 days                                            |                                                         | Oral gavage,<br>daily, 15 days           |          |                                                           |
| Androstenedione<br>production<br>(CYP 2B1/2 and<br>CYP2C11)                 |       |                                |                                | No changes                                                 |                                                         | Oral gavage,<br>daily, 14 or<br>28 days  |          |                                                           |

**Table 4.13 (continued)**

| End-point                        | Assay                         | Species, strain (sex)              | Tissue, cells            | Results <sup>a</sup>          | Dose or concentration (LED or HID, LEC or HIC) | Route, duration, dosing regimen                    | Comments                                                             | Reference                                |
|----------------------------------|-------------------------------|------------------------------------|--------------------------|-------------------------------|------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------|------------------------------------------|
| Estrogen metabolism              | Estrogen metabolism assay     | Mouse, B6C3F <sub>1</sub> (F)      | Hepatic liver microsomes | +                             | 1800 mg/kg per day                             | Gavage, daily, 3 days                              |                                                                      | <a href="#">Moser et al. (1996b)</a>     |
| Hepatic microsomal CYP           | Fluorometric method           |                                    |                          | ↑<br>(No change after 3 days) | 7814 ppm                                       | Inhalation, 6 h/day, 5 days/wk, 21 days            |                                                                      |                                          |
| Hepatic microsomal PROD activity | Fluorometric method           |                                    |                          | ↑                             |                                                | Inhalation, 6 h/day, 5 days/wk, both 3 and 21 days |                                                                      |                                          |
| Hepatic microsomal EROD activity | Fluorometric method           |                                    |                          | ↑                             |                                                | Inhalation, 6 h/day, 5 days/wk, both 3 and 21 days |                                                                      |                                          |
| <i>In vitro</i>                  |                               |                                    |                          |                               |                                                |                                                    |                                                                      |                                          |
| Testosterone production          | RIA                           | Rat, Leydig cells                  | Testis                   | ↓                             | 50 mM                                          | 3 h, after stimulation with hCG                    | hCG (2.0 IU/mL) was added last to stimulate testosterone production. | <a href="#">de Peyster et al. (2009)</a> |
| Testosterone production          | Chemiluminescence immunoassay | Rat, primary immature Leydig cells | Testis                   | ↓                             | 200 mM                                         | 0, 100, 200, or 300 mM for 24 h                    | Testosterone concentration measured in the medium.                   | <a href="#">Zhu et al. (2022)</a>        |

ABP, androgen-binding protein; CYP, cytochrome P450; EIA, enzyme immunoassay; EROD, 7-ethoxyresorufin *O*-deethylase; F, female(s); FSH, follicle-stimulating hormone; h, hour(s); hCG, human chorionic gonadotropin; HID, highest ineffective dose; HIC, highest ineffective concentration; IU, international units; LEC, lowest effective concentration; LED, lowest effective dose; LH, luteinizing hormone; M, male(s); MTBE, methyl *tert*-butyl ether; OHT, hydroxylated testosterone; ppm, parts per million; PROD, 7-pentoxoresorufin-*O*-dealkylase; RIA, radioimmunoassay; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; +, positive; or no change.

individuals with concomitant environmental and occupational exposure to solvents, glues, or metals were excluded. Controls had a similar age and racial distribution and lived in the same geographical area as the cases, and included 32 healthy individuals (17 men and 15 women). [The Working Group noted that the authors mentioned that the controls were not exposed to MTBE- and benzene-contaminated water, but did not describe how this was determined, especially considering that they were from the same geographical area as the cases.] Apoptosis was determined by TUNEL (terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling) assay in the lymphocytes of exposed participants (collected 10–12 months after the last episode of exposure, but it was not specified how this time was determined), from non-exposed participants, and in a lymphocytic cell line exposed to camptothecin (type I topoisomerase inhibitor, as positive control) or not exposed (negative control). [The Working Group noted that the TUNEL assay detects DNA fragmentation, a sign of apoptosis. The assay has the advantage of being very sensitive and fast, but also disadvantages, such as the risk of detecting false positives, since necrotic cells and cells undergoing DNA repair and active gene transcription are also detected ([Elmore, 2007](#)).] The frequency of apoptotic cells was significantly higher in the exposed group than in the control group, and it was also higher than in the technical positive control. Notably, some individuals from the control group showed a similar or higher frequency of apoptotic cells than did the positive control. Additionally, 80.5% of the cases showed a frequency of apoptotic cells that was above the median of the control group. Cell cycle analysis by flow cytometry revealed that exposed individuals had a higher proportion of cells in the S and G2/M phases and a lower proportion of cells in the G0/G1 phase, relative to controls. Although results were not shown individually for all cases, the authors stated that a correlation between abnormal apoptosis and a

shift in the cell cycle was detected in 85% of the patients. [The Working Group noted that statistical methods for cell cycle analysis were not properly described, thus limiting the strength of the conclusions.] According to the authors, the higher the frequency of apoptosis, the higher the shift from the G0/G1 phase to the S and G2/M phases, which might indicate increased mitogenic activity. [The Working Group noted that the main limitations of this study included the use of a single technique, the TUNEL assay, for evaluating apoptosis, especially because this method requires additional assessments to exclude potential false positives. Another limitation was the lack of details regarding the recruitment and exposure determination of the cases and controls. Additionally, blood sampling was performed at a single time-point, which added biases to the study, such as moment of collection (time of the day, period of the year), and sample processing (time between collection and analysis, transportation conditions), among others. Statistical methods were poorly described, especially for the cell cycle analysis.]

#### (b) *Experimental systems*

##### (i) *Non-human mammalian systems in vivo*

See [Table 4.14](#).

Short-term 3-day and 21-day exposures to MTBE were tested in female CD-1 and B6C3F<sub>1</sub> mice ([Moser et al., 1996b](#)). Exposure to MTBE at 8000 ppm led to increased relative liver weight in both mouse strains at both time points. Hepatocyte labelling with bromodeoxyuridine (BrdU) was also higher than the baseline at the same time points, except among B6C3F<sub>1</sub> mice exposed for 21 days, for which a reduction was observed. MTBE tumour-promoting effects in B6C3F<sub>1</sub> mice were also evaluated in the same study, with the initial administration of *N*-nitrosodiethylamine (DEN), followed by MTBE exposure (for 16 and 32 weeks). These data showed a decrease in total body weight



**Table 4.14 End-points relevant to cell proliferation, cell death, or nutrient supply in experimental systems and non-human mammalian systems in vivo and in vitro exposed to methyl *tert*-butyl ether**

| End-point                    | Assay          | Species, strain (sex), or cell line    | Tissue            | Results <sup>a</sup>                      | Dose (LED or HID) | Route, duration, dosing regimen                                                                                                                                                                                                                                                                             | Comments                                                                                            | Reference                            |
|------------------------------|----------------|----------------------------------------|-------------------|-------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------|
| Hepatocyte proliferation     | BrdU labelling | Mouse, CD-1 and B6C3F <sub>1</sub> (F) | Liver             | ↑ Cell proliferation                      | LED, 8000 ppm     | Short-term effects: inhalation of MTBE at 8000 ppm for 3 or 21 days, 5 days/week. Tumour-promoting model: female B6C3F <sub>1</sub> mice (age, 12 days) were initiated with a single i.p. injection of DEN (5 mg/kg, 7.1 mL/kg bw) or saline. Beginning at age 8 wk, mice were exposed to MTBE at 8000 ppm. | Only females were analysed.                                                                         | <a href="#">Moser et al. (1996a)</a> |
| TGFα and EGFR immunostaining | IHC            | Mouse, B6C3F <sub>1</sub> (F)          | Liver             | No change associated with MTBE treatment  |                   | Single i.p. injection of DEN (5 mg/kg, 7.1 mL/kg bw) or saline (initiation controls) and then treated with MTBE at 7814 ± 81 ppm for 6 h/day, 5 days/wk, for 4 or 8 mo.                                                                                                                                     | Only females were analysed.                                                                         | <a href="#">Moser et al. (1997)</a>  |
| Hyperplasia                  | Histopathology | Mouse, CD-1 (F)                        | Uterus            | ↓ Endometrial cell hyperplasia, incidence | LED, 3000 ppm     | Inhalation of MTBE vapour at 400, 3000, or 8000 ppm for 6 h/day, 5 days/wk, for 18 mo (mice) or 24 mo (rats).                                                                                                                                                                                               | Not relevant: spontaneous hyperplasia, too difficult to assess because of the nature of the tissue. | <a href="#">Bird et al. (1997)</a>   |
| Hyperplasia                  |                | Rats, F344, both sexes                 | Parathyroid gland | ↑                                         | LED, 3000 ppm     |                                                                                                                                                                                                                                                                                                             | Linked to nephropathy.                                                                              |                                      |
| Cell proliferation           | BrdU labelling | Rats, F344, both sexes                 | Kidney            | ↑<br>After day 5 and day 28 in male rats  | LED, 3000 ppm     | A 28-day vapour inhalation study with same dosing regimen as above ( <i>n</i> = 10/group).                                                                                                                                                                                                                  |                                                                                                     |                                      |
| Hepatocyte proliferation     | BrdU labelling | Mouse, CD-1 (F and M)                  | Liver             | ↑<br>At 5 days only in female mice        | LED, 8000 ppm     |                                                                                                                                                                                                                                                                                                             |                                                                                                     |                                      |

**Table 4.14 (continued)**

| End-point                        | Assay          | Species, strain (sex), or cell line         | Tissue                        | Results <sup>a</sup>                                                          | Dose (LED or HID)                                              | Route, duration, dosing regimen                                                                                                            | Comments                                                                                                       | Reference                               |
|----------------------------------|----------------|---------------------------------------------|-------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| Hyperplasia                      | Histopathology | Rat, F344, (M)                              | Thyroid gland<br>Nasal tissue | No change<br><br>No change (apparent increase of 4–6% in incidence)           |                                                                | Inhalation of vapour condensate from gasoline containing MTBE (21.3%) at 2, 10, or 20 g/m <sup>3</sup> for 6 h/day, 5 days/wk, for 104 wk. | Hyperplasia was not analysed in females. Did not evaluate the effects of MTBE alone.                           | <a href="#">Benson et al. (2011)</a>    |
| Cortical tubular hyperplasia     | Histopathology | Rat, Wistar (F and M)                       | Kidney                        | ↑ In female rats.<br>No change in males<br>No change                          | LED, 15 mg/mL (females)                                        | Drinking-water containing MTBE at concentrations of 0.5, 3, or 7.5 mg/mL (males) and 0.5, 3 or 15 mg/mL (females), 2 yr.                   | Data on hyperplasia were poorly described.                                                                     | <a href="#">Dodd et al. (2013)</a>      |
| Papillary epithelial hyperplasia |                |                                             |                               |                                                                               |                                                                |                                                                                                                                            |                                                                                                                |                                         |
| Cell proliferation               | BrdU labelling | Rat, Wistar (F and M)                       | Kidney                        | ↑ At 4 wk in males only at the highest dose<br>No changes in the other groups | LED, 15 mg/mL (males)                                          | Drinking-water containing MTBE at 0.5, 3, 7.5, or 15 mg/mL, 13 wk.                                                                         | Tubular cell regeneration in groups of males at 7.5 and 15 mg/mL.                                              | <a href="#">Bermudez et al. (2012)</a>  |
| Alterations in vasculature       | Histology      | Rat pups of exposed pregnant rats (F and M) | Vessels                       | No change                                                                     |                                                                | Gavage, once daily from day 6 to day 10 of gestation, at 500, 1000, 1200, or 1500 mg/kg per day.                                           |                                                                                                                | <a href="#">Kozlosky et al. (2013)</a>  |
| <i>PFKFB-3</i> mRNA expression   | qPCR           | Rat, Wistar, (M)                            | Lungs<br>Liver<br><br>Heart   | ↓<br>↓ (0.5 mg/kg)<br>↑ (5 and 50 mg/kg)<br>↑                                 | LED, 5 mg/kg<br>LED, 0.5 mg/kg<br>LED, 5 mg/kg<br>LED, 5 mg/kg | MTBE (0.5, 5, or 50 mg/kg bw), once daily for 2 mo.                                                                                        | The expression of <i>PFKFB-3</i> isoforms was analysed, but only by conventional PCR, limiting quantification. | <a href="#">Minchenko et al. (2009)</a> |

**Table 4.14 (continued)**

| End-point            | Assay | Species, strain (sex), or cell line | Tissue | Results <sup>a</sup>                | Dose (LED or HID)                  | Route, duration, dosing regimen | Comments                                                                                                 | Reference                                       |
|----------------------|-------|-------------------------------------|--------|-------------------------------------|------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| VEGF mRNA expression |       |                                     | Lungs  | ↑ (0.5 mg/kg)<br>↓ (5 and 50 mg/kg) | LED,<br>0.5 mg/kg;<br>LED, 5 mg/kg |                                 | The expression of <i>VEGF</i> isoforms was decreased in the lungs and increased in the liver (50 mg/kg). | <a href="#">Minchenko et al. (2009)</a> (cont.) |
|                      |       |                                     | Liver  | ↑                                   | LED,                               |                                 |                                                                                                          |                                                 |
|                      |       |                                     | Heart  | No change                           | 0.5 mg/kg                          |                                 |                                                                                                          |                                                 |

BrdU, bromodeoxyuridine; bw, body weight; DEN, *N*-nitrosodiethylamine; EGFR, endothelial growth factor receptor; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; IHC, immunohistochemistry; i.p., intraperitoneal; LED, lowest effective dose; M, male(s); mo, month(s); mRNA, messenger RNA; MTBE, methyl *tert*-butyl ether; TGFα, transforming growth factor alpha; *PFKFB*-3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; ppm, parts per million; VEGF, vascular endothelial growth factor; qPCR, quantitative polymerase chain reaction; wk, week(s); yr, year(s).

<sup>a</sup> ↑, increase; ↓, decrease; or no change.

and an increase in hepatocyte labelling at both time points. The findings were similar to those observed without pretreatment with DEN. Additionally, increased liver weight was observed after exposure to MTBE for 16 weeks, after either DEN or saline injection, or for 32 weeks after saline injection. [The Working Group noted that only females were analysed.]

Immunostaining of TGF $\alpha$  and EGFR was assessed in the liver of B6C3F<sub>1</sub> mice initiated with a single intraperitoneal injection of DEN (5 mg/kg, 7.1 mL/kg bw) or saline (initiation controls) and then exposed to MTBE at 7814  $\pm$  81 ppm for 6 hours per day, 5 days per week, for 4 or 8 months ([Moser et al., 1997](#)). Both EGFR and TNF $\alpha$  immunostaining were dependent on the phenotype (basophilic or acidophilic) of hepatocyte foci and hepatocellular tumours, irrespective of treatment. [The Working Group noted that only females were analysed.]

The carcinogenicity of MTBE vapour was tested in CD-1 mice and F-344 rats exposed for 18 and 24 months, respectively (see Section 3) ([Bird et al., 1997](#)). The incidence of endometrial cell hyperplasia was reduced after exposure at 3000 and 8000 ppm in mice. [The Working Group noted that endometrial cell hyperplasia is a spontaneous event and difficult to measure because of the characteristics of the tissue. Therefore, the Working Group considered this result to be uninformative.] Notably, the study was terminated earlier because of high mortality in male rats treated with MTBE at intermediate and higher doses. Nephropathy was less evident in female rats, but it was observed at the intermediate and higher doses. The nephropathy was associated with secondary lesions, such as hyperplasia of the parathyroid glands, in both sexes. [The Working Group noted that hyperplasia of the parathyroid gland was likely to be a consequence of nephropathy.] In addition, a 28-day study of vapour inhalation was conducted in both species. Increased BrdU labelling of hepatic cells was observed in female mice

treated with MTBE at 8000 ppm after 5 days. In the 28-day study on vapour inhalation, increased BrdU labelling was also reported in kidney cells in male rats treated with MTBE at 3000 ppm (day 5 and 31) and 8000 ppm (day 31) ([Bird et al., 1997](#)).

Another study showed no alteration in the frequency of nasal and thyroid hyperplasia in male F344 rats chronically exposed to gasoline containing MTBE for 2 years. There was an apparent (not statistically significant) increase in hyperplasia in the nasal turbinate level 2 sections in male rats, starting from the lowest dose (2 g/m<sup>3</sup>). The weight of selected organs (kidneys, testes or ovaries, lungs) was not affected by exposure, but terminal body weight was reduced in males and females exposed at the highest dose (20 g/m<sup>3</sup>) ([Benson et al., 2011](#)). [The Working Group noted as a limitation of the study protocol that hyperplasia was not analysed in the same tissues in males and females. For example, nasal sections and the thyroid gland, in which differences were observed among males, were not analysed in exposed females. A major limitation was the exposure to MTBE only in combination with gasoline, and not alone.]

The carcinogenicity of MTBE was addressed in Wistar rats exposed to the agent at 0.5, 3.0, or 15.0 mg/mL, via drinking-water, for 2 years (see Section 3) ([Dodd et al., 2013](#)). No differences in survival, body weight, or the weight of various organs (except for kidney) were observed between the exposed and non-exposed groups. The frequency of tubular hyperplasia was increased in the renal cortex of female rats, whereas the frequency of papillary epithelial hyperplasia did not significantly differ from that in the controls, at 24 months of exposure. [The Working Group noted that data on hyperplasia were poorly described.]

In a 13-week study, there was a decrease in total body weight in male Wistar rats treated via drinking-water with MTBE at the highest dose (15 mg/mL), although female rats were

unaffected ([Bermudez et al., 2012](#)). However, both males and females exhibited an increase in kidney relative weight. No significant increase in kidney cell proliferation was observed in either males or females, at any concentration of MTBE, after 1 week or 13 weeks. Only in males at the highest exposure at 4 weeks was there an increase in the labelling index in cortical proximal tubule cells. At 13 weeks, the kidneys of male rats showed an increase in the incidence and severity of tubular cell regeneration in the groups at 7.5 and 15 mg/mL.

[Kozlosky et al. \(2013\)](#) investigated whether MTBE would affect developing microcapillaries in utero pre- and postpartum. Pregnant female rats were dosed orally by gavage with MTBE in corn oil at 500 to 1500 mg/kg per day, from implantation (day 6 of pregnancy) through gestation. The treatment of pregnant Sprague-Dawley rats with MTBE did not lead to histological vascular alterations in the offspring. In the murine matrigel plug implantation assay, MTBE completely inhibited vessel invasion into the plugs containing endothelial cell growth supplement.

The effects of MTBE on 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB-3) and vascular endothelial growth factor (VEGF) mRNA expression were assessed by quantitative PCR in the lungs, liver, and heart of male Wistar rats treated once per day for 2 months at a dose of 0.5, 5, or 50 mg/kg bw ([Minchenko et al., 2009](#)). PFKFB-3 mRNA expression was decreased in the liver of rats at the lowest dose compared with controls. However, gene expression increased in the same organ in rats at the highest dose. In the other organs, only treatment at 5 mg/kg bw caused significant effects, leading to reduced expression in the lungs and increased expression in the heart. VEGF expression did not significantly vary in the heart after treatment at any dose, but it decreased in the lungs in rats treated with MTBE at 0.5 mg/kg bw and increased after treatment at 5 mg/kg bw. In the liver, MTBE at

all doses induced VEGF expression. The expression of a VEGF isoform (mRNA with a 14-base insert after exon 5) was also assessed and showed increased levels in the liver and decreased levels in the lungs of rats treated with MTBE at the highest dose. [The authors evaluated the expression of PFKFB-3 isoforms only by conventional PCR, and the Working Group noted that this was a limitation in the quantification of PFKFB-3 isoforms.]

#### (ii) *Non-human mammalian systems in vitro*

To investigate whether similar effects involving the cardiovascular system would be observed in mammals, [Kozlosky et al. \(2013\)](#) considered the in vitro formation on matrigel of capillary-like tubes by primary endothelial cells isolated from the brains of F344 rats. MTBE treatment resulted in a dose-dependent reduction in tube formation. Similarly, the authors of the same study reported the formation of tubes on matrigel by human primary vascular endothelial cells (HUVEC), which were narrower relative to those in controls in the presence of MTBE. [The Working Group noted that the study by Kozlosky et al. was the only one available in human primary cells.]

Other models have also been applied to investigate the effects of MTBE on cell the cell cycle ([Sgambato et al., 2009](#)). Rat fibroblasts treated with MTBE (0.084 mM or 0.84 mM) showed a reduction in viability associated with a cytostatic effect after 24 and 48 hours. Treatment at the highest concentration also promoted cell cycle arrest in S phase, and a reduction in the proportion of cells in G0/G1 and G2/M phases was observed in a time-dependent manner. Additionally, an increase in the number of hypodiploid cells was detected, indicating apoptosis. The molecular mechanisms potentially involved were investigated and suggested accumulation of pRb, p53, and p27<sup>KIP1</sup> as well as reduction in cyclin D1 at specific time points. Oxidative damage and DNA breaks were induced.

The effects of MTBE on cell proliferation were also addressed in normal rat fibroblasts (Rat-1). A dose-dependent reduction in cell survival was observed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and the median inhibitory concentration (IC<sub>50</sub>) was shown to be 0.84 mM. The trypan exclusion assay showed a reduced cell count after exposure to MTBE at 0.84 mM or 0.084 mM. The authors also reported a decrease in the percentage of cells in the G2/M phase, which was accompanied by an increase in the percentage of cells in S phase and in subG1, suggesting an increase in apoptosis ([Iavicoli et al., 2002](#)).

### (iii) Other models in vivo

Experiments with MTBE exposure have also been performed during embryonic development in zebrafish ([Bonventre et al., 2011](#)). First, the median lethal concentration (LC<sub>50</sub>) was defined at approximately 20 mM. Then, at sublethal concentrations, the authors observed a dose-dependent increase in the number of embryos exhibiting pooled blood in the common cardinal vein, cranial haemorrhages, abnormal intersegmental vessel formation, pericardial oedema, and reduced circulating erythrocytes. The mRNA expression of VEGFA, VEGFC, flk1/VEGFR2, and HIF1 $\alpha$ -like was reduced in embryos exposed to MTBE at 5 mM. Except for the cardiovascular system, no other organs were affected. In another study, the contribution of hypoxia inducible factor (HIF)-regulated pathways in anti-angiogenic effects caused by MTBE was confirmed by applying different strategies to block different players ([Bonventre et al., 2013](#)). VEGFA overexpression reduced the incidence of cranial haemorrhage and abnormal intersegmental vessel formation induced by MTBE. The inhibition of HIF degradation by *N*-oxaloylglycine or by the use of a morpholino (anti-sense oligomer) designed to knockdown a von Hippel–Lindau (VHL)-associated ubiquitin led to the reduction of pooled blood in the common cardinal

vein that was induced by MTBE. The incidence of cranial haemorrhage and abnormal intersegmental vessel formation induced by MTBE was also reduced by *N*-oxaloylglycine treatment.

### Synopsis

[The Working Group noted that the effects of MTBE exposure on cell proliferation were described in several studies, although with inconsistent findings. One study was available in exposed humans, but it presented important limitations, such as the use of nonspecific methodologies and the lack of precise exposure measurement. The available in vivo studies indicated that MTBE induced cell proliferation in the liver (in mice) and in the kidney (in rats), especially after short-term exposure. Data on effects on cell death and nutrient supply were scarce.]

### 4.2.10 Evaluation of high-throughput in vitro screening data

The mechanistic characteristics common to carcinogens (the 10 key characteristics of carcinogens) can be investigated through biochemical and cell-based assays run by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health Toxicity Forecaster/Toxicology in the 21st Century (ToxCast/Tox21) high-throughput screening programmes ([Chiu et al., 2018](#); [Guyton et al., 2018](#)). Since 2017, the *IARC Monographs* programme has described the results of high-throughput screening assays to compare activity across agents and other evidence in vitro and in vivo relevant to the key characteristics.

Of the six agents included in *IARC Monographs* Volume 138, four have been evaluated in the USEPA and United States National Institutes of Health high-throughput screening assays: MTBE (CAS No. 1634-04-4, DTXSID3020833), ETBE (CAS No. 637-92-3, DTXSID0025604), DIPE (CAS No. 108-20-3, DTXSID4021890), and TAME (CAS No. 994-05-8, DTXSID8024521).



Details of the results for specific assays (and end-points) run for MTBE, ETBE, DIPE, and TAME and mapping to the key characteristics of carcinogens can be found in Annex 4 (Supplementary material for Section 4, Mechanistic evidence, online only, available from: <https://publications.iarc.who.int/652>). It is important to note that some assays either lacked, or had uncharacterized and generally low, xenobiotic metabolism, limiting observations primarily to effects elicited by parent compounds. The strengths of the high-throughput screening battery of assays are the standardization of the protocols applied across compounds, allowing comparisons across compounds and the evaluation of specificity of assay end-points to the key characteristics, and ultimately to the apical outcome of carcinogenesis (Becker et al., 2017; Chiu et al., 2018; Watford et al., 2019). The 111 ToxCast/Tox21 assay end-points mapped to the key characteristics of carcinogens interrogated in this and other monographs were initially described by Chiu et al. (2018), with the most up-to-date mapping being described in detail in *IARC Monographs* Volume 123 (IARC, 2020). The kc-hits software package (Reisfeld et al., 2022) was used to map results from the US EPA CompTox Chemicals Dashboard v2.4.1 (April 2024) between 10 and 19 September 2024.

Results were available for 11 assay end-points mapped to key characteristics for MTBE. MTBE was considered to be active in four out of 60 assay end-points mapped to the key characteristic “alters cell proliferation, cell death, or nutrient supply” (KC10). Assays in which end-points were identified as active included TOX21\_RT\_HEK293\_FLO\_16hr\_viability, TOX21\_RT\_HEK293\_FLO\_32hr\_viability, and TOX21\_RT\_HEK293\_FLO\_40hr\_viability. The assays used a dead cell DNA stain for which an increase in measured fluorescence indicated a loss of cell viability. The labels “16hr”, “32hr”, and “40hr” indicate the times at which viability was measured after exposure. The other active

end-point assay was the TOX21\_ERb\_BLA\_Agonist\_viability, which is a type of viability reporter using which loss-of-signal activity can be used to understand changes in cell viability. These assays use HEK293T, a human embryonic kidney cell line, with chemical dosing in a 1536-well plate. All four active calls were noted with the flags “borderline active” and “less than 50% efficacy”.

[The Working Group noted that “flags” for active calls have been programmatically generated during the fit process to indicate characteristics of a curve that need extra attention, or to describe potential anomalies of the curve or data. Specific “flags” were noted here, when present, to provide interpretative context. The complete list of “flags” generated by the current hit calling pipeline is given by Feshuk et al. (2023). Not all “flags” are necessarily disqualifying, so the final active hit calls from kc-hits may include this subset.]

## 4.3 Other relevant evidence

### 4.3.1 Effects on metabolism

#### (a) Humans

Yang et al. (2016) assessed the relationship between exposure to MTBE and the prevalence of non-alcoholic fatty liver disease (NAFLD, now called metabolic dysfunction-associated steatotic liver disease, MASLD) among service station attendants in southern China. Personal exposure sampling for each participant (41 men and 30 women) was performed using a charcoal-based organic vapour monitor, followed by MTBE analysis using HS-SPME GC-MS. The mean exposure concentrations of MTBE were  $292.98 \pm 154.90 \mu\text{g}/\text{m}^3$  and  $286.64 \pm 122.28 \mu\text{g}/\text{m}^3$  in NAFLD and non-NAFLD groups, respectively. The prevalence of NAFLD was 15.49% among the participants. Multivariable logistic regression, adjusted for confounding factors, indicated no significant association between

four levels of MTBE exposure and NAFLD prevalence. Although the highest-level exposure was set as  $\geq 300 \mu\text{g}/\text{m}^3$ , none of the observed individual values exceeded the threshold limit value of 50 ppm ( $180\,500 \mu\text{g}/\text{m}^3$ ). [The Working Group noted that the findings of this study suggested that MTBE exposure was not a significant risk factor for NAFLD in this occupational cohort.]

[Guo et al. \(2024b\)](#) investigated the impact of exposure to MTBE on insulin function and glucose homeostasis in service station attendants ( $n=201$ ; all men) in Anhui Province, China. Levels of MTBE in the blood were measured using HS-SPME gas chromatography-high-resolution mass spectrometry. According to measured MTBE concentrations, participants were divided into two groups with high ( $n=100$ ; above the median; MTBE,  $0.71 \mu\text{g}/\text{L}$  in blood) or low ( $n=101$ ; below the median; MTBE,  $0.19 \mu\text{g}/\text{L}$ ) exposure levels. The study found a positive correlation between MTBE exposure and fasting plasma glucose levels, and a negative correlation between MTBE exposure and serum insulin levels (detected by ELISA), suggesting that MTBE inhibits insulin secretion. The results for fasting plasma glucose and insulin remained significant after adjusting for covariates in multiple linear regression models.

[Guo et al. \(2024a\)](#) explored the potential relationship between the exposure to MTBE and insulin resistance, using the triglyceride glucose (TyG) index as a surrogate marker, in the same population of service station attendants as described above ([Guo et al., 2024b](#)). In this study, participants were divided into two groups of occupationally exposed (service station attendants,  $n=83$ ; MTBE,  $0.73 \pm 0.51$  ppb) and non-occupationally exposed (other workers in the same company,  $n=118$ ; MTBE,  $0.26 \pm 0.15$  ppb). Levels of MTBE in the blood were measured using HS-SPME gas chromatography-high-resolution mass spectrometry. Using the defined exposure groups, MTBE levels were significantly higher in the occupationally exposed group than in the

non-occupationally exposed group. Considering the entire study population, significant findings included positive correlations between blood MTBE levels and both the TyG index and fasting plasma glucose. Multivariable logistic regression analysis identified MTBE exposure as an independent risk factor for a high TyG index (OR, 1.088), suggesting that MTBE might increase the risk of insulin resistance according to this surrogate marker.

#### (b) *Experimental systems*

[Guo et al. \(2023\)](#) investigated systemic metabolic effects and toxic mechanisms of MTBE exposure, with a focus on glutamine metabolism, in male Wistar rats. MTBE exposure was measured via intragastric administration of MTBE at doses of 0, 1, and 100 mg/kg bw daily for 24 weeks. Significant findings indicated that MTBE exposure reduced levels of serum high-density lipoprotein cholesterol (HDL-C) and increased levels of very low-density lipoprotein cholesterol (VLDL). Exposure disrupted glutamine metabolism by decreasing serum glutamine levels, increasing glutamate levels, and upregulating glutaminase 1 isoform (GLS1) expression in the liver, contributing to systemic lipid metabolism disturbances.

[Tang et al. \(2019\)](#) examined potential links between chronic exposure to gasoline-derived MTBE on adipogenesis and glucose metabolism in vitro and in vivo. MTBE was delivered by administering concentrations of up to  $100 \mu\text{mol}/\text{L}$  to mouse pre-adipocyte 3T3-L1 cells, and doses of  $100 \mu\text{g}/\text{kg}$  bw per day,  $1000 \mu\text{g}/\text{kg}$  bw per day, or  $100 \text{ mg}/\text{kg}$  bw per day through dietary exposure in mice for 14 weeks. Samples were divided into exposure groups according to MTBE level and diet types, including high-fat diet conditions. Both male and female mice were used, revealing sex-specific effects: male mice exhibited significant reductions in glucose tolerance and insulin sensitivity, whereas female mice showed increased insulin

sensitivity, without corresponding changes in glucose tolerance. MTBE exposure caused significant increases in adipocyte size, visceral white adipose tissue, and the expression of adipogenic marker genes *Fabp4* and *Pparg*.

[Valipour et al. \(2015\)](#) investigated the molecular interaction between MTBE and insulin in a sealed system, focusing on potential modifications and toxicity. MTBE was incubated under physiological conditions with commercial insulin, at concentrations of 8  $\mu$ M and 40  $\mu$ M; structural changes were assessed using spectroscopy and chemiluminescence methods. Samples were divided into two groups corresponding to MTBE concentration. Significant findings included the formation of a molten globule-like state of insulin, characterized by increased hydrophobic surface exposure and aggregation, with MTBE at 8  $\mu$ M. Additionally, MTBE induced ROS production, with higher levels at 8  $\mu$ M than at 40  $\mu$ M (see Section 4.2.5).

[Overall, the studies in experimental systems probed the potential for MTBE exposure to elicit metabolic effects. Significant findings included systemic metabolic disturbances, including altered lipid and glutamine metabolism and sex-specific metabolic effects.]

#### 4.3.2 Effects related to $\alpha_{2u}$ -globulin in the male rat kidney

Several studies investigated male rat-specific  $\alpha_{2u}$ -globulin nephropathy and its role in renal tumorigenesis to elucidate mechanisms related to the renal toxicity of MTBE in the male rat. These are described below.

[Poet and Borghoff \(1997\)](#) (see Section 4.1) studied the uptake and association constant of  $C^{14}$ -labelled MTBE in kidney homogenates of male and female F344 rats. The uptake of radio-labelled MTBE was five times higher in kidney homogenates from male rats than from female rats and was dependent on protein and chemical concentrations. In glomerular filtration, the radioactivity

coeluted with the total protein fraction, but no interaction between MTBE and proteins was found by dialysis or anion exchange chromatography. Using a two-compartment vial equilibration model, the dissociation constant for MTBE and  $\alpha_{2u}$ -globulin was estimated to be 0.215 mM; this value is in the range of that for other chemicals known to bind to  $\alpha_{2u}$ -globulin and cause  $\alpha_{2u}$ -globulin-mediated nephropathy, thus explaining the process that contributes to the uptake of MTBE into male rat kidney homogenate in vitro. [The Working Group noted that these data supported reversible binding of MTBE to  $\alpha_{2u}$ -globulin.]

[Leavens and Borghoff \(2009\)](#) explored the nephrotoxic and carcinogenic potential of MTBE using PBPK modelling in male F344 rats exposed by inhalation. The model is described in Section 4.1. The binding of MTBE and TBA to  $\alpha_{2u}$ -globulin was modelled as saturable and competitive and was assumed to affect only the rate of  $\alpha_{2u}$ -globulin hydrolysis in the kidney. The model could describe the concentrations of MTBE and TBA in the blood, kidney, and other tissues in male and female rats treated with MTBE by inhalation or repeated exposure to TBA. The model predicted differences in kidney concentrations of MTBE in male versus female rats, and it showed that the predicted binding affinity of MTBE to  $\alpha_{2u}$ -globulin was higher than that of TBA, and that the hydrolysis rate of chemically bound  $\alpha_{2u}$ -globulin was approximately 30% of that for the unbound protein. [The Working Group noted that the PBPK model supported the evidence that MTBE binds to the male rat-specific protein  $\alpha_{2u}$ -globulin, and that the PBPK model predictions closely matched measured values for most time points.]

[Prescott-Mathews et al. \(1997\)](#) exposed male and female F344 rats to MTBE at 0, 413, 1516, or 3013 ppm (6 hours per day) for 10 days. Labelling indices were increased in the male kidney at all exposure levels. In addition, proximal tubular necrosis and protein droplet accumulation

were observed at the two higher exposures. Immunohistochemical staining showed that  $\alpha_{2u}$ -globulin was present in and accumulated in protein droplets in the male rat kidney. A mild dose-related increase in  $\alpha_{2u}$ -globulin in the kidney was observed that was significant at the highest exposure level. The exposure level correlated strongly ( $r=0.994$ ) with cell proliferation and  $\alpha_{2u}$ -globulin concentration in the male rat kidney, with no correlation with cell proliferation in female rats (the assessment was conducted blind). No accumulation was observed for any protein except  $\alpha_{2u}$ -globulin. The study showed that MTBE induced  $\alpha_{2u}$ -globulin and enhanced renal cell proliferation (including in the renal cortex) in male F344 rats. In the same study, F344 rats were exposed to  $^{14}\text{C}$ -labelled MTBE by oral gavage ( $^{14}\text{C}$ ]-MTBE at 750 mg/kg bw in Emulphor once daily for 4 days) (Prescott-Mathews et al., 1999). The authors assessed the interaction between MTBE and  $\alpha_{2u}$ -globulin to understand the mechanisms underlying the development of MTBE-induced renal tumours. Samples were divided into groups of four rats per sex, with additional control groups that received the vehicle only. Male rats exhibited a statistically significant increase in renal  $\alpha_{2u}$ -globulin concentration (measured by ELISA), but no such effect was observed in female rats. In G-25 gel filtration, a slightly higher fraction of MTBE coeluted with the protein fraction in male rats but not in female rats. Only male rats showed a significant increase in MTBE levels measured by headspace chromatography in kidney samples incubated with *d*-limonene oxide (in comparison with those not incubated with *d*-limonene oxide), indicating that MTBE was associated in vivo with male-specific  $\alpha_{2u}$ -globulin, since *d*-limonene oxide has been shown to bind specifically to this protein. [The Working Group noted that the study showed that MTBE induced binding to  $\alpha_{2u}$ -globulin in the male rat kidney, and that binding was reversible.]

Clark et al. (2014) investigated subchronic toxicity and potential carcinogenicity of gasoline and of combined gasoline and MTBE vapour in Sprague-Dawley rats exposed by inhalation. Exposure was measured by administering vapour condensates containing MTBE at concentrations of 0, 2000, 10 000, and 20 000 mg/m<sup>3</sup> for 6 hours per day, 5 days per week, for 13 weeks. Samples were divided into four exposure groups, with 10 males and 10 females per group, and in recovery groups consisting of 20 animals at the highest exposure level and the control. Both male and female rats were used to assess sex-specific effects. Significant findings included increased kidney weights in male rats at all exposure levels, and in females at the intermediate and highest concentration; nephropathy (light hydrocarbon nephropathy) was also observed in males, in which microscopic lesions were consistent with changes associated with  $\alpha_{2u}$ -globulin accumulation.

Bermudez et al. (2012) (see Section 4.2.9) reported the development of CPN of minimal to mild severity in male rats, but not in females, after 1 year of exposure to MTBE through drinking-water. After 4 weeks, but not after 13 weeks of exposure, there was an increase in cell replication in the cortical proximal tubule cells only in male rats at the highest dose. After 4 weeks of exposure, minimal diffuse staining for  $\alpha_{2u}$ -globulin was evident in all males (but not in females) of all concentration groups, but tubular regeneration was not evident. After 13 weeks, diffuse  $\alpha_{2u}$ -globulin staining was found in all treated males, and tubular cell regeneration was increased in kidney from males exposed at 7.5 mg/mL or higher. The authors concluded that limited changes in the kidney were observed in male rats after 13 weeks of exposure to MTBE, together with an exacerbation in CPN in males after 1 year of exposure, thus suggestive of  $\alpha_{2u}$ -globulin nephropathy.

Robinson et al. (1990) described  $\alpha_{2u}$ -globulin nephropathy in male rats exposed to MTBE (at a dose of 100, 300, 900, or 1200 mg/kg) by



gavage for 90 consecutive days. In males, chronic nephropathy was common in the controls and in rats at the highest dose; however, tubular degenerative changes were more severe in MTBE-treated male rats that exhibited increased numbers of cytoplasmic hyaline droplets in the proximal tubular epithelial cells.

In some of the carcinogenicity studies described in Section 3, effects related to chronic nephropathy and development of kidney cancer were reported ([Bird et al., 1997](#); [Benson et al., 2011](#); [Dodd et al., 2013](#)). [Bird et al. \(1997\)](#) described a sustained increase in cell proliferation in proximal convoluted tubules in the kidney of male rats treated with MTBE at 3000 ppm after short-term exposure by inhalation (at day 5), and in the kidney of male rats treated with MTBE at 3000 or 8000 ppm at day 31 (see Section 4.2.9); an increase in protein accumulation, but not specific for  $\alpha_{2u}$ -globulin after immunohistochemical staining, in kidney tubular epithelial cells from male rats at 300 and 8000 ppm; and also a dose–response relation between kidney tumours and cell proliferation. [Dodd et al. \(2013\)](#) (see Section 3 and Section 4.2.9) described CPN in both male and female rats, with greater severity in males. The CPN was exacerbated at the highest dose of MTBE administered via drinking-water in a 2-year exposure study. In addition, cortical tubular and papillary epithelial hyperplasia were observed in female rats.

[Benson et al. \(2011\)](#) observed that the incidence of CPN among control males (88%) and control females (84%) rats was similar to that in rats exposed to emissions of gasoline containing MTBE.

[The Working Group noted that in the studies by [Dodd et al. \(2013\)](#) and [Benson et al. \(2011\)](#), CPN was observed in both male and female rats, indicating that nephropathy was not a male-specific pathology. The Working Group noted that nephropathy is frequently observed in aged rats, in both males and females.]

[The Working Group noted that, in several studies in the present section, findings regarding the  $\alpha_{2u}$ -globulin-associated response were reported. IARC has established seven criteria that should be fully met in order to conclude that an agent induces tumours of the kidney through an  $\alpha_{2u}$ -globulin-associated response ([IARC, 1999b](#)). Although such data can be informative in interpreting the relevance to humans of kidney tumours observed in rodents, the findings did not fulfil all the seven criteria required to conclude that the induction of renal tumours by MTBE operates solely via a mechanism associated with  $\alpha_{2u}$ -globulin in male rats ([IARC, 1999b](#)).]

## 5. Summary of Data Reported

### 5.1 Exposure characterization

Methyl *tert*-butyl ether (MTBE) is a volatile ether and a high-production-volume chemical produced from methanol and isobutylene. Its predominant use is as an additive in gasoline to increase combustion efficiency. In the 1990s, most MTBE was produced and used in the USA. Although no longer added to gasoline in the USA, MTBE continues to be used in gasoline in several countries throughout the world, at levels of up to 15% by volume. Minor uses are in the chemical industry. Contemporary information on environmental occurrence and exposure was limited, and no information was available from low- and middle-income countries (LMICs). MTBE is a common air pollutant because of traffic emissions. Contamination of water sources from leaking underground fuel tanks has been reported in areas where MTBE was used in gasoline. MTBE has a low odour threshold and an objectionable smell, which renders contaminated water undrinkable. Occupational exposure to MTBE has been measured among ship tanker workers, railroad tanker workers, service station

attendants, and car mechanics. The general population may be exposed via air pollution, and exposure to MTBE vapour can occur during car refuelling. Occupational standards exist for MTBE in many countries.

## 5.2 Cancer in humans

No data were available to the Working Group.

## 5.3 Cancer in experimental animals

Treatment with MTBE caused an increase in the incidence of malignant neoplasms and a combination of benign and malignant neoplasms in female mice and male rats in three studies conducted under Good Laboratory Practice (GLP).

In a well-conducted study that complied with GLP in male and female CD-1 mice exposed by inhalation (whole-body), MTBE caused an increase in the incidence of hepatocellular adenoma or carcinoma (combined) in females.

In a well-conducted study that complied with GLP in male and female Wistar rats (Han, GALAS) exposed by inhalation (whole-body), MTBE caused an increase in the incidence of brain astrocytoma in males.

In a well-conducted study that complied with GLP in male and female F344 CDF rats exposed by inhalation (whole-body), MTBE caused an increase in the incidence of renal tubular cell adenoma or carcinoma (combined) in males.

## 5.4 Mechanistic evidence

Data on the absorption, distribution, metabolism, and excretion of MTBE were available from studies in humans and in experimental systems.

In exposed humans, MTBE is readily absorbed via all relevant routes of exposure (inhalation, oral, dermal) and rapidly cleared from the blood; half-lives ranged from minutes to

several hours, reflecting distribution to other tissues, exhalation, and metabolism. A major portion of the absorbed dose is excreted as unchanged MTBE via exhaled breath, and a small fraction is excreted in the urine as MTBE or its metabolite *tert*-butyl alcohol (TBA). MTBE is metabolized mainly in the liver. MTBE is oxidatively demethylated to TBA, which is either conjugated or further metabolized, 2-hydroxyisobutyric acid (2-HIBA) and 2-methyl-1,2-propanediol (MPD) being the major metabolites. The conjugates and metabolites are excreted in the urine. Formaldehyde is formed both during the demethylation of MTBE to TBA, and in the breakdown of TBA.

The absorption and metabolism of MTBE are similar in exposed humans and rodents. Tissue distribution of MTBE metabolites has been demonstrated in the lung, liver, kidney, heart, brain, and testis. Additional *in vitro* experiments with human liver microsomes show that of the cytochrome P450s (CYPs), CYP2A6 is the major enzyme involved in the metabolism of MTBE, despite high interindividual variability, with a minor contribution from CYP3A4. A negligible role of CYP2E1 was reported in rats.

There is consistent and coherent evidence that MTBE exhibits one of the key characteristics of carcinogens.

MTBE induces oxidative stress. There was a paucity of data in exposed humans and human primary cells. In a single study in exposed humans, a significant correlation was reported between 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and urinary levels of MTBE in children living near a refinery compared with children living far from the refinery. One study in human peripheral blood lymphocytes showed that production of reactive oxygen species (ROS) resulted from *in vitro* exposure to MTBE. In the same study, a simultaneous increase in lipid peroxidation and decrease in the ratio of glutathione to glutathione disulfide (GSH:GSSG) was reported. The evidence is con-



sistent and coherent in experimental systems. The results of several studies in rodents showed that MTBE induces alterations in various oxidative end-points, including MDA and antioxidant enzyme activities (GSH levels; glutathione peroxidase, GPX1). Additional evidence of oxidative stress induced by MTBE came from several in vitro studies.

There is suggestive evidence that MTBE is electrophilic or can be metabolically activated to an electrophile. This was indicated by the formation of MTBE-derived DNA adducts in the kidney, liver, and lung of male mice exposed to a single dose of MTBE.

There is suggestive evidence that MTBE is genotoxic. There was a paucity of data in exposed humans. Increased levels of DNA strand breaks were observed in two in vitro studies, one in cultured human peripheral blood lymphocytes and one in a lung cancer cell line.

Induction of DNA strand breaks was also observed in the kidney, liver, and lung of rodents, as well as in rat fibroblasts, in the absence of metabolic activation. In vivo studies did not indicate DNA damage, except for the occurrence of sister-chromatid exchanges. MTBE was not mutagenic in non-human mammalian cells in vitro or in bacterial systems, except for in fibroblast cells in the absence of metabolic activation and in *Salmonella typhimurium* TA102 (a strain that is sensitive to oxidative stress) in the presence of metabolic activation.

There is suggestive evidence that MTBE modulates receptor-mediated effects. The suggestive evidence came from experimental systems in vivo and in vitro. Increased estrogen metabolism and decreased testosterone production were observed in rats and mice, and decreased testosterone production was observed in rat Leydig cells in vitro.

There is suggestive evidence that MTBE alters cell proliferation, cell death, or nutrient supply. The evidence came from a few studies using bromodeoxyuridine (BrdU) labelling in

experimental systems. There was induction of cell proliferation in the liver of female mice and in the kidney of male rats exposed to MTBE. Some evidence of hyperplasia was observed in the kidney of female and male rats and was considered to be associated with nephropathy.

For the other key characteristics of carcinogens – alters DNA repair or causes genomic instability, induces epigenetic alterations, induces chronic inflammation, is immunosuppressive, and causes immortalization – there was a paucity of available data.

MTBE was essentially without effects in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

## 6. Evaluation and Rationale

### 6.1 Cancer in humans

There is *inadequate* evidence in humans regarding the carcinogenicity of methyl *tert*-butyl ether.

### 6.2 Cancer in experimental animals

There is *sufficient* evidence in experimental animals for the carcinogenicity of methyl *tert*-butyl ether.

### 6.3 Mechanistic evidence

There is *limited* mechanistic evidence.

### 6.4 Overall evaluation

Methyl *tert*-butyl ether is *possibly carcinogenic to humans* (Group 2B).

## 6.5 Rationale

The Group 2B evaluation for methyl *tert*-butyl ether (MTBE) is based on *sufficient* evidence for cancer in experimental animals. This *sufficient* evidence is based on an increase in the incidence of malignant neoplasms and a combination of benign or malignant neoplasms in female mice and male rats in multiple well-conducted studies conducted under Good Laboratory Practice. There is *limited* mechanistic evidence for MTBE. Although there is consistent and coherent evidence that MTBE induces oxidative stress in experimental systems, there is only suggestive evidence that MTBE is electrophilic or can be metabolic activated to an electrophile; is genotoxic; modulates receptor-mediated effects; and alters cell proliferation, cell death, or nutrient supply. The evidence regarding cancer in humans is *inadequate* because no informative studies were available.

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# ETHYL *TERT*-BUTYL ETHER

## 1. Exposure Characterization

### 1.1 Identification of the agent

#### 1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 637-92-3 ([NCBI, 2025](#))

EC/List No.: 211-309-7 ([ECHA, 2025a](#))

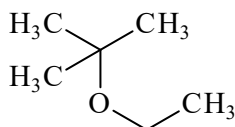
IUPAC systematic name: 2-ethoxy-2-methylpropane ([ECHA, 2025a](#))

Synonyms: ETBE; 2-methyl-2-ethoxypropane; *tert*-butyl ethyl ether; 2-ethoxy-2-methylpropane; ethyl 1,1-dimethylethyl ether; ethyl *tertiary* butyl ether; ([ECHA, 2025a](#); [NCBI, 2025](#)).

#### 1.1.2 Structural and molecular information

Molecular formula: C<sub>6</sub>H<sub>14</sub>O ([NCBI, 2025](#))

Molecular structure: ([ECHA, 2025a](#))



Relative molecular mass: 102.17 ([NCBI, 2025](#)).

#### 1.1.3 Chemical and physical properties

Description: clear, light yellow liquid ([NCBI, 2025](#))

Boiling-point: 72.6 °C ([NCBI, 2025](#))

Melting point: -94 °C at 101.3 kPa ([ECHA, 2025a](#))

Density: 0.75 g/mL at 15 °C ([ECHA, 2025a](#))

Vapour pressure: 17 kPa at 25 °C ([ECHA, 2025a](#))

Water solubility: 10–29.8 g/L at 5–40 °C ([ECHA, 2025a](#))

Octanol/water partition coefficient (P): log K<sub>ow</sub> = 1.48 at 25 °C ([ECHA, 2025a](#))

Henry's law constant: 1.64 × 10<sup>-3</sup> atm m<sup>3</sup>/mol at 25 °C ([NCBI, 2025](#))

Autoignition temperature: 392 °C at 100.34–100.92 kPa ([ECHA, 2025a](#))

Flash point: -19 °C at 101.3 kPa ([ECHA, 2025a](#))

Explosive limits: 1.24–7.7% vol in air ([NCBI, 2025](#))

Relative vapour density (air = 1): ~3.5 at 15 °C ([EFOA, 2006](#)).

#### 1.1.4 Technical products and impurities

The purity of ethyl *tert*-butyl ether (ETBE) commercial products is 90–100%, typically > 95% ([S&P Global, 2021](#); [TCI, 2025](#)). Impurities

may include C4 to C12 olefins, methyl, isopropyl alcohol and *tert*-butyl alcohol (TBA), methyl *sec*-butyl and methyl *tert*-amyl ethers, acetone, and methyl ethyl ketone ([ASTM International, 2025](#)).

## 1.2 Production and uses

### 1.2.1 Production

The common feedstocks for the manufacture of ETBE are ethanol, derived from agricultural products such as fermenting corn and other biomass (bioethanol), and isobutylene, derived from natural gas or petroleum refining ([Concawe, 2012](#)). [The Working Group noted that few data were publicly available on the composition and production volumes of gasoline and its additives. Because independently verifiable data were lacking, data published by Concawe, the science division of a trade organization, are presented.] There are different chemical processes that can be used to produce ETBE from these feedstocks. In commercial applications, acidic ion-exchange resin catalysts are used in the reaction of ethanol and isobutylene ([Weber de Menezes and Cataluña, 2008](#); [Yee et al., 2013](#)).

The Organisation for Economic Co-operation and Development (OECD) classifies ETBE as a high-production-volume chemical ([OECD, 2024](#)). The USA produces ETBE exclusively for export and is a major exporter of the compound ([US EPA, 2021](#)). Brazil has produced ETBE for export to Japan ([Noriega et al., 2023](#)). In Europe, production of ETBE initially took place primarily in Germany, France, and Spain. After the European Union issued its biofuel directive in 2009, other methyl *tert*-butyl ether (MTBE) plants in Europe were converted to ETBE plants. In 2025, data from the European Chemicals Agency indicated that 40 companies had active registrations to import or manufacture 1 tonne or more of ETBE, and 11 registered companies were no longer active ([ECHA, 2025b](#)). In 2020, global

production of ETBE was reported to be greater than 3 megatonnes ([Thornton et al., 2020](#)).

### 1.2.2 Uses

ETBE is used as a fuel oxygenate in gasoline to improve combustion efficiency, increase octane levels, and reduce air emissions (see also Section 1.1.4(b) of the monograph on gasoline in the present volume for more information on oxygenated additives in gasoline). ETBE is also used in diesel–ETBE-blended fuels ([Yee et al., 2013](#)). Its use as an additive in gasoline occurs primarily in western Europe ([US EPA, 2021](#)). France first began to use ETBE in 1992, and the use of ETBE later expanded to other countries. In Europe, a survey by the fuel industry from 2004 to 2009 found that the highest ETBE concentrations in premium unleaded gasoline ranged from 5 to 12 m/m% (wt%) in Austria, France, Hungary, and Spain ([Concawe, 2012](#)). After issuance of the European Union biofuel directive in 2009, use of ETBE increased ([Concawe, 2012](#)). In European gasoline, MTBE was the dominant oxygenate from 1999 to 2010, but ETBE was the dominant oxygenate from 2010 to 2015. Data collected by the fuel industry from 17 European countries between 2010 and 2015 showed marked variation in ETBE use by country. In 2015, the mean ETBE content in fuel was 1.67 wt%, the median was 0.10 wt%, and the maximum was 14.01 wt% (in Spain, for premium gasoline). In the European Union, ETBE content in gasoline decreased and ethanol content increased between 2010 and 2015 ([Concawe, 2018](#)). ETBE is also used in eastern Europe and Japan ([US EPA, 2021](#)).

In the USA, ETBE was used as a gasoline additive from around 1990 until 2006 but is no longer added to gasoline ([US EPA, 2021](#)) and is not used in biodiesel fuel ([US EIA, 2024](#)). In gasoline samples collected in states in the USA before the MTBE ban (from 1993–2002 or 2004), ETBE was not detected in 18 samples of oxyfuel

or reformulated gasoline, or in 57 samples of conventional gasoline. In samples collected after the MTBE ban (from 2004 to 2020), ETBE was detected in 10 out of 76 gasoline samples, at concentrations ranging from 16 to 320 mg/kg ([Stout and Healey, 2024](#)).

[The Working Group noted that no information on the use of ETBE in low- and middle-income countries (LMICs) was available.]

### 1.3 Analytical methods

For analytical methods for the detection and quantification of ETBE, see Section 1.3 of the monograph on MTBE in the present volume.

### 1.4 Occurrence and exposure

#### 1.4.1 Environmental occurrence

##### (a) Air

The half-life of ETBE in air has been estimated to be 2 days. In the USA, ETBE has been detected in the air at 73 contaminated sites in California between 2010 and 2013; most sites involved leaking underground storage tanks ([US EPA, 2021](#)). No empirical measurement data were available regarding ETBE in ambient air.

##### (b) Water

###### (i) Drinking-water

ETBE is a manufacturing by-product of cross-linked polyethylene (PEX) plastic, and ETBE has been detected in drinking-water from water systems in which new PEX pipes have been installed ([Lund et al., 2011](#); [Kelley et al., 2015](#)). In the USA, ETBE levels were measured at up to 175 µg/L in tap water during the first 3 days of use of PEX pipes and 74 µg/L after 30 days ([Venere, 2014](#)). Researchers in the Kingdom of the Netherlands (hereafter referred to as “the Netherlands”) created an “effort index” to characterize the difficulty of removing 18

micropollutants in drinking-water; the effort index for ETBE was 76, indicating that the chemical would have been difficult to remove by conventional water treatment methods ([Pronk et al., 2023](#)).

###### (ii) Surface water

In 2012, an industry trade association issued a report that provided limited data on surface water concentrations of ETBE in European countries. In Germany, 92.7% of samples in Bavaria did not exceed the LOD (range of LODs, 0.01–1 µg/L), and the highest measured value was 0.4 µg/L; in Saxony, nearly all values were below 0.2 µg/L, and the highest value was 1.2 µg/L. ETBE was not detected in highway run-off water in Switzerland, Germany, or the USA ([Concawe, 2012](#)). The surface water of the river Rhine, used for the production of drinking-water, has been monitored for ETBE since 2005 ([RIWA-Rijn, 2005](#)). In 2006, the mean concentration of ETBE was 0.231 µg/L (range, 0.014–28.7 µg/L) ([RIWA-Rijn, 2006](#)). The maximum annual concentrations decreased gradually from 5.78 µg/L in 2007 ([RIWA-Rijn, 2007](#)) to 0.26 µg/L in 2012 and were during many years below or around the limit of detection (0.03 µg/L) ([RIWA-Rijn, 2025](#)). The highest concentration of ETBE during recent years was 0.14 µg/L in 2020 ([RIWA-Rijn, 2020](#)).

###### (iii) Groundwater

In an analysis of groundwater at sites with leaking underground fuel tanks near community drinking-water sources in the Los Angeles region, California, USA, in 2000–2001, ETBE was detected at 8.9% of sites tested (77/868 sites). The geometric mean was 7 µg/L (range, 0.35–7500 µg/L) ([Shih et al., 2004](#)). A 2012 report provided limited data on the occurrence of ETBE in groundwater in Europe in 2000–2010, a period that largely pre-dated the 2009 European Union directive on biofuels and the expanded use of ETBE that followed ([Concawe, 2012](#)). In Switzerland, ETBE at concentrations ranging



from 11.8 to 13.1 µg/L was measured in groundwater in five contaminated groundwater wells in the municipality of Morbio Inferiore, and a maximum value of 1.02 µg/L in groundwater in the canton of St Gallen. In Germany, ETBE was detected in a small percentage of locations in Bavaria (maximum, 0.33 µg/L) and Saxony (maximum, 2.4 µg/L). In groundwater monitoring data from the Netherlands, the United Kingdom, Bologna in Italy, and Sweden, ETBE concentrations were characterized as being consistently below the LOD or below 1 µg/L.

In France, a study of a contaminated aquifer below a service station measured ETBE in groundwater in 2010 at concentrations greater than 300 mg/L ([Fayolle-Guichard et al., 2012](#)).

#### (c) Soil

Based on its carbon–water partitioning coefficient, ETBE is expected to move easily with water through soil, and biodegradation is not an important environmental fate process in soil ([US EPA, 2021](#)). The European Chemicals Agency estimated that the worst-case half-life of ETBE in soil is 89.5 days ([ECHA, 2023](#)).

In Sweden, ETBE levels in soil measured in 2008 were below the LOD at sites selected to represent background (<0.81 µg/kg), urban traffic (<0.69 µg/kg), a service station (<0.57 µg/kg), a refinery (<0.65 µg/kg), and an ETBE manufacturer (<0.89 µg/kg) ([Concawe, 2012](#)).

#### (d) Food and consumer products

No empirical measurement data were available for ETBE in food. Other than in gasoline (described in Section 1.2.2), no empirical measurement data were available for ETBE in consumer products.

### 1.4.2 Occupational exposure

ETBE is volatile at room temperature, as is gasoline. Consequently, exposure scenarios identified for gasoline – such as manufacturing,

blending, distribution and storage, and refuelling activities – will also by analogy pertain to ETBE exposures if gasoline contains this additive. See Section 1.4.2 of the monograph on automotive gasoline in the present volume for a detailed description of occupational exposure to gasoline. Workers in certain occupations may be exposed to ETBE through inhalation, skin contact, or accidental ingestion. [The Working Group noted that “gas station attendant”, “petrol station attendant”, “pump attendant”, “pump service attendant”, and “fuelling pump worker” can be used as job titles for the same job in some countries. The job title used in the present monograph is “service station attendant”.] Exposures depend on the specific tasks and work environment, including risk management measures already implemented. [The Working Group noted that the use of ETBE as an oxygenated gasoline additive is decreasing in many countries, and exposure to ETBE via gasoline is expected to change.]

In a study in Italy, 89 male service station attendants and 90 male control participants recruited from the general population living around Milan (25 km from Milan city centre) were investigated for exposure to ETBE ([Campo et al., 2016](#)). Personal air sampling was conducted during a mid-week shift. Urine spot samples were collected three times during the same week: (i) on Monday morning, before work began, as baseline; (ii) on the day of air sampling, before the start of the shift; and (iii) at the end of the shift when air sampling ended. Service station attendants had median exposures to airborne ETBE of 3.1 µg/m<sup>3</sup> (maximum concentration, 835 µg/m<sup>3</sup>). ETBE was detectable in a higher percentage of baseline and end of shift samples from workers than from controls ([Campo et al., 2016](#)). [The Working Group noted that significant positive correlations between exposure to airborne benzene and MTBE and the corresponding biological urinary markers were observed with Pearson correlation coefficient (*r*) values of 0.437 and 0.865 for benzene and MTBE, respectively.



The correlation between exposure to ETBE in air and urinary ETBE levels was low, which might be because of the low exposure levels in this study. However, the Working Group assumed that at greater ETBE exposures there would be a similar correlation for ETBE between concentrations in air and urine as for MTBE.]

Trace amounts of ETBE were found in urine samples from seven policemen in Italy ([Scibetta et al., 2007](#)). [The Working Group noted that this study was used to test a newly developed chemical analytical method. No further details were provided regarding occupational tasks performed by the policemen on the day of the sampling.]

In a study in Japan ([Eitaki et al., 2011](#)), four service stations selling ETBE-blended gasoline (7% ETBE) were investigated over 2 days in February and March 2009. None of the service stations investigated had introduced a stage II vapour recovery system, which captures gasoline vapours released during vehicle refuelling at service stations. Air concentrations were measured for four to six workers from each service station as well as for two gasoline-tanker truck drivers during unloading at a service station (approximately 1 hour). The geometric mean for the 8-hour time-weighted averages (TWAs) for ETBE for the 28 service station workers was  $[0.36 \text{ mg/m}^3]$  for all four service stations. In Japan, the pumps at full-service stations continue to pump gasoline even if the operators lose their grip after they insert the nozzle ([Eitaki et al., 2011](#)). One petroleum-tanker truck driver had an ETBE exposure of  $[4.95 \text{ mg/m}^3]$  during a 90-minute unloading operation, and the second driver had an air exposure of  $[3.6 \text{ mg/m}^3]$  during a 43-minute unloading operation. [The Working Group noted that the level of exposure of service station workers in Japan was more than 100 times that of the service station workers in Italy (i.e. 360 versus  $3 \text{ } \mu\text{g/m}^3$ ).]

[In addition to exposure via inhalation, the refinery and petrochemical plant workers might also be exposed via skin contact during gasoline blending, storage, or transfer. These exposures are expected to be fairly low at the present time given the different technical devices (e.g. use of vapour recovery systems) and automatic loading and unloading processes. Manual processes are more prone to spills and leaks.]

### 1.4.3 Exposure of the general population

The empirical measurement data regarding exposure of the general population to ETBE were very limited. [The Working Group noted that the general population is likely to be exposed to ETBE, primarily via inhalation in areas where ETBE is used in gasoline. Exposure via ingestion could also occur from contamination of drinking-water supplies by ETBE.]

#### (a) Air monitoring

In Milan, Italy, personal air exposure to ETBE and other volatile organic compounds was measured among schoolchildren aged 7–11 years during spring 2018 ( $n=82$ ) and winter 2019 ( $n=89$ ). Nearly all measures for ETBE were below the level of quantification ( $0.2 \text{ } \mu\text{g/m}^3$ ) ([Boniardi et al., 2023](#)).

In an earlier study conducted in Milan between 2007 and 2008, 108 adults (predominantly men) who were not occupationally exposed to ETBE wore passive air samplers for 5 hours during the day. In only 5% of the 108 air samples was the LOD for ETBE ( $0.8 \text{ } \mu\text{g/m}^3$ ) exceeded. On the basis of these findings, combined with the large proportion of concurrently measured urinary ETBE biomarkers that were below the LOD, the authors concluded that ETBE was not commonly present in gasoline in Italy at that time ([Fustinoni et al., 2010](#)).

*(b) Biomonitoring*

In central Italy, urinary levels of ETBE and three other gasoline oxygenates (MTBE; *tert*-amyl methyl ether, TAME; and diisopropyl ether, DIPE) were measured in a sample of Caucasian [White] schoolchildren aged 5–11 years. Samples were collected during summer 2017 ( $n=313$ ) and winter 2018 ( $n=263$ ) in two areas where the population and traffic density were characterized as low (rural) or medium (urban). Mean values of urinary ETBE were significantly higher during summer (423.5 ng/L) than during winter (66.2 ng/L). Multiple linear regression analyses showed that urinary ETBE levels were higher in children who spent  $\geq 1$  hour in vehicles on the sampling day during the summer and winter. Urinary ETBE levels did not vary by area of residence, an unexpected finding that the authors attributed to the fact that differences between ambient pollution levels in the different study areas were not large enough to significantly influence biomarker levels ([Antonucci et al., 2021](#)).

## 1.5 Exposure limits, regulations, and guidelines

### 1.5.1 Exposure limits and guidelines

Several countries have established occupation exposure limits for ETBE. Ireland has a TWA limit for occupational exposure to ETBE of 25 ppm [106.2 mg/m<sup>3</sup>], and Belgium, Canada (Ontario), Finland, and Spain all have a lower TWA limit of 5 ppm [21.2 mg/m<sup>3</sup>]. Poland has a TWA limit of 100 mg/m<sup>3</sup> and a 15-minute short-term limit of 200 mg/m<sup>3</sup> ([IFA, 2024](#)). In 2013, the ACGIH adopted its current recommended 8-hour TWA occupational exposure limit for ETBE of 25 ppm [106.2 mg/m<sup>3</sup>], to protect workers against respiratory irritation and central nervous impairment ([ACGIH, 2024](#)).

In the Netherlands, the Dutch Ministry of Housing, Spatial Planning and the Environment set a target level for remediation of contaminated drinking-water supplies for ETBE at 1 µg/L, similar to the target level set for MTBE, in accordance with the precautionary principle and the existing drinking-water legislation ([van Wezel et al., 2009](#)). In the state of California, USA, a secondary maximum concentration limit for ETBE in drinking-water was set at 5 µg/L, based on the taste and odour limit for MTBE ([California State Water Resources Control Board, 2024](#)).

In 2009, the Netherlands set updated maximum permissible risk levels for ETBE at 250 µg/kg body weight (bw) per day for oral exposure and 1900 µg/m<sup>3</sup> for exposure by inhalation ([Tiesjema and Baars, 2009](#)).

### 1.5.2 Regulations for use in gasoline

Within the European Union, standards allow ETBE to be added to gasoline at levels up to 22% by volume ([Michalopoulou et al., 2021](#)).

In several countries in Latin America and Asia, ETBE or ethanol can be added to gasoline to meet requirements for renewable fuel or oxygen content ([Koupal et al., 2024](#)). In Japan, oil refineries have used ETBE in gasoline to meet biofuel requirements. A maximum blend level of 22% ETBE is compatible with a gasoline standard of 10% bioethanol ([Sasatani, 2023](#)).

In the USA, five of the states that banned MTBE in 2000–2007 included bans on ETBE in the legislation ([Stout and Healey, 2024](#)).

## 2. Cancer in Humans

No data were available to the Working Group.

### 3. Cancer in Experimental Animals

#### 3.1 Rats

##### 3.1.1 Oral administration

See [Table 3.1](#).

In a study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female F344/DuCrIj rats (age, 6 weeks) were given drinking-water containing ETBE (purity, >99%) at concentrations of 0 (control), 625, 2500, or 10 000 ppm (w/w) for 104 weeks. Based on water consumption, the average daily intake of ETBE throughout the treatment period in the groups at 625, 2500, or 10 000 ppm was 28, 121, and 542 mg/kg bw for males and 46, 171, and 560 mg/kg bw for females ([JBRC, 2010a](#); also reported by [Suzuki et al., 2012](#)). There was no significant difference in survival rates between any ETBE-treated group of rats either sex or their respective controls. At study termination, survival was 38/50, 37/50, 34/50, and 34/50 in males, and 36/50, 37/50, 38/50, and 38/50 in females for the groups at 0 (control), 625, 2500, or 10 000 ppm, respectively. The body weights of males at 2500 and 10 000 ppm, and females at 10 000 ppm were significantly lower than those of the corresponding controls throughout the treatment period. The final body weights (as a percentage of those of the controls) of rats in the groups at 625, 2500, and 10 000 ppm were 96%, 93%, 91% for males and 90%, 89%, and 83% for females, respectively. All rats underwent complete necropsy. The kidney sections (for males in the control and group at 10 000 ppm, and for females in all groups) were histopathologically reviewed by a pathology working group.

No significant increases in the incidence of neoplastic lesions were found in any of the treatment groups of either sex.

Regarding non-neoplastic lesions, ETBE caused an increase in the average severity, but not the incidence, of chronic progressive nephropathy (CPN) in both males and females at 10 000 ppm. In addition, the incidence of urothelial hyperplasia of the pelvis and of mineral deposition in the renal papilla was significantly increased in males at groups at 2500 and 10 000 ppm. [The Working Group noted this was a well-conducted study that complied with GLP, used multiple concentrations, included both males and females, had an adequate number of animals per group, and had an adequate duration of exposure and observation.]

In a study of chronic toxicity and carcinogenicity that complied with GLP, groups of 60 male and 60 female Sprague-Dawley rats (CRC/RF colony; age, 8 weeks) were treated with ETBE (purity, >94%, the main impurities being ethyl alcohol (ethanol), 2.88%; TBA, 1.59%; MTBE, 1.01%; 2-ethoxy-butane, 0.12%; olefin C8, 0.11%; *tert*-butyl-isopropyl ether, 0.09%; and methyl alcohol (methanol), 0.01%). The compound was administered by gavage in extra virgin olive oil solution at doses of 0, 250, and 1000 mg/kg bw 4 days per week for 104 weeks ([Maltoni et al., 1999](#)). The rats were kept under observation until natural death. Upon death, the rats underwent a systematic necropsy. There was no significant effect of treatment on water and food consumption or body weight, but the survival rate was low starting from week 40. There was a dose-correlated increase in the mortality rate in males, starting from week 40 until the end of the experiment, and in females from week 40 to week 88 of the biophase. By week 104, survival was approximately 25%, 25%, and 13% in males, and approximately 29%, 29%, and 35% in females for the groups at 0 (control), 250, and 1000 mg/kg bw per day, respectively. [The Working Group assessed the survival rates using Figs 7 and 8 of the publication.]

**Table 3.1 Studies of carcinogenicity in experimental animals exposed to ethyl *tert*-butyl ether**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                          | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                        | Tumour<br>incidence                                            | Significance | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Rat, F344 (F344/<br>DuCr1Cr1j) (M)<br>6 wk<br>104 wk<br><a href="#">JBRC (2010a)</a> ,<br>also reported<br>by <a href="#">Suzuki et al. (2012)</a> | Oral administration (drinking-water)<br>ETBE, > 99%<br>Drinking-water<br>0, 625, 2500, 10 000 ppm<br>(approximately, 28, 121, and<br>542 mg/kg bw per day)<br>for 104 wk<br>50, 50, 50, 50<br>38, 37, 34, 34                                                                                      | All sites: No significant increase in the incidence of tumours |              | <i>Principal strengths:</i> Well-conducted GLP study, adequate number of animals per group, adequate duration of exposure and observation, multiple concentrations used, male and female animals used.                                                                                                                                                                                                                                                                                              |
| Full carcinogenicity<br>Rat, F344 (F344/<br>DuCr1Cr1j) (F)<br>6 wk<br>104 wk<br><a href="#">JBRC (2010a)</a> ,<br>also reported<br>by <a href="#">Suzuki et al. (2012)</a> | Oral administration (drinking-water)<br>ETBE, > 99%<br>Drinking-water<br>0, 625, 2500, 10 000 ppm<br>(approximately, 46, 171, and<br>560 mg/kg bw per day)<br>for 104 wk<br>50, 50, 50, 50<br>36, 37, 38, 38                                                                                      | All sites: No significant increase in the incidence of tumours |              | <i>Principal strengths:</i> Well-conducted GLP study, adequate number of animals per group, adequate duration of exposure and observation, multiple concentrations used, male and female animals used.                                                                                                                                                                                                                                                                                              |
| Full carcinogenicity<br>Rat, Sprague-Dawley (CRC/RF colony) (M)<br>8 wk<br>104 wk<br><a href="#">Maltoni et al. (1999)</a>                                                 | Oral administration (gavage)<br>ETBE, > 94% (main impurities:<br>EA, 2.88%; TBA, 1.59%; MTBE,<br>1.01%; 2-EtOBu, 0.12%; olefin C8,<br>0.11%; tBuOiPr, 0.09%; MeOH,<br>0.01%)<br>Olive oil<br>0, 250, 1000 mg/kg bw<br>4×/wk (Monday, Tuesday,<br>Thursday, Friday) for 104 wk<br>60, 60, 60<br>NR | All sites: No significant increase in the incidence of tumour  |              | <i>Principal strengths:</i> Well-conducted GLP study. Long-term study (> 2 years), adequate number of animals per group, male and female animals used, two doses used.<br><br><i>Principal limitations:</i> Unusual observation period (lifetime), low survival rate of animals in all groups at 104 wk.<br><br><i>Other comments:</i> The Working Group assessed the survival rates from Figs 7 and 8 of the publication. Histological re-analysis by <a href="#">Malarkey and Bucher (2011)</a> . |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                         | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                        | Tumour<br>incidence                                                                                                                                                | Significance                                                                                                                                                                                          | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, Sprague-<br>Dawley (CRC/RF<br>colony) (F)<br>8 wk<br>104 wk<br><a href="#">Maltoni et al. (1999)</a>                                      | Oral administration (gavage)<br>ETBE, > 94% (main impurities:<br>EA, 2.88%; TBA, 1.59%; MTBE,<br>1.01%; 2-EtOBu, 0.12%; olefin C8,<br>0.11%; tBuOiPr, 0.09%; MeOH,<br>0.01%)<br>Olive oil<br>0, 250, 1000 mg/kg bw<br>4×/wk (Monday, Tuesday,<br>Thursday, Friday) for 104 wk<br>60, 60, 60<br>NR | <i>Uterus</i><br>Carcinoma<br>1/60, 2/60, 0/60<br>Sarcoma (malignant schwannoma)<br>1/60, 8/60*, 2/60<br><br>Carcinoma or sarcoma (combined)<br>2/60, 10/60*, 2/60 | NS<br><br>$*P \leq 0.05$ , $\chi^2$ test;<br>[ $P = 0.0473$ , one-tailed<br>Fisher exact test]<br><br>$*P \leq 0.05$ , $\chi^2$ test; [ $P = 0.0147$ , one-tailed<br>Fisher exact test]               | <i>Principal strengths:</i> Well-conducted GLP study, long-term study (> 2 years), adequate number of animals per group, male and female animals used, two doses used.<br><br><i>Principal limitations:</i> Unusual observation period (lifetime), low survival rate of animals in all groups at 104 wk.<br><br><i>Other comments:</i> The Working Group assessed the survival rates from Fig. 7 and 8 of the publication. Histological re-analysis by <a href="#">Malarkey and Bucher (2011)</a> : Uterus-vagina: leiomyoma and leiomyosarcoma (combined), 1/60, 0/60, 3/60; malignant schwannoma, 0/60, 7/60*, 2/60; uterine carcinoma, 0/60, 1/60, 0/60; histiocytic sarcoma, 1/60, 0/60, 1/60. Malignant schwannomas in the uterus are rare tumours. |
| Full<br>carcinogenicity<br>Rat, F344 (F344/<br>DuCrlCrlj) (M)<br>6 wk<br>104 wk<br><a href="#">JBRC (2010b)</a> , also<br>reported by <a href="#">Saito et al. (2013)</a> | Inhalation (whole-body exposure)<br>ETBE, > 99%<br>Filtered air<br>0, 500, 1500, 5000 ppm<br>6 h/day, 5 days/wk for 104 wk<br>50, 50, 50, 50<br>44, 38, 39, 30                                                                                                                                    | <i>Liver</i><br>Hepatocellular adenoma<br>0/50, 2/50, 1/49,<br>9/50*<br><br>Hepatocellular carcinoma<br>0/50, 0/50, 0/49,<br>1/50                                  | $P < 0.01$ , Peto trend<br>test; [ $P = 0.0001$ ,<br>Cochran–Armitage<br>trend test]<br>$*P < 0.01$ , two-tailed<br>Fisher exact test;<br>[ $P = 0.0013$ , one-tailed<br>Fisher exact test]<br><br>NS | <i>Principal strengths:</i> Well-conducted GLP study, adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, multiple doses used.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                                                         | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                                                                                                                                                                                                              | Tumour<br>incidence                                                                                                                                                                                                                                                                                                                                                                           | Significance                                                                                                                                                                                                                                                                                                          | Comments                                                                                                                                                                                                |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Rat, F344 (F344/<br>DuCrlCrIj) (F)<br>6 wk<br>104 wk<br><a href="#">JBRC (2010b)</a> , also<br>reported by <a href="#">Saito<br/>et al. (2013)</a>                                | Inhalation (whole-body exposure)<br>ETBE, > 99%<br>Filtered air<br>0, 500, 1500, 5000 ppm<br>6 h/day, 5 days/wk for 104 wk<br>50, 50, 50, 50<br>38, 39, 30, 30                                                                                                                                                                                                                                                                                                                          | All sites: No tumours observed                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                       | <i>Principal strengths:</i> Well-conducted GLP study, adequate duration of exposure and observation, adequate number of animals per group, male and female animals used, multiple doses used.           |
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, F344 (F344/<br>DuCrlCrIj) (M)<br>6 wk<br>29 wk<br><a href="#">JBRC (2008a)</a><br>also reported by<br><a href="#">Hagiwara et al.<br/>(2011)</a> | Oral administration (gavage)<br>ETBE, 99.3%<br>Distilled water<br>DMBDD (group 1), DMBDD<br>+ 300 mg/kg per day ETBE<br>(group 2), DMBDD + 1000 mg/kg<br>per day ETBE (group 3), 0 ETBE<br>(group 4), 1000 mg/kg per day<br>ETBE (group 5)<br>After 4-wk DMBDD treatment<br>(see detail in main text) and 1-wk<br>non-treatment period, rats were<br>treated with ETBE at 0 (control),<br>300, or 1000 mg/kg per day by<br>gavage for 23 wk<br>30, 30, 30, 12, 12<br>30, 26, 30, 12, 12 | <i>Thyroid</i><br>Adenoma<br>Tumour<br>incidence: 7/30,<br>13/30, 19/30*, 0, 0<br><br>Tumour<br>multiplicity:<br>0.3 ± 0.5, 0.6 ± 0.8,<br>1.0 ± 1.1*, 0, 0<br>Carcinoma<br>Tumour<br>incidence: 3/30,<br>5/30, 4/30, 0, 0<br>Tumour<br>multiplicity:<br>0.1 ± 0.4, 0.2 ± 0.5,<br>0.1 ± 0.3, 0, 0<br>Adenoma or carcinoma (combined)<br>Tumour<br>incidence: 8/30,<br>17/30*, 20/30**,<br>0, 0 | <br><br>* <i>P</i> < 0.01, two-tailed<br>Fisher exact test;<br>[ <i>P</i> = 0.0019, one-tailed<br>Fisher exact test]<br><br>* <i>P</i> < 0.01, Dunnett test<br>(two-tailed)<br><br>NS<br><br>NS<br><br><br>* <i>P</i> < 0.05, two-tailed<br>Fisher exact test;<br>** <i>P</i> < 0.01, two-tailed<br>Fisher exact test | <i>Principal strengths:</i> Well-conducted GLP study, two doses used, high number of animals per group, adequate duration of exposure and observation.<br><br><i>Principal limitations:</i> Males only. |



**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                                                                     | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals | Tumour<br>incidence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Significance                                                                                                                                                                                                                                                                                                                 | Comments |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, F344 (F344/<br>DuCrI CrIj) (M)<br>6 wk<br>29 wk<br><a href="#">JBRC (2008a)</a><br>also reported by<br><a href="#">Hagiwara et al.<br/>(2011)</a><br>(cont.) |                                                                                                            | Tumour<br>multiplicity:<br>0.4 ± 0.8,<br>0.8 ± 0.8*,<br>1.1 ± 1.2**, 0, 0<br><i>Colon</i><br>Adenocarcinoma<br>Tumour<br>incidence: 20/30,<br>18/30, 24/30, 0, 0<br>Tumour<br>multiplicity:<br>0.9 ± 0.9,<br>1.0 ± 1.10,<br>1.8 ± 1.3*, 0, 0<br>Adenoma or adenocarcinoma (combined)<br>Tumour<br>incidence: 25/30,<br>21/30, 28/30, 0, 0<br>Tumour<br>multiplicity:<br>1.8 ± 1.3, 1.4 ± 1.3,<br>3.0 ± 1.7*, 0, 0<br><i>Liver</i><br>Hepatocellular adenoma<br>Tumour<br>incidence: 0/30,<br>1/30, 6/30*, 0, 0<br><i>Forestomach</i><br>Squamous cell papilloma<br>Tumour<br>incidence: 0/30,<br>4/30, 3/30, 0, 0 | * <i>P</i> < 0.05 by Dunnett<br>test (two-tailed);<br>** <i>P</i> < 0.01 by Dunnett<br>test (two-tailed)<br><br>NS<br><br>* <i>P</i> < 0.01 by Dunnett<br>test (two-tailed)<br><br>NS<br><br>* <i>P</i> < 0.01 by Dunnett<br>test (two-tailed)<br><br>NS<br><br>* <i>P</i> < 0.05, two-tailed<br>Fisher exact test<br><br>NS |          |

**Table 3.1 (continued)**[illegible]

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                                                     | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                                                                                                                                                                                                                  | Tumour<br>incidence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Significance                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Comments                                                                                                                                                                                                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, Wistar<br>(CrIj:WI) (M)<br>6 wk<br>22 wk<br><a href="#">JBRC (2008b)</a> ,<br>also reported by<br><a href="#">Hagiwara et al.<br/>(2015)</a> | Oral administration (gavage)<br>ETBE, 99.3%<br>Distilled water<br>EHEN (group 1), EHEN +<br>100 mg/kg per day ETBE<br>(group 2), EHEN + 300 mg/kg<br>per day ETBE (group 3), EHEN<br>+ 500 mg/kg per day ETBE<br>(group 4), EHEN + 1000 mg/kg<br>per day ETBE (group 5)<br>7 days/wk for 19 wk after 2 wk of<br>EHEN treatment (500 ppm with<br>drinking-water)<br>30, 30, 30, 30, 30<br>30, 30, 30, 30, 30 | <i>Liver</i><br>Hepatocellular adenoma<br>Tumour<br>incidence: 4/30,<br>5/30, 8/30, 8/30,<br>15/30*<br><br>Tumour<br>multiplicity:<br>0.2 ± 0.8, 0.2 ± 0.6,<br>0.5 ± 0.9, 0.4 ± 0.7,<br>0.6 ± 0.7<br>Hepatocellular carcinoma<br>Tumour<br>incidence: 0/30,<br>2/30, 0/30, 3/30,<br>5/30*<br>Tumour<br>multiplicity:<br>0.0 ± 0.0, 0.1 ± 0.3,<br>0.0 ± 0.0, 0.1 ± 0.3,<br>0.3 ± 0.7*<br>Hepatocellular adenoma or carcinoma<br>(combined)<br>Tumour<br>incidence: 4/30,<br>7/30, 8/30, 10/30,<br>17/30* | <i>P</i> < 0.01, Cochran–<br>Armitage trend test<br>* <i>P</i> < 0.01, two-tailed<br>Fisher exact test;<br>[ <i>P</i> = 0.0024, one-tailed<br>Fisher exact test]<br>NS<br><br><i>P</i> < 0.01, Cochran–<br>Armitage trend test<br>[* <i>P</i> = 0.0261, one-tailed<br>Fisher exact test]<br>* <i>P</i> < 0.05, Dunnett test<br>(two-tailed)<br><br><i>P</i> < 0.01, Cochran–<br>Armitage test<br>* <i>P</i> < 0.01, two-tailed<br>Fisher exact test;<br>[ <i>P</i> = 0.0005, one-tailed<br>Fisher exact test] | <i>Principal strengths:</i> Well-conducted GLP study, multiple<br>concentrations used, adequate number of animals per<br>group, adequate duration of exposure and observation.<br><i>Principal limitations:</i> Males only. |

**Table 3.1 (continued)**

| <b>Study design</b>                                                                                                                                                                                            | <b>Route</b>                    | <b>Tumour incidence</b>                                                                                                                                                                                                                                                  | <b>Significance</b>                                                                                                                                                                                                                                                                                     | <b>Comments</b> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| <b>Species, strain (sex)</b>                                                                                                                                                                                   | <b>Agent tested, purity</b>     |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                         |                 |
| <b>Age at start</b>                                                                                                                                                                                            | <b>Vehicle</b>                  |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                         |                 |
| <b>Duration</b>                                                                                                                                                                                                | <b>Dose(s)</b>                  |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                         |                 |
| <b>Reference</b>                                                                                                                                                                                               | <b>No. of animals at start</b>  |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                         |                 |
|                                                                                                                                                                                                                | <b>No. of surviving animals</b> |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                         |                 |
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, Wistar<br>(CrIj;WI) (M)<br>6 wk<br>22 wk<br><a href="#">JBRC (2008b)</a><br>also reported by<br><a href="#">Hagiwara et al.<br/>(2015)</a><br>(cont.) |                                 | Tumour<br>multiplicity:<br>0.2 ± 0.8, 0.3 ± 0.6,<br>0.5 ± 0.9, 0.5 ± 0.7,<br>0.9 ± 1.1**<br><i>Kidney</i><br>Renal tubule adenoma<br>Tumour<br>incidence: 17/30,<br>22/30, 24/30,<br>25/30*, 26/30**                                                                     | **P < 0.01, Dunnett<br>test (two-tailed)<br><br><br><br><br>P < 0.01, Cochran–<br>Armitage trend test;<br>*P < 0.05, two-tailed<br>Fisher exact test;<br>[P = 0.0235, one-tailed<br>Fisher exact test]<br>**P < 0.05, two-tailed<br>Fisher exact test;<br>[P = 0.0102, one-tailed<br>Fisher exact test] |                 |
|                                                                                                                                                                                                                |                                 | Tumour<br>multiplicity:<br>2.0 ± 2.8, 1.9 ± 1.8,<br>2.8 ± 3.3, 2.2 ± 1.6,<br>3.4 ± 4.0<br>Renal tubule carcinoma<br>Tumour<br>incidence: 5/30,<br>10/30, 14/30*,<br>8/30, 7/30<br>Tumour<br>multiplicity:<br>0.3 ± 0.7, 0.5 ± 0.9,<br>0.7 ± 0.8, 0.4 ± 0.7,<br>0.4 ± 0.8 | NS<br><br><br><br><br><br><br><br>*P < 0.05, two-tailed<br>Fisher exact test;<br>[P = 0.0125, one-tailed<br>Fisher exact test]<br>NS                                                                                                                                                                    |                 |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                                                              | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals | Tumour<br>incidence                                                                                                                                                                                       | Significance                                               | Comments |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|----------|
| Initiation–<br>promotion (tested<br>as promoter)<br>Rat, Wistar<br>(Crlj:WI) (M)<br>6 wk<br>22 wk<br><a href="#">JBRC (2008b)</a><br>also reported by<br><a href="#">Hagiwara et al.<br/>(2015)</a><br>(cont.) |                                                                                                            | Renal tubule adenoma or carcinoma<br>(combined)<br>Tumour<br>incidence: 18/30,<br>23/30, 25/30,<br>26/30, 26/30<br>Tumour<br>multiplicity:<br>2.3 ± 2.9, 2.4 ± 2.1,<br>3.5 ± 3.8, 2.6 ± 1.8,<br>3.8 ± 4.3 | <i>P</i> < 0.05, Cochran–<br>Armitage trend test<br><br>NS |          |

bw, body weight; BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DMBDD, diethylnitrosamine, *N*-methyl-*N*-nitrosourea, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, 1,2-dimethylhydrazine dihydrochloride, and *N*-bis(2-hydroxypropyl)nitrosamine; EA, ethyl alcohol (ethanol); EHEN, *N*-ethyl-*N*-2-hydroxyethyl nitrosamine; ETBE, ethyl *tert*-butyl ether; 2-EtOBu, 2-ethoxy-butane; F, female(s); F344, Fischer 344; GLP, Good Laboratory Practice; h, hour(s); M, male(s); MeOH, methyl alcohol (methanol); MTBE, methyl *tert*-butyl ether; NR, not reported; NS, not significant; ppm, parts per million; TBA, *tert*-butyl alcohol; tBuOiPr, *tert*-butyl-isopropyl ether; wk, week(s).

In females, there was a significant increase ( $P \leq 0.05$ ,  $\chi^2$  test; [ $P = 0.0473$ , Fisher exact test]) in the incidence of sarcoma of the uterus – 1/60, 8/60, and 2/60 in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively) [4 and 2 of these sarcomas were characterized as malignant schwannomas in the groups at 250 and 1000 mg/kg bw, respectively.] There was a significant increase in the incidence of carcinoma or sarcoma (combined) of the uterus in the group at 250 mg/kg bw, incidence being 2/60, 10/60, and 2/60 for the groups at 0 (control), 250, and 1000 mg/kg bw, respectively ( $P \leq 0.05$ ,  $\chi^2$  test; [ $P = 0.0147$ , Fisher exact test]). In 2011, the histopathological data were reviewed by the Pathology Working Group of the United States National Toxicology Program (NTP) ([Malarkey and Bucher, 2011](#)), and all sections of the oral cavity, uterus, and vagina were re-examined. After re-examination, it was found that ETBE caused a significant increase in incidence only for malignant schwannoma of the uterus in the group at 250 mg/kg bw (7/60; 12%) compared with the control group (0/60). [The Working Group noted that malignant schwannoma of the uterus is a rare tumour.]

There was no statistical difference in the incidence of any tumour in treated males compared with controls.

Regarding non-neoplastic lesions, a significantly increased incidence of squamous hyperplasia of the oral cavity was found in the group of males at 250 mg/kg bw and in both groups of females treated with ETBE.

[The Working Group noted that this was a well-conducted study that complied with GLP, and that it was strengthened by the use of an adequate number of animals per group, both males and females, two doses, and an adequate exposure period. However, the study was limited by a critically low survival rate of animals in all groups at 104 weeks. The Working Group also noted that the study observation period (lifetime) increased uncertainty in interpreting the

incidence of malignant tumours, which did not show a dose–response relationship. In addition, after the histopathological re-analysis by the NTP, malignant tumours were determined to be of multiple tissue origins (e.g. epithelial, muscle, and peripheral neural cells). The Working Group therefore considered the data from the histopathological re-analysis to be more suitable for the present evaluation.]

### 3.1.2 Inhalation

In a study of chronic toxicity and carcinogenicity that complied with GLP, groups of 50 male and 50 female F344/DuCrIj rats (age, 6 weeks) were exposed to ETBE (purity, > 99%) at a concentration of 0, 500, 1500, or 5000 ppm (v/v) in whole-body inhalation chambers for 6 hours per day, 5 days per week, for 104 weeks ([JBRC, 2010b](#); also reported by [Saito et al., 2013](#)). At study termination, survival was 44/50, 38/50, 39/50, and 30/50 in males, and 38/50, 39/50, 30/50, and 30/50 in females for the groups at 0 (control), 500, 1500, or 5000 ppm, respectively. A decreased survival rate was found in males at 5000 ppm (60%) and in females at 1500 (60%) and 5000 ppm (60%) compared with the respective control groups (males, 88%; females, 76%). Terminal body weights (compared with those of controls) in the groups at 500, 1500, and 5000 ppm were 94%, 94%, and 75% for males and 95%, 91%, and 78% for females, respectively. All rats underwent a complete necropsy.

In males, there was a significant positive trend in the incidence of hepatocellular adenoma ( $P < 0.01$ , Peto trend test; [ $P = 0.0001$ , Cochran–Armitage trend test]), and there was a significant increase in incidence ( $P < 0.01$ , Fisher exact test; [ $P = 0.0013$ , one-tailed Fisher exact test]) in the group at 5000 ppm compared with the vehicle control group – 0/50, 2/50, 1/49, and 9/50 in the groups at 0 (vehicle control), 500, 1500, and 5000 ppm, respectively. Hepatocellular carcinoma was found in only one male rat in the



group at 5000 ppm. No effects on the incidence of tumours or proliferative lesions were observed in the liver in females in any treated group. No treatment-related significant increase in the incidence of neoplastic lesions in other organs or tissues apart from the liver was found in males or females.

Regarding preneoplastic lesions, a significant increase in the incidence of liver eosinophilic cell foci and a significant increase in the incidence of liver basophilic cell foci were found in males at 5000 ppm. ETBE caused a significant increase in the degree of CPN in males and females in the group at 5000 ppm compared with the respective control groups. In males, mineral deposition in the renal papilla in the group at 5000 ppm was significantly elevated. A significant increase in the incidence of urothelial hyperplasia of the renal pelvis was also found at 1500 and 5000 ppm. [The Working Group noted that this was a well-conducted study that complied with GLP and was strengthened by the use of multiple doses, males and females, and an adequate number of animals per group, and had an adequate duration of exposure and observation.]

### 3.1.3 Initiation–promotion studies

A medium-term multiorgan carcinogenesis bioassay that complied with GLP was conducted to investigate the promoting potential of ETBE on tumour development in male F344/DuCrIj rats ([JBRC, 2008a](#); also reported by [Hagiwara et al., 2011](#)). Rats (age, 6 weeks) were allocated to five groups (30 animals each in groups 1–3; 12 animals each in groups 4 and 5). Rats in groups 1–3 were sequentially treated with five agents (abbreviated as “DMBDD”) as follows: diethylnitrosamine (DEN) (100 mg/kg bw, single intraperitoneal dose by injection at start); *N*-methyl-*N*-nitrosourea (20 mg/kg bw, by intraperitoneal injection on each of experimental days 4, 7, 11, and 14); *N*-butyl-*N*-(4-hydroxybutyl)ni-

trosamine (BBN) (0.05%, in the drinking-water, during weeks 1 and 2); 1,2-dimethylhydrazine dihydrochloride (40 mg/kg bw, by subcutaneous injection on each of experimental days 18, 21, 25, and 28); and *N*-bis(2-hydroxypropyl) nitrosamine (0.1%, in the drinking-water, during weeks 3 and 4) – to initiate tumour development in the target tissues. Groups 4 and 5 received vehicle without carcinogens during the first step treatment period (4 weeks). [The Working Group noted that the schedule of treatment with vehicle was not reported in the article.] After 4 weeks of treatment with DMBDD and a 1-week non-treatment period, animals in groups 1–3 were treated by gavage with ETBE (purity, 99.3%) at a dose of 0 (group 1, control), 300 (group 2), or 1000 (group 3) mg/kg per day, respectively, for 23 weeks. Rats that were not treated with DMBDD were treated by gavage with ETBE at doses of 0 (group 4) and 1000 (group 5) mg/kg per day for the same period. At study termination, the survival rates were 87% (26/30) and 100% (30/30) for groups 2 and 3, respectively, and there were no treatment-related changes compared with survival in the control group (ETBE, 0 mg/kg per day), which was 100% (30/30). In groups 4 and 5, the survival rates were both 100% (12/12). After ETBE treatment (29 weeks), all surviving rats were euthanized for histopathological examination.

There was a significant increase in the incidence of follicular cell adenoma of the thyroid gland (7/30, 13/30, and 19/30 in groups 1, 2, and 3, respectively) with ETBE at 1000 mg/kg per day after DMBDD treatment (group 3) in comparison with group 1 ( $P < 0.01$ , two-tailed Fisher exact test; [ $P = 0.0019$ , one-tailed Fisher exact test]). The multiplicity of follicular cell adenoma was  $0.3 \pm 0.5$ ,  $0.6 \pm 0.8$ , and  $1.0 \pm 1.1$  in groups 1, 2, and 3, respectively, with a significant increase ( $P < 0.01$ , Dunnett test, two-sided) in group 3 compared with group 1. There was a significant increase in the incidence of follicular cell adenoma or carcinoma (combined) in group 2 ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0176$ ,

one-tailed Fisher exact test]) and group 3 ( $P < 0.01$ , two-tailed Fisher exact test; [ $P = 0.0020$ , one-tailed Fisher exact test]) in comparison with group 1 – 8/30 (27%), 17/30 (57%), and 20/30 (67%) in groups 1, 2, and 3, respectively. The multiplicity of follicular cell adenoma and carcinoma (combined) was  $0.4 \pm 0.8$ ,  $0.8 \pm 0.8$ , and  $1.1 \pm 1.2$  in groups 1, 2, and 3, respectively, and there was a significant increase in group 2 ( $P < 0.05$ , Dunnett test, two-sided) and group 3 ( $P < 0.01$ , Dunnett test, two-sided) in comparison with group 1. The incidence of carcinoma of the thyroid gland was unchanged in both treated groups compared with controls. In the colon, there was no significant change in the incidence of adenocarcinoma in either treated group compared with controls (20/30, 18/30, and 24/30 in groups 1, 2, and 3, respectively). However, there was a significant increase in the multiplicity of colon adenocarcinoma ( $0.9 \pm 0.9$ ,  $1.0 \pm 1.10$ , and  $1.8 \pm 1.3$  in groups 1, 2, and 3, respectively) in group 3 in comparison with group 1 (the control group) ( $P < 0.01$ , Dunnett test, two-sided). Although the incidence of adenoma or adenocarcinoma (combined) – 25/30, 21/30, and 28/30 in groups 1, 2, and 3, respectively – was unchanged, the average number of neoplastic lesions – adenoma or adenocarcinoma (combined),  $1.8 \pm 1.3$ ,  $1.4 \pm 1.3$ , and  $3.0 \pm 1.7$  in groups 1, 2, and 3, respectively – was significantly increased in group 3 compared with group 1 ( $P < 0.01$ , Dunnett test, two-sided). In the forestomach, the incidence of focal squamous cell papilloma was slightly but not significantly increased in the treated groups compared with controls. The combined incidence of squamous cell hyperplasia and papilloma of the forestomach was significantly increased in groups 2 and 3 (6/30 and 6/30, respectively, in comparison with group 1, 0/30;  $P < 0.05$ ). [The Working Group considered that it was not appropriate to combine preneoplastic and neoplastic lesions since hyperplasia and papilloma are different end-points when assessing carcinogenicity.] The

incidence of hepatocellular adenoma (0/30, 1/30, and 6/30 in groups 1, 2, and 3, respectively) significantly increased ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0019$ , one-tailed Fisher exact test]) in group 3 compared with group 1. No hyperplastic and neoplastic lesions were found in the controls, groups 4 and 5.

Regarding preneoplastic lesions, there was a significant increase in the incidence of follicular cell hyperplasia of the thyroid gland. The average number of foci that were positive for glutathione S-transferase (GST) per unit area of liver section was statistically significantly increased in group 3 compared with group 1. The multiplicity of atypical hyperplasia of the renal tubules was significantly increased in group 3 compared with the control groups. An increase in the incidence of papillomatosis of the urinary bladder was found in rats treated with ETBE at 1000 mg/kg per day in group 5.

[The Working Group noted that this was a well-conducted study that complied with GLP, and that it was strengthened by the use of two doses and an adequate number of animals per group, and had an adequate duration of exposure and observation. However, it was limited by the use of males only.]

In another study that complied with GLP by the same scientific group, the tumour-promoting effects of ETBE were investigated in a two-stage bioassay for hepatic and renal carcinogenesis ([JBRC, 2008b](#); also reported by [Hagiwara et al., 2015](#)). One hundred and fifty male Crlj:WI Wistar rats (age, 6 weeks) were randomized into five groups (30/group) and given drinking-water containing *N*-ethyl-*N*-(2-hydroxyethyl)nitrosamine (EHEN) at a concentration of 500 ppm for 2 weeks. One week later, the rats were treated with ETBE (purity, 99.3%) at doses of 0, 100, 300, 500, and 1000 mg/kg per day (groups 1–5, respectively) by gavage, daily, 7 days per week, for 19 weeks. No animals died during the experiment. Final body weights were comparable among all the groups. At experimental

week 23, all animals were euthanized for the examination of preneoplastic and neoplastic lesion development in the liver and kidney.

There was a significant positive trend ( $P < 0.01$ , Cochran–Armitage trend test) in the incidence of hepatocellular adenoma (4/30, 5/30, 8/30, 8/30, and 15/30 in groups 1–5, respectively), and the incidence was significantly increased in group 5 ( $P < 0.01$ , two-tailed Fisher exact test; [ $P = 0.0024$ , one-tailed Fisher exact test]). In addition, the Cochran–Armitage trend test indicated a significant positive trend in the incidence of hepatocellular carcinoma ( $P < 0.01$ , Cochran–Armitage trend test), the incidence (0/30, 2/30, 0/30, 3/30, and 5/30 in groups 1–5, respectively) being significantly increased [ $P = 0.0261$ , Fisher exact test] in group 5. There was a significant positive trend ( $P < 0.01$ , Cochran–Armitage trend test) in the incidence of hepatocellular adenoma or carcinoma (combined) (4/30, 7/30, 8/30, 10/30, and 17/30 in groups 1–5, respectively), and the incidence was significantly increased in group 5 ( $P < 0.01$ , two-tailed Fisher exact test; [ $P = 0.0005$ , one-tailed Fisher exact test]). There was a significant increase in group 5 in the multiplicity of hepatocellular carcinoma ( $0.0 \pm 0.0$ ,  $0.1 \pm 0.3$ ,  $0.0 \pm 0.0$ ,  $0.1 \pm 0.3$ , and  $0.3 \pm 0.7$  in groups 1–5, respectively;  $P < 0.05$ , Dunnett test, two-sided) and of hepatocellular adenoma or carcinoma (combined) ( $0.2 \pm 0.8$ ,  $0.3 \pm 0.6$ ,  $0.5 \pm 0.9$ ,  $0.5 \pm 0.7$ , and  $0.9 \pm 1.1$  in groups 1–5, respectively;  $P < 0.01$ , Dunnett test, two-sided), but not in groups 2–4, when compared with the controls, group 1.

For the kidney, there was a significant positive trend ( $P < 0.01$ , Cochran–Armitage trend test) in the incidence of renal tubule adenoma, and the incidence (17/30, 22/30, 24/30, 25/30, and 26/30 in groups 1–5, respectively) was significantly increased in group 4 ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0235$ , one-tailed Fisher exact test]) and group 5 ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0102$ , one-tailed Fisher exact test]). The incidence of renal tubule carcinoma (5/30, 10/30, 14/30, 8/30, and 7/30 in

groups 1–5, respectively) was also significantly increased in group 3 ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0125$ , one-tailed Fisher exact test]). There was a significant positive trend ( $P < 0.05$ , Cochran–Armitage trend test) in the incidence of renal tubule adenoma or carcinoma (combined) (18/30, 23/30, 25/30, 26/30, and 26/30 in groups 1–5, respectively).

Regarding preneoplastic lesions, atypical tubule hyperplasia of the kidney was observed in all rats in each group, including the control group. The multiplicity of atypical tubule hyperplasia was significantly increased with ETBE at 1000 mg/kg per day when compared with the control value. [The Working Group noted that this was a well-conducted study that complied with GLP, and that it was strengthened by the use of multiple doses and an adequate number of animals per group, and had an adequate duration of exposure and observation. However, it was limited by the use of males only.]

In another initiation–promotion study that complied with GLP, the effects of ETBE on two-stage carcinogenesis in the urinary bladder were investigated in male rats initiated with BBN (JBRC, 2010c; also reported by Hagiwara et al., 2013). Male F344/DuCrIj rats (age, 6 weeks) were randomized to five groups (30/group) and were given drinking-water containing BBN at 500 ppm for 4 weeks. One week later, the rats were treated with ETBE (purity, > 99%) at a dose of 0, 100, 300, 500, or 1000 mg/kg bw per day by gavage, 7 days per week, for 31 weeks. At experimental week 37, all surviving animals were euthanized for examination of the development of preneoplastic and neoplastic lesions in the urinary bladder.

At the end of the experiment, the survival rates in groups treated with ETBE at 100, 300, 500, and 1000 mg/kg bw per day were 97% (29/30), 97% (29/30), 100% (30/30) and 100% (30/30), respectively, and there were no treatment-related changes when compared with the control value of 100% (30/30). During the BBN

treatment period (weeks 1–4) and non-treatment period (week 5), no statistically significant body-weight changes were found in groups 2–5 compared with the controls (group 1). During the ETBE treatment period (weeks 6–36), mean body weights in the group treated with ETBE at 1000 mg/kg bw per day (group 5) were significantly lower than the control values from week 32 until the end of the experiment. Mean body weights in the group treated with ETBE at 500 mg/kg bw per day (group 4) were comparable to the control values. Mean body weights in the groups treated with ETBE at 100 and 300 mg/kg bw per day (groups 2 and 3) were significantly lower than the control values from weeks 16 and 18, respectively, to the end of the experiment.

The incidences of papilloma of the urinary bladder in the treated groups (21/30, 13/30, 17/30, 17/30, and 21/26 in groups 1–5, respectively) were comparable to the control values. The incidence of carcinoma of the urinary bladder (5/30, 7/30, 6/30, 14/30, and 9/26 in groups 1–5, respectively) was significantly increased ( $P < 0.05$ , two-tailed Fisher exact test; [ $P = 0.0125$ , one-tailed Fisher exact test]) in group 4. In addition, the tumour multiplicity ( $0.15 \pm 0.35$ ,  $0.30 \pm 0.62$ ,  $0.24 \pm 0.51$ ,  $0.50 \pm 0.58$ , and  $0.40 \pm 0.71$  in groups 1–5, respectively) was significantly increased ( $P < 0.05$ , two-tailed Fisher exact test) in group 4 ( $0.5 \pm 0.58$ ) compared with the control group 1 ( $0.15 \pm 0.35$ ). The incidence of papilloma or carcinoma (combined) in groups 2–5 treated with ETBE at 100–1000 mg/kg per day was comparable to that in the control group 1 (24/30, 18/30, 20/30, 25/30, and 21/26 in groups 1–5, respectively).

Regarding preneoplastic lesions, in the urinary bladder, diffuse papillary hyperplasia of the epithelium was observed in rats in group 5 (13%) but not in groups 1–4. The incidence of papillary and nodular hyperplasia was statistically significantly decreased in groups 2–4, but not in group 5, compared with the control value. [The Working Group noted that this was a well-conducted study that complied with GLP

and that it was strengthened by the use of multiple doses and an adequate number of animals per group, and had an adequate duration of exposure and observation. However, it was limited by the use of males only.]

### 3.2 Evidence synthesis for cancer in experimental animals

The carcinogenicity of ETBE has been assessed in three well-conducted GLP studies in male and female F344/DuCrIj rats treated by oral administration (drinking-water) ([JBRC, 2010a](#); also reported by [Suzuki et al., 2012](#)); in male and female Sprague-Dawley rats treated by oral administration (gavage) ([Maltoni et al., 1999](#)) [histopathological re-analysis by [Malarkey and Bucher, 2011](#)]; and in male and female F344/DuCrIj rats treated by inhalation (whole-body) ([JBRC, 2010b](#); also reported by [Saito et al., 2013](#)). In addition, there were three well-conducted GLP initiation–promotion studies in male F344/DuCrIj rats treated by oral administration (gavage) ([JBRC, 2008a](#); also reported by [Hagiwara et al., 2011](#); [JBRC, 2010c](#); also reported by [Hagiwara et al., 2013](#)) and in male CrIj:WI Wistar rats ([JBRC, 2008b](#); also reported by [Hagiwara et al., 2015](#)).

In the well-conducted GLP study in male and female Sprague-Dawley rats treated by oral administration (gavage) ([Maltoni et al., 1999](#)), there was a significant increase in the incidence of sarcoma and of carcinoma or sarcoma (combined) of the uterus. The histopathological data were reviewed by the NTP Pathology Working Group ([Malarkey and Bucher, 2011](#)), including re-examination of all sections of the oral cavity, uterus, and vagina. The re-analysis showed a significant increase in the incidence of malignant schwannoma of the uterus. [The Working Group noted that malignant schwannoma of the uterus is a rare tumour. The Working Group also noted that the study observation period (lifetime)



increased uncertainty in interpreting the incidence of malignant tumours, which did not show a dose–response relationship. In addition, after the histopathological re-analysis by the NTP, malignant tumours were determined to be of multiple tissue origins (e.g. epithelial, muscle, and peripheral neural cells).]

In the well-conducted GLP study in male and female F344/DuCrIjCrIj rats treated by inhalation (whole-body) ([JBRC, 2010b](#); also reported by [Saito et al., 2013](#)), there was a significant positive trend in the incidence of hepatocellular adenoma in males, and the incidence was significantly increased. A uterine schwannoma was found in one rat in the group at the highest concentration.

In the well-conducted GLP initiation–promotion study in male F344/DuCrIjCrIj rats treated by oral administration (gavage) ([JBRC, 2008a](#); also reported by [Hagiwara et al., 2011](#)), there was a significant increase in the incidence and multiplicity of follicular cell adenoma of the thyroid gland. There was a significant increase in the incidence and multiplicity of follicular cell adenoma or carcinoma (combined) of the thyroid gland. There was a significant increase in the incidence of hepatocellular adenoma. There was a significant increase in the multiplicity of adenocarcinoma and of adenoma or carcinoma (combined) of the colon.

In the well-conducted GLP initiation–promotion study in male F344/DuCrIjCrIj rats treated by oral administration (gavage) ([JBRC, 2010c](#); also reported by [Hagiwara et al., 2013](#)), there was a significant increase in the incidence and multiplicity of carcinoma of the urinary bladder.

In the well-conducted GLP initiation–promotion study in male CrIj:WI Wistar rats treated by oral administration (gavage) ([JBRC, 2008b](#); also reported by [Hagiwara et al., 2015](#)), there was a significant positive trend in the incidence of hepatocellular adenoma, and the incidence was significantly increased in group 5. In addition, the Cochran–Armitage trend test indicated a significant positive trend in the

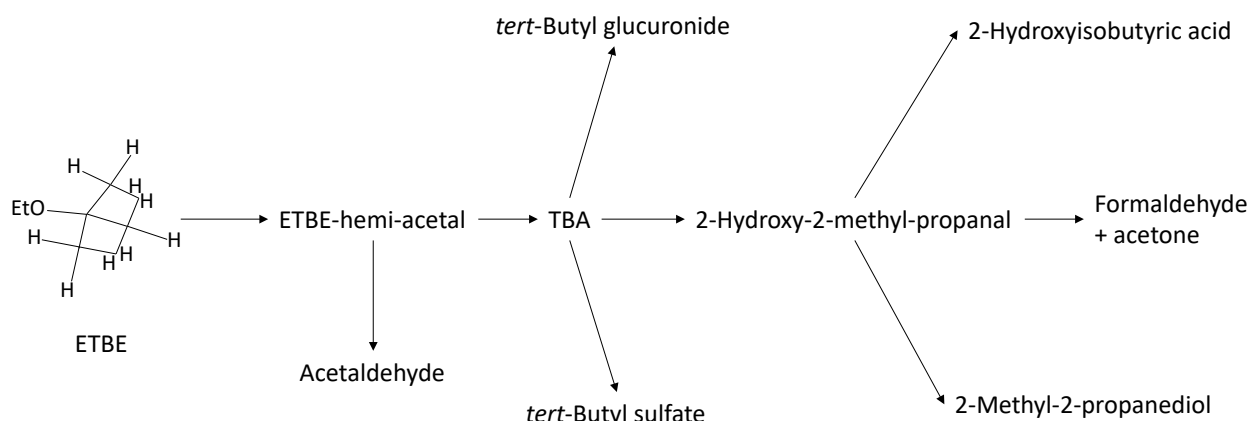
incidence of hepatocellular carcinoma, and the incidence was significantly increased in group 5. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined), and the incidence was significantly increased in group 5. The multiplicity of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined) was significantly increased in group 5. For the kidney, there was a significant positive trend in the incidence of adenoma of the renal tubules, and the incidence was significantly increased in groups 4 and 5. The incidence of carcinoma of the renal tubules was also significantly increased in group 3. There was a significant positive trend in the incidence of adenoma or carcinoma (combined) of the renal tubules.

There was no statistical difference in the incidence of any tumour in the well-conducted GLP study in male and female F344/DuCrIjCrIj rats treated by oral administration (drinking-water) ([JBRC, 2010a](#); also reported by [Suzuki et al., 2012](#)).

## 4. Mechanistic Evidence

### 4.1 Absorption, distribution, metabolism, and excretion

ETBE can be absorbed via both inhalation and ingestion. [The Working Group noted that the metabolism of ETBE is very similar to that of MTBE (also reviewed in the present volume), and the two agents differ in chemical structure only by the presence of an ethyl group instead of a methyl group.] The first step in the metabolism of ETBE is oxidation, resulting in the formation of acetaldehyde and an unstable hemi-acetal that decomposes to the alcohol TBA ([Fig. 4.1](#)). TBA is then conjugated or further oxidized to diols that are then conjugated or, in the case of 2-methyl-1,2-butanediol, oxidized to the corresponding acid.

**Fig. 4.1 Metabolism of ethyl *tert*-butyl ether**

ETBE, ethyl *tert*-butyl ether; TBA, *tert*-butyl alcohol.  
Adapted from [US EPA \(2021\)](#).

#### 4.1.1 Humans

##### Exposed humans

[Nihlén et al. \(1998a\)](#) studied the uptake and disposition of ETBE vapour in eight healthy male volunteers (aged between 21 and 41 years, with a body-weight range of 70–97 kg) during 2 hours of light physical exercise (50 W) in an exposure chamber. The participants were exposed to ETBE at 0 (control), 5, 25, and 50 ppm on four different occasions. ETBE, TBA, and acetone were measured in exhaled air, blood, and urine using head-space gas chromatography (HS-GC). The respiratory uptake during exposure was 32–34% of the inhaled ETBE, and the post-exposure exhalation of ETBE and TBA was 45–50% and 1.4–3.8% (on equimolar basis), respectively. The post-exposure decline in ETBE in the blood was described by four phases with average half-lives of 2 minutes, 18 minutes, 1.7 hours, and 28 hours, whereas that in the urine was described by two half-lives of 8 minutes and 8.6 hours. Average concentrations of 1.1, 5.4, and 10  $\mu\text{M}$  were reached after 2 hours at 5, 25, and 50 ppm, respectively. Post-exposure

half-lives of TBA in the blood and urine were on average 12 and 8 hours, respectively. Urinary excretion of ETBE and TBA was below 1% of the ETBE uptake, indicating further metabolism of TBA or other routes of metabolism and elimination. The kinetics of ETBE and TBA were linear at concentrations up to 50 ppm. The authors suggested that TBA is a more appropriate marker of exposure for ETBE than the parent ether itself. Acetone levels in the blood increased after ETBE exposure compared with control exposure, the increase being more prominent after exposure at 50 ppm. [The Working Group noted that the kinetics of ETBE in this study were very similar to those in a previous study on MTBE that had the same design and was carried out by the same group ([Nihlén et al., 1998b](#)) (see Section 4.1 of the monograph on MTBE in the present volume).]

Six volunteers (three men, and three women) were exposed to ETBE at 4 and 40 ppm. Average concentrations of ETBE in the blood at the end of the exposure were 1.3 and 12.1  $\mu\text{M}$ , respectively. The corresponding concentrations of TBA were 1.8 and 13.9  $\mu\text{M}$ . TBA was cleared from human blood with an average half-life of 9.8 hours at



40 ppm. Low concentrations of TBA, 2-methyl-1,2-propane diol, and 2-hydroxyisobutyric acid (2-HIBA) were detected in the urine. The metabolites were rapidly eliminated, with half-lives in the urine of between 10.2 and 28.3 hours. Between 41% and 53% of the retained ETBE was recovered as metabolites in urine ([Amberg et al., 2000](#)). [The Working Group noted that the same research group had also conducted a study on MTBE using the same design ([Amberg et al., 1999](#)) and that the two studies showed a similar toxicokinetic profile.]

[The Working Group noted that, overall, the two studies on experimental inhalation showed a consistent kinetic pattern with respect to the inhalation uptake, concentrations of ETBE and TBA in the blood, and excretion in the urine. The data showed that the kinetics of ETBE and TBA are linear up to 50 ppm. Variations in ETBE concentration in the blood – 10  $\mu$ M after 2 hours at 50 ppm, and 50  $\mu$ M at work versus 13.9  $\mu$ M after 4 hours at rest – were caused by differences in the duration of exposure and workload. The two studies also showed similar kinetics to those shown by MTBE in previous inhalation studies.]

#### 4.1.2 Experimental systems

##### (a) Non-human mammalian systems in vivo

[Bernauer et al. \(1998\)](#) studied the biotransformation of  $^{13}\text{C}$ -labelled ETBE in rats exposed at 2000 ppm for 6 hours. Biotransformation of the initial metabolite, TBA, was studied after oral exposure to 250 mg/kg bw, by gavage. Urinary metabolites were identified by  $^{13}\text{C}$  nuclear magnetic resonance (NMR) and GC-MS. TBA, a TBA conjugate (probably a glucuronide), and acetone were detected in the urine. 2-Methyl-1,2-propanediol, 2-HIBA, and another unidentified conjugate of TBA, most probably a sulfate, were the major urinary metabolites of MTBE and ETBE, as assessed by NMR. In rats dosed with  $^{13}\text{C}$ -labelled TBA,  $^{13}\text{C}$ -labelled acetone, TBA, and its glucuronide represented minor urinary

metabolites, whereas 2-methyl-1,2-propanediol, 2-HIBA, and presumably TBA sulfate were major metabolites.

[Amberg et al. \(2000\)](#) exposed F344 NH rats (five males, five females) to ETBE at 4 ppm or 40 ppm for 4 hours in a dynamic whole-body chamber. ETBE, TBA, 2-methyl-1,2-propane diol, and 2-HIBA were analysed in urine samples collected for 72 hours; ETBE and TBA were also assessed in blood samples collected for up to 48 hours. The average concentrations of ETBE after 4 hours, at 4 and 40 ppm, were 1.0  $\mu$ M and 5.3  $\mu$ M, respectively, and the corresponding values for TBA in the blood were 5.7  $\mu$ M and 21.7  $\mu$ M, respectively. Notably, TBA was also found (at a concentration of 1.1  $\mu$ M) in the blood of non-exposed control rats. 2-HIBA was the major metabolite in the urine, whereas TBA and 2-methyl-1,2-propanediol were present in lesser proportions. The excretion half-lives for the urinary metabolites were between 2.6 and 4.7 hours. In non-exposed control rats, the three metabolites were also found in the urine, and TBA was found in the blood. Metabolite recovery in the urine was estimated to be 50% at 4 ppm, and 53% at 40 ppm. [The Working Group concluded that the biotransformation and excretion of ETBE were similar in rats and in humans and that ETBE and its metabolites were rapidly excreted in both species.]

Fischer 344 (F344) male rats were exposed to ETBE at a dose of 0, 150, or 1000 mg/kg bw, twice daily, by gavage, for 1 and 2 weeks. The total content of cytochrome P450 (CYP) and levels of hydroxyl radicals ( $\cdot\text{OH}$ ) were found to increase significantly at both doses and over both durations of exposure. There was an increase in the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) after 2 weeks of exposure, accompanied by an accumulation of CYP2B1/2B2, CYP3A1/3A2, and CYP2C6. Upregulation of CYP2E1 and CYP1A1 mRNA expression was observed at the highest dose, after week 1 and week 2 (see Sections 4.2.1 and 4.2.2) ([Takehashi et al., 2013](#)).

[Lin et al. \(2020\)](#) exposed wildtype (WT) and *Aldh2* knockout (KO) (*Aldh2*<sup>-/-</sup>) male C57BL/6 mice to ETBE at 500 ppm for 6 hours. The exposure resulted in the formation of three metabolites – TBA, 2-methyl-1,2-propanediol, and ethanol – the levels of which were significantly higher in the liver, brain, fat, and testis of the KO mice compared with the WT mice. Treatment with TBA at 20 mg/mL, in drinking-water, caused significant changes in organ weights and histopathology, and increased levels of genetic damage in both mouse strains. Similar results were observed in KO mice exposed to TBA at 5 mg/mL, associated with an increase in markers of oxidative stress (reduced glutathione, GSH; and reduced GSH:glutathione disulfide ratio, GSH:GSSG ratio) in serum. In addition, the findings indicated indirectly that the enzyme aldehyde dehydrogenase 2 (ALDH2) was involved in the metabolism of both ETBE and TBA, and that deficiency could greatly increase sensitivity to TBA-induced toxicity.

[The Working Group noted that the above studies in vivo supported the metabolic profile described in [Fig. 4.1](#). Also, both the metabolic profile and the excretion pattern were similar in rats and in humans. One study confirmed the distribution of TBA and 2-methyl-1,2-propanediol to the liver and brain tissue.]

(b) *Human and non-human mammalian systems in vitro*

(i) *Tissue partitioning*

[Nihlén et al. \(1995\)](#) determined liquid:air partition coefficients in studies in vitro using HS-GC for three ethers added to gasoline (MTBE, ETBE, TAME) and the metabolite TBA. The average values for ETBE were 11.7 in human blood, 8.39 in physiological saline, and 190 in olive oil, and those for TBA were 462, 603, and 168, respectively. No significant sex-specific differences were observed with respect to the blood:air partition coefficients.

[Kaneko et al. \(2000\)](#) determined liquid:air partition coefficients for the same three ethers as well as the main metabolites, TBA, and TAA. Results were similar to those of [Nihlén et al. \(1995\)](#). For ETBE, the coefficients were 11.6 in rat blood, 11.0 in physiological saline, and 189 in olive oil. Additionally, Kaneko et al. reported rat tissue:air partition coefficients for ETBE of 22.4 in muscle, 19.5 in brain, 127 in kidney, 33.2 in liver, 25.2 in testis, and 136 in fat. Similar results were also obtained for TBA, the liquid:air partition coefficients being equal to 531 in blood, 638 in physiological saline, and 198 in olive oil, and the tissue:air partition coefficients being 790 in muscle, 617 in brain, 658 in kidney, 608 in liver, 641 in testis, and 129 in fat.

[The Working Group noted the similar values of partition coefficients for blood:air (humans and rats), saline:air, and olive oil:air for ETBE. The study by [Kaneko et al. \(2000\)](#) indicated that the distribution of ETBE and TBA to various tissues varied depending on tissue composition and the physical properties of the two agents.]

(ii) *Metabolism*

The metabolism of ETBE was investigated using different microsomal preparations from various tissues from Sprague-Dawley male rats. The highest metabolic activity was observed in preparations of olfactory mucosa (37 times as high as in preparations from liver), followed by nasal respiratory mucosa (four times as high). No detectable activity was observed in microsomal preparations from the lung, kidney, or olfactory bulb. The apparent  $K_m$  and  $V_{max}$  coefficient values in olfactory microsomes were 125  $\mu$ M and 11.7 nmol/min per mg of protein, respectively. The addition of TAME to the preparation inhibited the metabolism of ETBE and vice versa. Coumarin at a concentration of 50  $\mu$ M inhibited the metabolism of TAME and ETBE by approximately 87% ([Hong et al., 1997](#)). [The Working Group noted that coumarin is predominantly metabolized by CYP2A6 in the human liver.]

[Turini et al. \(1998\)](#) studied induction and inhibition of the metabolism of MTBE and ETBE in rat liver microsomes and in purified hepatic CYP isoenzymes (2B1, 2E1, 2C11, and 1A1). Oral exposure to ETBE at a dose of 2 mL/kg bw, for 2 days, induced the activity of hepatic microsomal CYP2E1 and CYP2B1/2. Intraperitoneal exposure to TBA at a dose of 200 or at 400 mg/kg bw, for 4 days, induced the activity of all liver microsomal CYPs. ETBE was oxidatively deethylated to yield acetaldehyde, and the dealkylation rate increased fourfold in rats that were pre-treated with phenobarbital. An increase in dealkylation was also observed in rats that were pre-treated with pyrazole, an inducer of CYP2E1. Of the purified CYPs, CYP2B1 exhibited the highest activity towards ETBE, whereas CYPs 1A1, 2C1, and 2E1 were only slightly active. Metyrapone, a potent inhibitor of CYP2B, inhibited the deethylation of ETBE in microsomes from phenobarbital-treated rats, whereas 4-methylpyrazole (a probe inhibitor of CYP2E1) and anti-CYP2E1 immunoglobulin G (IgG), did not inhibit ETBE deethylation. [The Working Group noted that inhibition studies suggested that rat CYP2A1 might also exert an important role in the oxidation of MTBE and ETBE. According to Turini et al., the results indicated that CYP2B1, when expressed, was the major enzyme involved in the oxidation of ETBE.]

[Hong et al. \(1999b\)](#) used liver microsomes from CYP2E1 KO (2E1<sup>-/-</sup>) female mice to investigate the contribution of this cytochrome to the metabolism of the gasoline ethers MTBE, ETBE, and TAME. The microsomes were able to metabolize all three ethers. The metabolizing activity (in nmol/min per mg) detected in the liver microsomes from the KO mice was similar to that observed in female, age-matched, WT (2E1<sup>+/+</sup>) C57BL/6N and 129/Sv mice. [The Working Group noted that, according to the above data, CYP2E1 played a negligible role in the metabolism of these ethers in the mouse liver.]

The metabolism of gasoline ethers was studied in human liver microsomes and in the presence of heterologously expressed human CYP enzymes. [Hong et al. \(1999a\)](#) observed large interindividual variation in the metabolism of three ethers in 15 samples of human liver microsomes. The activities of microsomes in metabolizing MTBE, ETBE, and TAME were highly correlated ( $r=0.91-0.96$ ), suggesting that these ethers are metabolized by the same enzyme(s). The highest degree of correlation was with human CYP2A6 ( $r=0.90-0.95$ ), which is known to be a polymorphic enzyme. Of a series of human CYP enzymes, expressed in human B-lymphoblastoid cells, CYP2A6 exhibited the highest turnover in metabolizing gasoline ethers. Addition of coumarin caused a dramatic inhibition of the metabolism of the three ethers, whereas the addition of anti-CYP2E1 IgG did not exert any effect. [The Working Group noted that the evidence indicated that CYP2A6 could be the major enzyme responsible for the metabolism of the three ethers ([Hong et al., 1999a](#)).]

[Le Gal et al. \(2001\)](#) studied the metabolism of the gasoline ethers MTBE, ETBE, and TAME in microsomes prepared from 12 human livers. Two apparent  $K_m$  values were identified for ETBE, averaging about 0.11 and 5.0 mM. The authors concluded that the data on kinetics, correlation studies, chemical inhibition, and metabolism through heterologously expressed human CYPs supported the hypothesis that the major enzyme involved in metabolism of these ethers is CYP2A6, with a minor contribution of CYP3A4 at low substrate concentrations.

[The Working Group noted that experiments with microsomal preparations from rat olfactory mucosa and from human liver samples indicated that CYP2A6 is the major enzyme involved in the deethylation of ETBE. The experiments also indicated that there is large interindividual variation in metabolic activity and that the microsomal activities involved in metabolizing ETBE, MTBE and TAME are highly correlated. Studies

with liver microsomes from CYP2E1 KO mice showed that CYP2E1 plays a minor role in the metabolism of ETBE.]

(c) *Physiologically based pharmacokinetic modelling*

[Nihlén and Johanson \(1999\)](#) used data from experiments in humans within an exposure chamber, published in a previous study ([Nihlén et al., 1998a](#)), to develop a physiologically based pharmacokinetic (PBPK) model for ETBE. The PBPK model was used to predict levels of ETBE and TBA in the blood, urine, and exhaled air under various exposure conditions, which included long durations of exposure, fluctuating exposure, and exposure during various degrees of physical activity. Interindividual variability in biomarker levels was also predicted, on the basis of experimental data from eight volunteers. Increasing physical activity, from rest to heavy exercise, increased all biomarker levels by approximately twofold at the end of an 8-hour work shift, and threefold the following morning. A small accumulation of all biomarkers was observed over 1 week (8 hours per day for 5 days) of simulated exposure. Other simulations suggested that interindividual variability in biomarker levels would be higher the morning after the exposure than at the end of the work shift and also that they would be higher for TBA than for ETBE. Monte Carlo simulations suggested that ETBE levels in the blood and exhaled air at the end of an 8-hour work shift were highly sensitive to exposure fluctuations, whereas ETBE levels the following morning were less sensitive, as were TBA levels in the urine and blood. [The Working Group noted that the simulations indicated that levels of TBA in the urine were a suitable biomarker for exposure to ETBE and gasoline vapour.]

[Salazar et al. \(2015\)](#) developed a PBPK model for ETBE and TBA to calculate internal doses of TBA after exposure to TBA or ETBE in rats. The model was based on earlier PBPK models for

MTBE that considered the metabolism of ETBE to TBA, and further metabolism of TBA in the liver. It was used to evaluate whether effects in the kidney and liver were consistent with different routes of exposure (inhalation, intravenous, or oral) and with the predicted internal dose of ETBE or TBA. [The Working Group noted that potential effects caused by binding to the male rat-specific protein  $\alpha_{2u}$ -globulin were not included in the model.] For effects on the kidney that were not directly linked to cancer (such as organ-weight changes, urothelial hyperplasia, and CPN) there were consistent dose-response relationships (with dose expressed as the concentration of TBA in the blood). There was also a consistent dose-response relationship for relative liver weight on the basis of the metabolism of TBA.

[Borghoff et al. \(2017\)](#) developed a rat PBPK model for ETBE and TBA that was based on a previous model for MTBE. The model included binding of ETBE and TBA to the male rat-specific protein  $\alpha_{2u}$ -globulin (see Section 4.3). The metabolism of ETBE and of TBA was described as a single, saturable pathway in the liver. The model predicted similar area-under-the-curve (AUC) values for TBA in the kidney under various exposure conditions in cancer bioassays testing ETBE and TBA. In addition, the model predicted nonlinear ETBE kinetics, based on blood AUC data for ETBE and TBA, at concentrations above approximately 2000 ppm (the inflection point) after exposure by inhalation. [The Working Group noted that, according to the authors, the results indicated the occurrence of metabolic saturation at the exposure level at which liver tumours were observed in rats (ETBE, 5000 ppm).]

[Watanabe-Matsumoto et al. \(2022\)](#) used a human PBPK model for ETBE, combined with quantitative structure-property relationship (QSPR) modelling, that considered only metabolism in the liver, to calculate partition coefficients,  $V_{max}$ , and  $K_m$ . Partition coefficients were



estimated from Henry's law constants and log octanol:water partition coefficients ( $K_{ow}$ ) using linear regression based on data on 181 industrial organic chemicals.  $V_{max}$  and  $K_m$  were obtained by calculating the sum of the contribution of structural fragments of the molecule (Free-Wilson approach) for the same 181 chemicals. The predicted concentration profile of ETBE in the blood was similar to that reported by [Nihlén et al. \(1998a\)](#) using data from human volunteers. [The Working Group noted that the study illustrated an approach to predicting the toxicokinetics of chemicals with unknown or limited input data. It included sensitivity analyses and uncertainty estimates. However, experimental data in humans and in rats were also available for ETBE.]

[The Working Group noted that these PBPK models were developed for ETBE and TBA in humans and in rats assuming metabolism only in the liver. No metabolites other than TBA were modelled. The rat model predicted nonlinear kinetics (saturation of the metabolism of ETBE) at around 2000 ppm.]

### Synopsis

[Overall, the Working Group noted that ETBE is easily absorbed after exposure via inhalation or ingestion. The available data, including in vivo studies in humans and rodents, in vitro studies of metabolism with microsomal preparations, and PBPK modelling, support the main metabolic pathways depicted in [Fig. 4.1](#). A central step in the biotransformation of ETBE is its deethylation to TBA. TBA is either conjugated or further metabolized to 2-HIBA, 2-methyl-2-propanediol, and formaldehyde plus acetone, before excretion in the urine. Small fractions of the absorbed dose are excreted as ETBE and TBA. ETBE and its metabolites are readily distributed in the body. The pattern and rates of metabolism of ETBE and TBA are qualitatively and quantitatively similar in humans and rats.

Furthermore, the kinetics of ETBE are similar to those of MTBE. Experimental data in humans showed linear kinetics for ETBE and TBA at up to at least 50 ppm. Experimental data in rats, combined with PBPK modelling, showed saturation of ETBE metabolism, beginning at around 2000 ppm.]

## 4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens ([Smith et al., 2016](#)) encompassed by the agent ETBE.

Evidence was available for the key characteristics “is genotoxic”, “induces oxidative stress”, “is immunosuppressive”, “modulates receptor-mediated effects”, and “alters cell proliferation, cell death, or nutrient supply”. There was a paucity of data for the remaining key characteristics of carcinogens.

The evidence was available only in non-human mammalian or non-mammalian experimental systems.

### 4.2.1 *Is genotoxic*

#### (a) *Non-human mammalian systems in vivo*

See [Table 4.1](#).

Induction of DNA strand breaks, as measured by alkaline comet assay, was observed in the leukocytes of *Aldh2* KO (*Aldh2*<sup>-/-</sup>) and WT (*Aldh2*<sup>+/+</sup>) male and female C57BL/6 mice exposed to ETBE at various doses via inhalation for 6 hours per day, 5 days per week, for 13 weeks. The damage was observed at 500, 1750, and 5000 ppm in *Aldh2*<sup>-/-</sup> male mice, at 5000 ppm in *Aldh2*<sup>+/+</sup> male mice, and at 5000 ppm in *Aldh2*<sup>-/-</sup> female mice. No differences in DNA strand breaks were observed in *Aldh2*<sup>+/+</sup> female mice at any exposure ([Weng et al., 2011](#)).

**Table 4.1 End-points relevant to genotoxicity in non-human mammalian systems in vivo exposed to ethyl *tert*-butyl ether**

| End-point (assay)                            | Species, strain (sex)                        | Tissue                       | Results <sup>a</sup>                                                              | Dose (LED or HID)                         | Route, duration, dosing regimen                               | Comments                                                                                                                                                                                                                                                                                                                                                                                               | Reference                                    |
|----------------------------------------------|----------------------------------------------|------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| Gene mutation ( <i>cII</i> mutant frequency) | Rat, F344 Big Blue homozygous transgenic (M) | Liver and bone marrow        | –                                                                                 | 5000 ppm                                  | Inhalation; 6 h/day, 28 consecutive days                      | The study followed OECD TG 488. Animals per exposure group, <i>n</i> = 5. Animals were euthanized 3 days after the final exposure. The positive control group was exposed orally to ethyl nitrosourea. While positive controls were significantly higher than the exposed groups, they were not significantly different to the laboratory historical negative control for <i>cII</i> mutant frequency. | <a href="#">Gollapudi and Rushton (2023)</a> |
| Micronucleus formation                       | Rat, Sprague-Dawley (M, F)                   | Bone marrow from femurs      | –                                                                                 | 20 000 mg/m <sup>3</sup>                  | Inhalation; 6 h/day, 5 days/wk, 4 wk                          | Exposure to gasoline containing ETBE. There was a reproducibility issue between different slide sets. Animals per exposure group, <i>n</i> = 5 per sex per group.                                                                                                                                                                                                                                      | <a href="#">Schreiner et al. (2014)</a>      |
| Sister-chromatid exchange                    | Rat, Sprague-Dawley (M, F)                   | Blood                        | + Only in females at 10 000 mg/m <sup>3</sup><br>– All other dosing groups (M, F) | 0, 2000, 10 000, 20 000 mg/m <sup>3</sup> | Inhalation; 6 h/day, 5 days/wk, 4 wk                          | Exposed to gasoline with ETBE. In female rats, there was no dose–response relation. Animals per exposure group, <i>n</i> = 5 per sex per group.                                                                                                                                                                                                                                                        |                                              |
| Micronucleus formation                       | Rat, F344/DuCr1Cr1j (M, F)                   | Bone marrow cells from femur | –                                                                                 | 10 000 ppm                                | Oral (drinking-water); 13 wk                                  | Drinking-water was replaced once per week. Animals per exposure group, <i>n</i> = 10 per sex per group.                                                                                                                                                                                                                                                                                                | <a href="#">Noguchi et al. (2013)</a>        |
| Micronucleus formation                       | Rat, F344/DuCr1Cr1j (M, F)                   | Bone marrow cells from femur | –                                                                                 | 5000 ppm                                  | Inhalation; 6 h/day, 5 days/wk, 13 wk                         | Animals per exposure group, <i>n</i> = 10 per sex per group.                                                                                                                                                                                                                                                                                                                                           |                                              |
| Micronucleus formation                       | Rat, F344/DuCr1Cr1j (M, F)                   | Bone marrow cells from femur | –                                                                                 | 1000 mg/kg per day                        | Intraperitoneal injection; 2 consecutive days, one dose daily | Animals per exposure group, <i>n</i> = 5 per sex per group.                                                                                                                                                                                                                                                                                                                                            |                                              |
| Micronucleus formation                       | Mouse, B6C3F <sub>1</sub> (M)                | Bone marrow                  | –                                                                                 | 1700 mg/kg per day                        | Intraperitoneal injection, three doses                        | Summary report.                                                                                                                                                                                                                                                                                                                                                                                        | <a href="#">NTP (1996a)</a>                  |



**Table 4.1 (continued)**

| End-point (assay)                           | Species, strain (sex)                                                                           | Tissue                           | Results <sup>a</sup> | Dose (LED or HID)                                              | Route, duration, dosing regimen            | Comments                                                                                  | Reference                             |
|---------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------|----------------------|----------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------------|
| Micronucleus formation                      | Rat, F344 (M)                                                                                   | Bone marrow                      | –                    | 2500 mg/kg per day                                             | Intraperitoneal injection, three doses     | Summary report.                                                                           | <a href="#">NTP (1996b)</a>           |
| Micronucleus formation                      | Mouse, CD-1 mice (M, F)                                                                         | Bone marrow cells from femur     | –                    | 5000 ppm                                                       | Inhalation; 6 h/day for 5 consecutive days |                                                                                           | <a href="#">US EPA (1995b)</a>        |
| Micronucleus formation                      | Rat, F344 (M, F)                                                                                | Bone marrow                      | –                    | 10 000 ppm                                                     | Oral (drinking-water), 13 wk               |                                                                                           | <a href="#">JBRC (2007a)</a> (N 7046) |
| Micronucleus formation                      | Rat, F344 (M, F)                                                                                | Bone marrow                      | –                    | 2000 mg/kg                                                     | Oral (gavage), daily, 2 days               |                                                                                           | <a href="#">JBRC (2007b)</a> (N 7049) |
| DNA damage (alkaline comet assay)           | Mouse, C57BL/6 KO ( <i>Aldh2</i> <sup>-/-</sup> ) or WT ( <i>Aldh2</i> <sup>+/+</sup> ) (M, F)  | Blood (leukocytes)               | +                    | 500 ppm for KO males<br>5000 ppm for KO females or WT males    | Inhalation; 6 h/day, 5 days/wk, 13 wk      | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure. | <a href="#">Weng et al. (2011)</a>    |
| DNA strand breaks (alkaline comet assay)    | Mouse, C57BL/6 KO ( <i>Aldh2</i> <sup>-/-</sup> ) and WT ( <i>Aldh2</i> <sup>+/+</sup> ) (M, F) | Liver                            | +                    | 500 ppm for KO females<br>5000 ppm for WT males and KO females | Inhalation; 6 h/day, 5 days/wk, 13 wk      | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure. | <a href="#">Weng et al. (2012)</a>    |
| Micronucleus formation (chromosomal damage) | Mouse, C57BL/6 KO ( <i>Aldh2</i> <sup>-/-</sup> ) and WT ( <i>Aldh2</i> <sup>+/+</sup> ) (M, F) | Blood (peripheral)               | +                    | 5000 ppm in WT males and KO females<br>1750 ppm in KO males    | Inhalation; 6 h/day, 5 days/wk, 13 wk      | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure. | <a href="#">Weng et al. (2013)</a>    |
| DNA strand breaks (alkaline comet assay)    | Mouse, C57BL/6 KO ( <i>Aldh2</i> <sup>-/-</sup> ) and WT ( <i>Aldh2</i> <sup>+/+</sup> ) (M)    | Sperm from left cauda epididymis | +                    | 1750 ppm                                                       | Inhalation; 6 h/day, 5 days/wk, 13 wk      | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure. | <a href="#">Weng et al. (2014)</a>    |

**Table 4.1 (continued)**

| End-point (assay)                        | Species, strain (sex)                                                                             | Tissue                           | Results <sup>a</sup> | Dose (LED or HID)                                 | Route, duration, dosing regimen      | Comments                                                                                                  | Reference                          |
|------------------------------------------|---------------------------------------------------------------------------------------------------|----------------------------------|----------------------|---------------------------------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------------------|
| DNA strand breaks (alkaline comet assay) | Mouse, C57BL/6 KO ( <i>Aldh2</i> <sup>-/-</sup> ), WT ( <i>Aldh2</i> <sup>+/+</sup> ), and HT (M) | Sperm from left cauda epididymis | +                    | 200 ppm in HT and KO mice<br>No change in WT mice | Inhalation; 6 h/day, 5 days/wk, 9 wk | Animals per exposure group, <i>n</i> = 5–9.<br>Limitation was high exposure.                              | <a href="#">Weng et al. (2014)</a> |
| DNA strand breaks (alkaline comet assay) | Mouse, C57BL/6 KO ( <i>Aldh2</i> <sup>-/-</sup> ), WT ( <i>Aldh2</i> <sup>+/+</sup> ), and HT (M) | Blood and liver                  | +                    | 200 ppm in HT and KO mice<br>No change in WT mice | Inhalation; 6 h/day, 5 days/wk, 9 wk | Presence of centrilobular hypertrophy and diffuse vacuolar degeneration that could indicate liver damage. | <a href="#">Weng et al. (2019)</a> |

*Aldh2*, aldehyde dehydrogenase 2; *cII*, gene of lambda bacteriophage; ETBE, ethyl *tert*-butyl ether; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; HT, heterozygous; KO, knockout; LED, lowest effective dose; M, male(s); OECD, Organisation for Economic Co-operation and Development; ppm, parts per million; TG, test guideline; wk, week(s); WT, wildtype.

<sup>a</sup> +, positive; –, negative; or no change.

In another study ([Weng et al., 2012](#)), DNA strand breaks were observed in the liver of *Aldh2* KO and WT male and female mice after exposure via inhalation to ETBE at 500, 1750, and 5000 ppm. Whereas significant increases in the frequency of DNA strand breaks were observed at all doses in KO male mice, DNA strand breaks were observed only at the highest dose (5000 ppm) in WT male mice. In females, no significant increase in DNA strand breaks was reported at any dose in WT mice compared with controls, whereas a significant increase was observed at the highest dose (5000 ppm) in KO mice when compared with the respective control animals.

Micronucleus formation, specifically in micronucleated reticulocytes and in mature normochromic erythrocytes, was assessed in *Aldh2* KO (*Aldh2*<sup>-/-</sup>) and WT (*Aldh2*<sup>+/+</sup>) male and female mice exposed via inhalation to ETBE at 0, 500, 1750, or 5000 ppm ([Weng et al., 2013](#)). Significant increases in the frequency of micronucleus formation was observed in reticulocytes from male KO mice at 1750 and 5000 ppm, and in male WT mice only at 5000 ppm. No effects were observed in either WT or KO female mice. Micronucleus frequency was significantly increased in normochromic erythrocytes in KO male mice at all doses, and in WT male mice at 5000 ppm. A significant increase in micronucleus frequency was observed only in KO female mice after exposure to ETBE at 5000 ppm, but not in WT mice.

The formation of DNA strand breaks was assessed in the sperm of *Aldh2* KO, WT, or heterozygous (HT) mice exposed to ETBE via inhalation at a dose of 0, 500, 1750, or 5000 ppm (6 hours per day, 5 days per week, for 13 weeks), or at 50, 200 or 500 ppm (6 hours per day, 5 days per week, for 9 weeks) ([Weng et al., 2014](#)). An increase in DNA strand breaks was observed only at the higher doses of 1750 and 5000 ppm after 13 weeks exposure in WT mice and at doses of 500, 1750, and 5000 ppm in KO mice, after

13 weeks. After 9 weeks, an increase in DNA strand breaks was observed at doses of 200 and 500 ppm in HT mice and in KO mice, but not in WT mice.

In a follow-up study, [Weng et al. \(2019\)](#) assessed the formation of DNA strand breaks in the liver and in blood of *Aldh2* KO, WT, or HT male mice exposed to ETBE at 0, 50, 200, or 500 ppm via inhalation for 9 weeks. Significant increases in DNA strand breaks, as measured by the alkaline comet assay, were observed in the liver and blood of HT and KO male mice at the two higher doses, but not in WT mice.

[The Working Group noted that the above studies from the group of [Weng et al. \(2011, 2012, 2013, 2014, and 2019\)](#) were all conducted in the same model of *Aldh2* KO, HT, or WT C57BL/6 mice, which were exposed to similar high doses via inhalation, thus limiting the interpretation of the findings.]

[Henley et al. \(2014\)](#) described a method to generate vapours of gasoline that did or did not contain fuel oxygenates with a similar composition to that of refuelling vapours originating from an automotive fuel tank at near maximum in-use temperatures; these vapours were used in inhalation toxicology studies to mimic environmental exposure(s). The authors generated seven different test materials, including base gasoline alone, gasoline plus several ethers (MTBE, ETBE, TAME, or DIPE), or gasoline plus an alcohol (ethanol or TBA).

[Schreiner et al. \(2014\)](#) assessed the formation of micronuclei in the bone marrow of rats exposed to gasoline blended with ETBE (as described above by [Henley et al., 2014](#)) at 0, 2000, 10 000, or 20 000 mg/m<sup>3</sup> via inhalation for 4 weeks, 6 hours per day, 5 days per week. No consistent increase in the frequency of micronuclei was observed in the bone marrow at 24 hours after the final exposure. In the same study, sister-chromatid exchange was measured in peripheral blood samples collected from the abdominal vein. Although there was a significant

increase in sister-chromatid exchange observed at 10 000 mg/m<sup>3</sup> in female rats, no statistical increase was observed in male rats, and there was no evidence for a dose–response trend in either males or females. [The Working Group noted that the findings for sister-chromatid exchange were inconclusive, and data for the evaluation of the frequency of micronucleated immature erythrocytes were not reproducible.]

[Noguchi et al. \(2013\)](#) assessed the formation of micronuclei in male and female F344/DuCrIj rats exposed to ETBE via oral exposure (drinking-water; 0, 1600, 4000, and 10 000 ppm), inhalation (0, 500, 1500, and 5000 ppm), or intraperitoneal injection (0, 250, 500, and 1000 mg/kg per day). No significant increase in micronucleus frequency was observed in the bone marrow of either male or female rats at any tested dose, regardless of exposure route.

Several other negative results for micronucleus formation in bone marrow of rats exposed to ETBE by intraperitoneal injection, gavage, or via drinking-water were reported ([NTP, 1996b](#); [JBRC, 2007a, b](#)), as well as for mice exposed to ETBE by intraperitoneal injection or inhalation ([US EPA, 1995b](#); [NTP, 1996a](#)).

ETBE did not induce gene mutations in the liver or bone marrow of homozygous transgenic male F344 Big Blue rats exposed by inhalation to ETBE at doses of up to 5000 ppm for 28 days ([Gollapudi and Rushton, 2023](#)).

#### (b) *Non-human mammalian systems in vitro*

The treatment of cultured Chinese hamster ovary cells (CHO-K1-BH4) with ETBE (0.10, 0.30, 1.0, 3.0, and 5.0 mg/mL) did not result in significant or concentration-related increases in the frequency of chromosome aberrations either in the presence or the absence of metabolic activation from rat liver S9 (9000×g supernatant) ([US EPA, 1995a](#)).

#### (c) *Non-mammalian systems*

Gene mutation was investigated in various bacterial strains (*Salmonella typhimurium* TA97, TA98, TA100, and TA1535). ETBE did not induce mutagenicity at concentrations of 100, 333, 1000, 3333, or up to 10 000 µg/plate in any of the tested strains ([Zeiger et al., 1992](#)).

#### 4.2.2 *Induces oxidative stress*

##### *Non-human mammalian systems in vivo*

See [Table 4.2](#).

Several studies investigated the potential association between ETBE and oxidative damage to DNA by measuring levels of 8-oxoguanine (8-oxoGua) using the human OGG1 (hOGG1)-modified comet assay ([Smith et al., 2006](#)). The measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by HPLC in the liver and in urine samples from rodents exposed to ETBE via inhalation.

8-oxoGua and 8-OHdG were evaluated in the liver of *Aldh2* KO and WT male and female mice exposed via inhalation to ETBE at a dose of 0, 500, 1750, or 5000 ppm ([Weng et al., 2012](#)). Significantly higher levels of total oxidative DNA damage (8-oxoGua) were observed in KO male mice, compared with the controls, after exposure to ETBE at 500, 1750, and 5000 ppm. In KO female and WT male mice, only in the groups at 5000 ppm was there a significant increase in the end-point; no increase was observed in WT female mice at any dose level. In the same study, 8-OHdG was measured by HPLC with an electrochemical detector (HPLC-ECD) in the liver of KO mice. Levels of 8-OHdG were higher (8-OHdG/10<sup>6</sup>dG, up to 12) in the groups at 500, 1750, and 5000 ppm compared with the control group (8-OHdG/10<sup>6</sup>dG, ~4). In KO female mice, levels of 8-OHdG in the DNA of liver from groups of KO mice at 1750 and 5000 ppm were higher than those in the control group.

**Table 4.2 End-points relevant to oxidative stress in non-human mammalian systems in vivo exposed to ethyl tert-butyl ether**

| End-point | Assay                      | Species, strain (sex)                                                                                               | Tissue                             | Results <sup>a</sup>                                                          | Dose (LED or HID)                                                        | Route, duration, dosing regimen                                 | Comments                                                                                                                                          | Reference                          |
|-----------|----------------------------|---------------------------------------------------------------------------------------------------------------------|------------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| 8-oxoGua  | hOGGI-modified comet assay | Mouse, C57BL/6 <i>Aldh2</i> KO ( <i>Aldh2</i> <sup>-/-</sup> ) and WT ( <i>Aldh2</i> <sup>+/+</sup> ) (M, F)        | Liver                              | ↑                                                                             | 500 ppm in male KO mice; 5000 ppm in male WT mice and female KO mice     | Inhalation; 6 h/day, 5 days/wk, 13 wk                           | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure. The authors used the term “total DNA oxidative damage”. | <a href="#">Weng et al. (2012)</a> |
| 8-OHdG    | HPLC-EDC                   |                                                                                                                     | Liver                              | ↑                                                                             | 500 ppm in male KO mice; 5000 ppm in male WT mice; 1750 ppm in female KO |                                                                 |                                                                                                                                                   |                                    |
| 8-oxoGua  | hOGGI-modified comet assay | Mouse, C57BL/6 <i>Aldh2</i> KO ( <i>Aldh2</i> <sup>-/-</sup> ) and WT ( <i>Aldh2</i> <sup>+/+</sup> ) (M, F)        | Blood (from postcaval vein)        | ↑ Only in male KO mice<br>No change in male WT, female KO, and female WT mice | 5000 ppm                                                                 | Inhalation; 6 h/day, 5 days/wk, 13 wk                           | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure.                                                         | <a href="#">Weng et al. (2013)</a> |
| 8-oxoGua  | hOGGI-modified comet assay | Mouse, C57BL/6 <i>Aldh2</i> KO ( <i>Aldh2</i> <sup>-/-</sup> ), WT ( <i>Aldh2</i> <sup>+/+</sup> ), and HT mice (M) | Sperm (from left cauda epididymis) | ↑                                                                             | 500 ppm for KO mice<br>1750 ppm for WT mice                              | Inhalation; 6 h/day, 5 days/wk, 13 wk (500, 1750, and 5000 ppm) | Animals per exposure group, <i>n</i> = 5 per sex per group. Limitation was high exposure.                                                         | <a href="#">Weng et al. (2014)</a> |
| 8-oxoGua  | hOGGI-modified comet assay |                                                                                                                     |                                    | ↑                                                                             | 500 ppm (LED) for KO and HT mice                                         | Inhalation; 6 h/day, 5 days/wk, 9 wk (50, 200, 500 ppm)         | Limitation was very high exposure.                                                                                                                | <a href="#">Weng et al. (2014)</a> |

**Table 4.2 (continued)**

| End-point                                                                            | Assay                      | Species, strain (sex)                                                                                          | Tissue                                       | Results <sup>a</sup>                                                                                                 | Dose (LED or HID)          | Route, duration, dosing regimen                            | Comments                                  | Reference                               |
|--------------------------------------------------------------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------|----------------------------------------------|----------------------------------------------------------------------------------------------------------------------|----------------------------|------------------------------------------------------------|-------------------------------------------|-----------------------------------------|
| 8-oxoGua                                                                             | hOGG1-modified comet assay | Mouse, C57BL/6 <i>Aldh2</i> KO ( <i>Aldh2</i> <sup>-/-</sup> ), WT ( <i>Aldh2</i> <sup>+/+</sup> ), and HT (M) | Liver and blood (hepatocytes and leukocytes) | ↑                                                                                                                    | 200 ppm in HT and KO mice  | Inhalation; 6 h/day, 5 days/wk, 9 wk (0, 50, 200, 500 ppm) |                                           | <a href="#">Weng et al. (2019)</a>      |
| 8-OHdG                                                                               | HPLC-EDC                   | Mouse, C57BL/6 <i>Aldh2</i> KO ( <i>Aldh2</i> <sup>-/-</sup> ), WT ( <i>Aldh2</i> <sup>+/+</sup> ), and HT (M) | Liver                                        | ↑                                                                                                                    | 200 ppm in HT and KO mice  |                                                            |                                           |                                         |
| 8-OHdG                                                                               |                            |                                                                                                                | Urine                                        | ↑                                                                                                                    | 200 ppm in HT and KO mice  |                                                            |                                           |                                         |
| Hydroxyl radicals (·OH)                                                              | ESR                        | Rat, F344/DuCrIcrlj (M)                                                                                        | Liver microsomal fraction                    | ↑                                                                                                                    | 1000 mg/kg                 | Oral, twice per day (6-h intervals), 1–2 wk                | Animals per exposure group, <i>n</i> = 20 | <a href="#">Kakehashi et al. (2013)</a> |
| mRNA expression of CYP isoenzyme genes associated with formation of oxidative stress | qPCR                       |                                                                                                                | Liver                                        | ↑ Accumulation of CYP2B1/2B2, CYP3A1/3A2 and CYP2C6<br>Upregulation of CYP2E1 and CYP1A1<br>↓ Downregulation of OGG1 | 1000 mg/kg at week 1 and 2 |                                                            |                                           |                                         |
| 8-OHdG                                                                               | HPLC-EDC                   |                                                                                                                | Liver                                        | ↑                                                                                                                    | 1000 mg/kg at week 2       |                                                            |                                           |                                         |

8-OHdG, 8-hydroxy-2'-deoxyguanosine; 8-oxoGua, 8-oxoguanine; CYP, cytochrome P450; ESR, electron spin resonance; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; hOGG1, human 8-oxoguanine DNA glycosylase 1; HPLC-EDC, high-performance liquid chromatography-electrochemical detection; HT, heterozygous; KO, knockout; LED, lowest effective dose; M, male(s); mRNA, messenger RNA; OH, hydroxyl; qPCR, quantitative polymerase chain reaction; ppm, parts per million; WT, wildtype; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; or no change.



In general, 8-OHdG levels were lower in female mice than in male mice.

Net oxidative DNA damage (8-oxoGua) was evaluated using the hOGG1-modified comet assay in the blood of male and female C57BL/6 *Aldh2* KO and WT mice after exposure by inhalation to ETBE at doses of 0, 500, 1750, and 5000 ppm ([Weng et al., 2013](#)). While significant increases were observed in KO male mice at 5000 ppm compared with the controls, no significant increases were observed in WT male, KO female, and WT female mice at any dose.

Similarly, net oxidative DNA damage (8-oxoGua), measured by the hOGG1-modified comet assay, was evaluated in the sperm of KO, WT, or HT mice ([Weng et al., 2014](#)) after exposure via inhalation to ETBE at 500, 1750, or 5000 ppm (for 6 hours per day, 5 days per week, 13 weeks) or 200 and 500 ppm (6 hours per day, 5 days per week, 9 weeks). A significant increase in 8-oxoGua levels was observed in KO mice (at 500 ppm for 9 weeks and at 500, 1750, and 5000 ppm for 13 weeks), HT (only at 500 ppm for 9 weeks), and WT mice (only at 1750 and 5000 ppm for 13 weeks).

Oxidative DNA damage (hOGG1-modified comet assay) was also evaluated in the liver and in blood of KO, WT, and HT male mice exposed via inhalation to ETBE at 0, 50, 200, or 500 ppm for 9 weeks ([Weng et al., 2019](#)). It was reported that HT and KO male mice showed a significant increase in levels of 8-oxoGua in the liver and blood when exposed to ETBE at 200 and 500 ppm, but no significant difference was observed in WT male mice compared with the controls. In the same study, 8-OHdG was also determined by HPLC-ECD in the liver and in urine. Similarly to the above findings, elevated levels of 8-OHdG were observed in the liver and urine of HT and KO male mice exposed via inhalation to ETBE at 200 and 500 ppm. [The Working Group noted that the studies by Weng et al. investigated end-points of oxidative stress along with end-points of genotoxicity (see also

Section 4.2.2); the limitations of these studies, as reported earlier in the present monograph, interfered with the interpretation of the results.]

[Kakehashi et al. \(2013\)](#) investigated the potential induction of oxidative stress in F344 rats exposed orally to ETBE at a dose of 0, 150, or 1000 mg/kg bw for 1 or 2 weeks. The authors observed a significant increase in levels of hydroxyl radicals ( $\cdot\text{OH}$ ) in liver microsomal fractions, assessed by electron spin resonance, and of levels of 8-OHdG in the liver, as assessed by HPLC-ECD, after 2 weeks of exposure. These findings were accompanied by an accumulation of CYP2B1/2B2, CYP3A1/3A2, and CYP2C6 (measured by quantitative real-time polymerase chain reaction and immunohistochemistry), downregulation of OGG1, and also upregulation of CYP2E1 and CYP1A1 after exposure to ETBE at 1000 mg/kg bw for 1 or 2 weeks.

#### 4.2.3 *Is immunosuppressive*

##### (a) *Non-human mammalian systems in vivo*

[Banton et al. \(2011\)](#) investigated the potential immunotoxicity effects in female Crl:CD(SD) rats exposed to ETBE at a dose of 0, 250, 500, or 1000 mg/kg bw per day, once daily, for 28 days. In addition, the authors assessed the T-cell-dependent antibody response in rats sensitized with sheep red blood cells (SRBCs), using the splenic antibody-forming cell (AFC) assay. No ETBE-related effects were observed on the specific activity (AFC/ $10^6$  spleen cells) or total activity (AFC/spleen) of splenic IgM AFCs towards the T-cell-dependent antigen, SRBC.

[Li et al. \(2011\)](#) investigated the effects of ETBE on splenocytes from male C57BL/6J mice after exposed by inhalation at 0, 500, 1750, or 5000 ppm, for 6 hours per day, 5 days per week, 6 or 13 weeks. Several cell phenotypes (B cells (PerCP-Cy5.5-CD45R/B220); T cells (PerCP-Cy5-CD3e); T-cell subsets (FITC-CD4 and PE-CD8a); natural killer cells; and macrophages) were quantified by flow cytometry. Splenic

natural killer cells, B cells, macrophages, and the total number of splenocytes were not affected by ETBE exposure, but a significant decrease was observed in the percentage of splenic CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> T cells, and CD4<sup>+</sup> T cells, and there was a dose-dependent decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratio.

[White et al. \(2014\)](#) investigated the immunotoxicity of gasoline or various mixtures of gasoline combined with fuel oxygenates (including ETBE) in female Sprague-Dawley rats exposed via inhalation to the various vapour condensates at 0, 2000, 10 000, or 20 000 mg/mg<sup>3</sup>, for 6 hours per day, 5 days per week, for 4 weeks. The characterization of the various test materials was described by [Henley et al. \(2014\)](#). The AFC response to the T-dependent antigen, SRBC, was used to determine the effects of the vapour condensates on the humoral components of the immune system. Exposure to gasoline combined with ETBE at 10 000 and 20 000 mg/m<sup>3</sup> resulted in a significant dose-related decrease in the AFC (cells forming IgM antibody to the T-dependent antigen SRBC) response. The exposure to gasoline vapour condensate alone did not result in significant changes in the IgM AFC response to SRBC, either in the specific activity (AFC/10<sup>6</sup> spleen cells) or the total spleen activity (AFC/spleen).

[The Working Group noted that only vapour condensates from a mixed blend of gasoline with ETBE elicited the humoral immune response, suggesting that the addition of ETBE was capable of altering the rat humoral response. A similar alteration was observed after the addition of the fuel oxygenates ethanol or DIPE; in contrast, the addition of MTBE, TAME, or TBA did not affect humoral immunity in rats.]

#### (b) *Non-human mammalian systems in vitro*

One study in the rat RBL2H3 cultured mast cell line was available to the Working Group ([Yamaki and Yoshino, 2009](#)). The effects of ETBE on IgE-dependent mast cell activation were

investigated by the evaluation of IgE-dependent degranulation (measured by the release of  $\beta$ -hexosaminidase), calcium ion (Ca<sup>2+</sup>) mobilization (assessed by cytometry), phosphorylation of Akt serine/threonine kinase 1 (as an indicator of phosphatidylinositol 3-kinase, PI3K, activity (assessed by immunoblotting), and global cellular tyrosine phosphorylation (assessed using monoclonal antibodies for phosphorylated tyrosine). RBL2H3 cells were treated with ETBE (0.1, 0.3, or 1.0%). ETBE inhibited Fc $\epsilon$ RI-mediated mast cell degranulation through the activation of protein tyrosine kinase and PI3K, and calcium mobilization.

#### 4.2.4 *Modulates receptor-mediated effects*

##### (a) *Non-human mammalian systems in vivo*

See [Table 4.3](#).

[de Peyster et al. \(2009\)](#) investigated reproductive steroids (testosterone and 17 $\beta$ -estradiol) and hormone-dependent reproductive organs in male rats exposed orally by gavage to ETBE at a dose of 0, 600, 1200, or 1800 mg/kg per day in corn oil for 14 days. No significant alteration in testosterone serum levels was observed in peripheral blood samples (collected 1 hour after treatment) at any dose tested. Estradiol levels were significantly higher in rats at the higher doses of 1200 and 1800 mg/kg than in untreated rats. No abnormal histopathology was observed in the testis, and there was no significant alteration in the weight of hormone-dependent reproductive organs.

[Takehashi et al. \(2013\)](#) investigated cellular proliferation, apoptosis, and upstream regulators of differentially expressed genes and proteins in F344 rats exposed orally to ETBE at 0, 150, or 1000 mg/kg bw per day for 1 or 2 weeks. No treatment-related macroscopic tissue changes were observed except in the liver tissue. A significant increase in CYP total content in liver microsomes at all tested doses, combined with a significant accumulation of CYP2B1/2B2, CYP3A1/3A2,

**Table 4.3 End-points relevant to modulation of receptor-mediated effects in non-human mammalian systems in vivo exposed to ethyl tert-butyl ether**

| End-point                                                         | Assay                    | Species, strain (sex)   | Tissue                     | Results <sup>a</sup>                                                   | Dose (LED or HID)                   | Route, duration, dosing regimen           | Comments                                  | Reference                                |
|-------------------------------------------------------------------|--------------------------|-------------------------|----------------------------|------------------------------------------------------------------------|-------------------------------------|-------------------------------------------|-------------------------------------------|------------------------------------------|
| Testosterone production and 17 $\beta$ -estradiol level           | Hormone radioimmunoassay | Rat, F344 (M)           | Blood, reproductive organs | No change in testosterone<br>↑ For estradiol (LED, 1200 mg/kg per day) | 0, 600, 1200, or 1800 mg/kg per day | Oral (gavage) in corn oil for 14 days     | Animals per exposure group, <i>n</i> = 12 | <a href="#">de Peyster et al. (2009)</a> |
| PPAR $\alpha$ mRNA, PPAR $\alpha$ -related Cyp4a1 and Cyp4a2 mRNA | Real-time PCR            |                         | Liver                      | ↑                                                                      | 1000 mg/kg per day group at week 1  |                                           | <i>n</i> = 20                             | <a href="#">Kakehashi et al. (2013)</a>  |
| Peroxisome proliferation                                          | TEM analysis             |                         |                            | ↑                                                                      | 1000 mg/kg per day group at week 2  |                                           |                                           |                                          |
| CAR                                                               | IPA analysis             | Rat, F344/DuCrlCrIj (M) | Liver microsomal fraction  | ↑ (IPA prediction)                                                     | 0, 150, 1000 mg/kg                  | Oral, twice a day (6-h intervals), 1–2 wk |                                           |                                          |
| PXR                                                               |                          | Rat, F344/DuCrlCrIj (M) | Liver microsomal fraction  | ↑ (IPA prediction)                                                     | 0, 150, 1000 mg/kg                  |                                           |                                           |                                          |
| PPARs                                                             |                          | Rat, F344/DuCrlCrIj (M) | Liver microsomal fraction  | ↑ (IPA prediction)                                                     | 1000 mg/kg                          |                                           |                                           |                                          |
| T3, free T3, T4, free T4                                          | Blood measurement        | Rat, F344 (M)           | Blood                      | No change at wk 2<br>↓ For all at wk 4                                 | 1000 mg/kg per day                  | Oral (gavage) for 2 or 4 wk               | Animals per exposure group, <i>n</i> = 30 | <a href="#">JBRC (2008c)</a>             |

CAR, constitutive androstane receptor; CYP, cytochrome P450; F344, Fischer 344; h, hour(s); HID, highest ineffective dose; IPA, ingenuity pathway analysis; LED, lowest effective dose; M, male(s); mRNA, messenger RNA; PCR, polymerase chain reaction; PPAR, peroxisome proliferator receptor; PXR, pregnane X receptor; T3, triiodothyronine; T4, thyroxine; TEM, transmission electron microscopy; wk, week(s).

<sup>a</sup> ↑, increase; ↓, decrease; or no change.

and CYP2C6, was also observed. Upregulation of CYP2E1 and CYP1A1 at weeks 1 and 2, and peroxisome proliferation at week 2 were observed at the highest dose. Although mRNA levels of peroxisome proliferator-activated receptor (PPAR $\alpha$ ), PPAR $\alpha$ -related CYP4A1, and CYP4A2 increased at the highest dose after week 1, it was reported to return to baseline levels at week 2.

PPAR $\alpha$  activation, in terms of peroxisome proliferation in hepatocytes of F344 rats exposed to ETBE for 2 weeks, was further assessed using transmission electron microscopy analysis. The average number of peroxisomes in hepatocytes was increased at both doses when compared with that in hepatocytes from untreated rats. In addition, induction of apoptosis and cell cycle arrest were also reported in hepatocytes of treated rats. Protein function analysis, as assessed by ingenuity pathway analysis (IPA) and in combination with proteome analysis, predicted the activation of upstream regulators, including constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptors (PPAR $\gamma$ ,  $\delta$ , and possibly  $\alpha$ ).

In another study, the authors reported a significant reduction in serum levels of triiodothyronine (T3), free T3, thyroxine (T4), and free T4 hormones in F344 rats exposed orally by gavage to ETBE at a dose of 0, 100, 300, or 1000 mg/kg per day for up to 4 weeks ([JBRC, 2008c](#)).

[The Working Group identified two additional studies on endocrine disruption that investigated end-points more relevant to toxicity than to reproduction ([Fujii et al., 2010](#); [Gray et al., 2014](#)). No major alterations were observed for end-points related to fertility, gestation, delivery, or other reproduction-related end-points, except for some increases in various organ relative weights in both studies.]

#### (b) *Non-human mammalian systems in vitro*

In the study by [de Peyster et al. \(2009\)](#), the effects of ETBE (50 and 100 mM) on testosterone production after human chorionic gonadotropin (hCG) stimulation in a model in isolated rat Leydig cells were also evaluated. ETBE at both concentrations inhibited testosterone production by 67% and 54%, respectively, compared with controls.

#### 4.2.5 *Alters cell proliferation, cell death, or nutrient supply*

##### (a) *Non-human mammalian systems in vivo*

See [Table 4.4](#).

[Medinsky et al. \(1999\)](#) investigated the potential toxicological effects of ETBE in male and female F344 rats and CD-1 mice exposed by inhalation to ETBE at doses of 0, 500, 1750, or 5000 ppm (6 hours per day, 5 days per week) for 13 weeks. In rats, increases in the weight of the kidney, adrenal gland, and heart (females only) were observed. A dose-related increase in liver weight was also observed in male and female rats and mice. In addition, signs of  $\alpha_{2u}$ -globulin nephropathy, which included increases in regenerative foci, rate of renal cell proliferation, and  $\alpha_{2u}$ -globulin-containing protein droplets, were observed in the kidneys of male rats at all tested doses. [The Working Group noted that an increase in cortical epithelial cell proliferation, also called “regenerative proliferation”, in the kidney could be a consequence of the observed nephropathy in rats and may be not related to carcinogenesis.] Increases in the incidence of centrilobular hepatocyte hypertrophy and rate of hepatocyte cell proliferation were observed in male and female mice exposed to ETBE at the highest concentration of 5000 ppm. [The Working Group noted that the bromodeoxyuridine (BrdU)-labelling method used to measure cell proliferation was adequate. The Working Group also noted that the increase in hepatocyte

**Table 4.4 End-points relevant to cell proliferation, cell death, or nutrient supply in non-human mammalian systems in vivo exposed to ethyl tert-butyl ether**

| End-point        | Assay                                  | Species, strain (sex)      | Tissue                             | Results <sup>a</sup>      | Dose (LED or HID)                                                       | Route, duration, dosing regimen               | Comments                                                                                                                                                                                                                                                                                                                                                                               | Reference                                                           |
|------------------|----------------------------------------|----------------------------|------------------------------------|---------------------------|-------------------------------------------------------------------------|-----------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
| Cell replication | BrdU labelling in immunohistochemistry | Mouse, CD-1 (M, F)         | Liver (hepatocytes)                | ↑                         | 1750 ppm at 1 and 13 wk (F) and at 1 and 4 wk (M)                       | Inhalation, 6 h/day, 5 days/wk, 1, 4 or 13 wk | Animals per exposure group, <i>n</i> = 10–15 rats or mice per sex. Signs of $\alpha_2$ -globulin nephropathy were reported only in kidney of male rats. Incidence of centrilobular hepatocyte hypertrophy in mice. The increase in cortical epithelial cell proliferation, also called “regenerative proliferation”, in kidney could be a consequence of the observed rat nephropathy. | <a href="#">Medinsky et al. (1999)</a>                              |
|                  |                                        | Rat, F344 (M, F)           | Kidney (cortical epithelial cells) | ↑                         | 500 ppm after 13 wk (M) and 1 wk (F) 5000 ppm after 1 or 4 wk (F and M) |                                               |                                                                                                                                                                                                                                                                                                                                                                                        |                                                                     |
| Hyperplasia      | Histopathology                         | Rat, F344/DuCr1Cr1j (M, F) | Ureter                             | ↑, Urothelial hyperplasia | 1500 ppm only in M                                                      | Inhalation, 6 h/day, 5 days/wk, 104 wk        | Animals per exposure group, <i>n</i> = 50 per sex per dose. The study followed OECD TG 451. Deposition in the renal papilla in male kidney was observed in males. CPN in both sexes at 5000 ppm.                                                                                                                                                                                       | <a href="#">IBRC (2010b)</a> ; <a href="#">Saito et al. (2013)</a>  |
| Hyperplasia      | Histopathology                         | Rat, F344/DuCr1Cr1j (M, F) | Ureter                             | ↑, Urothelial hyperplasia | 2500 ppm only in males                                                  | Drinking-water, 2 year (104 wk)               | Deposition in the renal papilla in male kidney was observed in males. CPN in both sexes.                                                                                                                                                                                                                                                                                               | <a href="#">IBRC (2010a)</a> ; <a href="#">Suzuki et al. (2012)</a> |

**Table 4.4 (continued)**

| End-point                       | Assay                                  | Species, strain (sex)   | Tissue                                           | Results <sup>a</sup>                            | Dose (LED or HID)       | Route, duration, dosing regimen            | Comments                                                                                                                                                                                                                                                                                                                                                                  | Reference                                            |
|---------------------------------|----------------------------------------|-------------------------|--------------------------------------------------|-------------------------------------------------|-------------------------|--------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| Hyperplasia                     | Pathological examination               | Rat, F344/DuCr1Cr1j (M) | Thyroid gland, liver, kidney, adrenal and testis | No change                                       | 1000 mg/kg per day      | Gavage 28 wk (0 or 1000 mg/kg per day)     | Animals per exposure group, <i>n</i> = 30. Other groups of animals were sequentially given five carcinogens with different target sites in the first 4 wk for multiorgan initiation and after 1 wk they received ETBE by gavage (300 or 1000 mg/kg per day until experimental wk 28). These animals had increased incidence of hyperplasia in the kidney and forestomach. | <a href="#">JBRC (2008a); Hagiwara et al. (2011)</a> |
| Hyperplasia                     | Histopathology                         | Rat, Wistar (M)         | Kidney                                           | ↑, Atypical tubule                              | 1000 mg/kg bw           | Gavage, daily, for 19 wk                   | Animals per exposure group, <i>n</i> = 30. Rats were exposed to drinking-water containing EHEN, as an initiator, at a dose of 500 ppm before ETBE (see Section 3). Atypical tubule hyperplasia can be considered a preneoplastic lesion.                                                                                                                                  | <a href="#">JBRC (2008b); Hagiwara et al. (2015)</a> |
| Hyperplasia                     | Histopathology                         | Rat, F344/DuCr1Cr1j (M) | Urinary bladder                                  | +, Extensive papillary hyperplasia in 4/30 rats | 1000 mg/kg bw           | Drinking-water, daily, 7 days/wk for 36 wk | <i>n</i> = 30. Rats were initiated with BBN to test the effect of ETBE on two-stage urinary bladder carcinogenesis (see Section 3).                                                                                                                                                                                                                                       | <a href="#">JBRC (2010c); Hagiwara et al. (2013)</a> |
| Regenerative cell proliferation | BrdU labelling in immunohistochemistry | Rat, F344 (M)           | Liver                                            | ↑                                               | 1000 mg/kg bw at day 28 | Gavage for 3, 10, 17, and 28 days          | Early induction of cell proliferation activity at day 3.                                                                                                                                                                                                                                                                                                                  | <a href="#">Takehashi et al. (2015)</a>              |



**Table 4.4 (continued)**

| End-point          | Assay                                  | Species,<br>strain (sex) | Tissue      | Results <sup>a</sup> | Dose<br>(LED or<br>HID)  | Route,<br>duration,<br>dosing<br>regimen | Comments                                                                                                                                                         | Reference                    |
|--------------------|----------------------------------------|--------------------------|-------------|----------------------|--------------------------|------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Cell proliferation | BrdU labelling in immunohistochemistry | Rat, F344 (M)            | Forestomach | (↑)                  | 1000 mg/kg bw after 1 wk | Gavage, daily, for 1 or 4 wk             | There was a non-significant increasing trend in BrdU in squamous cells of the forestomach.<br>One rat had focal squamous cell hyperplasia at 300 mg/kg for 4 wk. | <a href="#">IBRC (2008d)</a> |

BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; bw, body weight; BrdU, bromodeoxyuridine; CPN, chronic progressive nephropathy; EHEN, *N*-ethyl-*N*-(2-hydroxyethyl)nitrosamine; ETBE, ethyl *tert*-butyl ether; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; M, male(s); OECD, Organisation for Economic Co-operation and Development; ppm, parts per million; TG, test guideline; wk, week(s).

<sup>a</sup> +, positive; ↑, increase; ↓, decrease; (), findings in a study of limited quality; or no change.

cell proliferation accompanied by hypertrophy and increase in liver weight may lead to hepatocarcinogenesis. The Working Group noted that these results were supported by the finding of a significant positive trend in the incidence of hepatocellular adenoma (see Section 3) (JBRC, 2008b; also reported by Hagiwara et al., 2015).]

In another study (JBRC, 2010b; Saito et al., 2013), an increased incidence of urothelial hyperplasia and mineral deposition in the renal papilla of the pelvis was observed in male rats exposed to ETBE at 1500 and 5000 ppm. Preneoplastic lesions of the liver and hepatocellular adenoma (see Section 3), and an increase in the severity of CPN, were reported in male and female F344 rats after a 2-year exposure, via inhalation, to ETBE at 5000 ppm.

Suzuki et al. (2012) (also reported by JBRC, 2010a) evaluated the carcinogenic potential of ETBE in male and female F344 rats exposed orally, via drinking-water, to ETBE at a concentration of 0, 625, 2500, or 10 000 ppm for 2 years. As in the previous studies, ETBE at 2500 and 10 000 ppm significantly increased the incidence of urothelial hyperplasia of the pelvis and mineral deposition in the renal papilla in the kidney in males. An increase in the incidence of CPN was reported in the kidney of both male and female rats at 10 000 ppm. [The Working Group noted that urothelial hyperplasia is an informative end-point and was associated with the exposure.]

In a carcinogenesis bioassay, promotion of hepatocarcinogenesis was evaluated in male F344 rats exposed to ETBE at a dose of 0, 300, or 1000 mg/kg per day by gavage for 23 weeks (JBRC, 2008a; Hagiwara et al., 2011). Significant increases in the relative weights of the thyroid gland, liver, kidney, adrenal gland, and testis were reported after exposure at the higher dose; at the same high dose, no increase in the incidence of hyperplasia of the kidney or the urinary bladder was observed. The same authors conducted another initiation–promotion study on the

effects of ETBE on urinary bladder carcinogenesis initiated by BBN in rats (JBRC, 2010c; Hagiwara et al., 2013). Papillomatosis (extensive papillary hyperplasia) in the urinary bladder was observed in 13% of rats (4/30) at 1000 mg/kg per day, but not at lower doses of 100–500 mg/kg per day. [The Working Group noted that extensive papillary hyperplasia could be considered an informative end-point.]

In an additional study, the same authors investigated the tumour-promoting effects of ETBE in an initiation–promotion study on hepatic and renal carcinogenesis in rats (see Section 3). Atypical tubule hyperplasia of the kidney was observed in all animals in each group, including the control group. The multiplicity of atypical tubule hyperplasia was significantly increased in rats treated with ETBE at 1000 mg/kg per day compared with rats in the control group (JBRC, 2008b; Hagiwara et al., 2015). [The Working Group noted that atypical tubule hyperplasia can be considered to be a preneoplastic lesion.]

In another study, F344 rats were exposed by gavage to ETBE at a dose of 0 or 1000 mg/kg bw, twice per day, for 3, 10, 17, or 28 days. An increase in regenerative cell proliferation as a result of ETBE treatment was observed at day 3 and day 28, followed by induction of cell cycle arrest and apoptosis by day 14 (Kakehashi et al., 2015).

Cell proliferation was investigated in the forestomach of rats exposed to ETBE (0, 100, 300, 500, or 100 mg/kg per day) by gavage for 1 or 4 weeks. In rats at 1000 mg/kg per day, there was a non-significant increasing trend in BrdU in squamous cells of the forestomach, and one rat at 300 mg/kg per day had focal squamous cell hyperplasia at 4 weeks (JBRC, 2008d).

#### (b) *Other non-mammalian systems in vivo*

In zebra fish embryos, Bonventre et al. (2012) investigated developmental lesions and potential toxicity associated with ETBE exposure,

examining the gene transcript levels of vascular endothelial growth factor (vegf), as well as multiple isoforms from the wnt and matrix metalloproteinase (mmp) families. The median lethal concentration (LC<sub>50</sub>) was calculated based on ETBE exposure in the range of 2.5 to 40.0 mM; in the groups at 30 and 40 mM, fish embryos died. The observed lesions included enlarged heart, cranial haemorrhage, and yolk dysmorphogenesis. Toxicity was evaluated in a dose-response study (0.625–10 mM) in developing zebrafish. Abnormal craniofacial structures and whole-body oedema were observed. The authors also observed a reduction in levels of circulating erythrocytes, and a decrease in mRNA transcripts of mmp9 with ETBE at 5 mM. A significant decrease in wnt and mmp was also reported, but the mRNA transcript of vegf was not altered.

#### 4.2.6 Evaluation of high-throughput *in vitro* screening data

The mechanistic characteristics common to carcinogens (the 10 key characteristics of carcinogens) can be investigated through biochemical and cell-based assays run by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health Toxicity Forecaster/Toxicology in the 21st Century (ToxCast/Tox21) high-throughput screening programmes (Chiu *et al.*, 2018; Guyton *et al.*, 2018) (see Section 4.2.10 of the monograph on MTBE in the present volume, and Annex 4, Supplementary material for Section 4 Mechanistic evidence, online only, available from: <https://publications.iarc.who.int/652>). ETBE (CAS No. 637-92-3, DTXSID0025604) has been evaluated in the US EPA and United States National Institutes of Health high-throughput screening assays.

Results were available for 111 assay end-points mapped to the key characteristics for ETBE. ETBE was considered active in one out of four assay end-points mapped to the key

characteristic “induces oxidative stress” (KC5). The assay in which the end-point was identified as active was TOX21\_ARE\_BLA\_Agonist\_ratio. TOX21\_ARE\_BLA\_Agonist\_ratio is an assay readout measuring reporter gene activity via receptor activity and uses an inducible reporter (beta-lactamase) detected by the GAL4 beta-lactamase reporter gene. It is designed to target transcription factor activity, specifically mapping to *NFE2L2* gene(s) using the positive control beta-naphthoflavone. These assays use HepG2, a human hepatoma cell line, and the chemical is applied in a 1536-well plate. The active call was noted with the flags “Borderline active” and “Less than 50% efficacy”.

## 5. Summary of Data Reported

### 5.1 Exposure characterization

Ethyl *tert*-butyl ether (ETBE) is a volatile ether and a high-production-volume chemical that is manufactured from ethanol and isobutylene. Its predominant use is as an additive in gasoline to increase combustion efficiency and as an additive in renewable fuel to reduce carbon emissions from motor vehicles. The use of ETBE is most prominent in Europe and Japan. ETBE is an air pollutant via traffic emissions and has been detected in groundwater near sources of leaking underground fuel tanks. ETBE also has been detected in drinking-water from water systems in which new plastic pipes have been installed. Occupational exposure is expected to occur mainly during production and use in gasoline production as well as through gasoline vapour. Workers in certain occupations, such as service station attendants, tanker drivers, and workers in petroleum production, may be exposed to ETBE through inhalation, skin contact, or accidental ingestion. The general population may be exposed via air pollution. Several countries in

Europe and Canada have occupational exposure limits for ETBE.

## 5.2 Cancer in humans

No data were available to the Working Group.

## 5.3 Cancer in experimental animals

Treatment with ETBE caused an increase in the incidence of benign and of malignant neoplasms in both sexes of a single species (rat) in two studies that complied with Good Laboratory Practice (GLP).

In a well-conducted study that complied with GLP in male and female F344/DuCrIj rats exposed by inhalation (whole-body), ETBE caused hepatocellular adenoma in males. A hepatocellular carcinoma was found in one male of the 50 males exposed.

In a well-conducted study that complied with GLP in male and female Sprague-Dawley rats treated by oral administration (gavage), there was a significant increase in the incidence of malignant schwannoma of the uterus.

## 5.4 Mechanistic evidence

Regarding the absorption, distribution, metabolism, and excretion of ETBE, data were available from studies in humans and from experimental systems.

In exposed humans, ETBE is absorbed rapidly after inhalation and shows a consistent kinetic pattern with respect to inhalation uptake. ETBE is exhaled or metabolized to *tert*-butyl alcohol (TBA), which is conjugated or further oxidized to various diols. ETBE is rapidly cleared from the blood. Metabolites of ETBE are found in the urine.

In rodents, ETBE can be absorbed via inhalation or ingestion. ETBE and its metabolites are distributed through the body. Metabolic patterns

and rates for ETBE are similar in humans and rats. ETBE is first oxidized, generating acetaldehyde and an unstable hemiacetal, which decomposes to TBA. TBA can be further conjugated or oxidized to diols, which are subsequently conjugated or, in the case of 2-methyl-1,2-butanediol, oxidized to the corresponding acid.

In vitro studies using microsomal preparations from rat olfactory mucosa indicate that CYP2A6 enzyme, and not CYP2E1, is the major enzyme involved in the deacetylation of ETBE. The involvement of CYP2A6 was observed also in human liver samples.

There is consistent and coherent evidence that ETBE exhibits the key characteristics of carcinogens. ETBE induces cell proliferation, cell death, or nutrient supply. No data were available in exposed humans or in human primary cells or tissues. ETBE induces cell proliferation in the liver of mice exposed via inhalation, and in rats via gavage. Urothelial hyperplasia was reported in two long-term studies in rats exposed to ETBE via inhalation or drinking-water. In addition, atypical tubule hyperplasia of the kidney or extensive papillary hyperplasia of the urinary bladder were observed in male rats exposed via oral administration in two initiation–promotion studies of carcinogenicity.

For the other key characteristics of carcinogens, there was a paucity of data.

ETBE was essentially without effects in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

# 6. Evaluation and Rationale

## 6.1 Cancer in humans

There is *inadequate* evidence in humans regarding the carcinogenicity of ethyl *tert*-butyl ether.

## 6.2 Cancer in experimental animals

There is *sufficient* evidence in experimental animals for the carcinogenicity of ethyl *tert*-butyl ether.

## 6.3 Mechanistic evidence

There is *strong* mechanistic evidence for the key characteristics of carcinogens in experimental systems.

## 6.4 Overall evaluation

Ethyl *tert*-butyl ether is *possibly carcinogenic to humans* (Group 2B).

## 6.5 Rationale

The Group 2B evaluation for ethyl *tert*-butyl ether (ETBE) is based on *sufficient* evidence for cancer in experimental animals and on *strong* mechanistic evidence in experimental systems. The *sufficient* evidence for cancer in experimental animals is based on an increase in the incidence of benign and malignant neoplasms in both sexes of a single species (rat) in two studies carried out under Good Laboratory Practice. The mechanistic evidence is *strong* because there is consistent and coherent evidence that ETBE alters cell proliferation, cell death, or nutrient supply in experimental systems. The evidence regarding cancer in humans is *inadequate* because no studies were available.

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# TERT-BUTYL ALCOHOL

## 1. Exposure Characterization

### 1.1 Identification of the agent

#### 1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 75-65-0 ([ECHA, 2025a](#))

EC/List No.: 211-309-7 ([ECHA, 2025a](#))

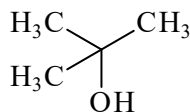
IUPAC systematic name: 2-methylpropan-2-ol ([NCBI, 2025](#))

Synonyms: 2-methyl-2-propanol; trimethyl carbinol; TBA; *tert*-butanol; *t*-butanol; *tertiary*-butanol; 1,1-dimethylethanol, trimethylmethanol, Arconol ([ECHA, 2025a](#); [NCBI, 2025](#)).

#### 1.1.2 Structural and molecular information

Molecular formula: C<sub>4</sub>H<sub>10</sub>O ([ECHA, 2025a](#))

Molecular structure: ([ECHA, 2025a](#))



Relative molecular mass: 74.14 ([OSHA, 2024](#)).

#### 1.1.3 Chemical and physical properties

*Description:* at 20 °C and 1013 hPa, *tert*-butyl alcohol (TBA) is a colourless oily liquid with a sharp alcohol odour ([NCBI, 2025](#); [ECHA, 2025a](#))

*Boiling-point:* 82.41 °C at 101.3 kPa ([ECHA, 2025a](#))

*Melting point:* 25.7 °C at 101.3 kPa ([ECHA, 2025a](#))

*Density:* 0.79 g/mL at 20 °C ([ECHA, 2025a](#))

*Vapour pressure:* 54.13 hPa at 25 °C ([ECHA, 2025a](#))

*Water solubility:* 1000 g/L at 25 °C ([ECHA, 2025a](#))

*Octanol/water partition coefficient (P):* log K<sub>ow</sub> = 0.317 at 22.5 °C ([ECHA, 2025a](#))

*Henry's law constant:* 9.05 × 10<sup>-6</sup> atm·m<sup>3</sup>/mol at 25 °C ([NCBI, 2025](#))

*Autoignition temperature:* 470 °C ([ECHA, 2025a](#))

*Flash point:* [11.1 °C] ([OSHA, 2024](#)); 15–23 °C ([ECHA, 2025a](#))

*Explosive limits:* 2.4–8% by volume in air ([NCBI, 2025](#))

*Relative vapour density:* (air = 1.0): 2.55 ([OSHA, 2024](#)).

### 1.1.4 Technical products and impurities

Commercial products have a purity of more than 99% or more than 99.8 wt% ([NCBI, 2025](#); [TCI, 2025](#)). Possible impurities or low level contaminants include ethanol, isopropanol, and *n*-propanol ([Pharmco, 2009](#)).

## 1.2 Production and uses

### 1.2.1 Production

The precursor chemicals used to manufacture TBA include 1-butene, isobutylene, acetyl chloride, and dimethyl zinc, and *tert*-butyl hydroperoxide ([US EPA, 2021a](#)). TBA is a co-product of the synthesis of propylene oxide and can be dehydrated to produce isobutene ([Di Girolamo et al., 2021](#)). In one conventional process for the production of propylene oxide, the first step involves noncatalytic liquid-phase oxidation of isobutane to form *tert*-butyl hydroperoxide. TBA is produced as a major co-product in this step of the process ([Ghanta et al., 2013](#)).

The Organisation for Economic Co-operation and Development has classified TBA as a high-production-volume chemical ([OECD, 2024](#)). Manufacturers of TBA are found in many countries throughout the world, including the United States of America (USA), China, Japan, Europe (including Germany and the Kingdom of the Netherlands), and the Republic of Korea. The market size for TBA in 2022 was estimated to be 1850 000 tonnes ([ChemAnalyst, 2023](#)). In Europe, six companies held active registrations to import or manufacture  $\geq 1$  tonne of TBA in 2025 ([ECHA, 2025a](#)). In the USA, production of TBA in 2019 was reported to be in the range of [453 592 to 2 267 962 tonnes] ([NCBI, 2024](#)). Data on total global production volumes were not available from public sources.

### 1.2.2 Uses

#### (a) Chemical feedstock

TBA is used to produce isobutylene, a feedstock for synthetic rubber, and the gasoline additives methyl *tert*-butyl ether (MTBE) and methyl *tert*-butyl ether (ETBE) ([McGregor, 2010](#)).

#### (b) Gasoline

Although TBA has been used as a gasoline oxygenate and octane booster in the past, larger amounts have been used for the manufacture of MTBE and ETBE ([US EPA, 2021a](#)). TBA has also been used in bioethanol as a stabilizer ([Concawe, 2012](#)). [The Working Group noted that few data were publicly available on the composition and production volumes of gasoline and its additives. Because independently verifiable data were lacking, data published by Concawe, the science division of a trade organization, are presented.] When not directly added to gasoline, TBA may be present as an impurity or degradation product of the additives MTBE or ETBE ([Concawe, 2012](#); [Toumari and Suffet, 2021](#)).

In Europe, a survey by the fuel industry noted that trace amounts of TBA had been measured in gasoline samples collected from multiple countries between 1999 and 2009. Among 1239 gasoline samples, the mean TBA concentration was 0.03 m/m% (wt%) and the median concentration was 0.01 wt%. The maximum concentration detected was 1.03 wt% in super premium gasoline from Switzerland in winter 2000/2001 ([Concawe, 2012](#)). In more recent data from the fuel industry covering 2010–2015 and a much smaller number of gasoline samples, the average TBA concentrations ranged from 0.02 to 0.04 wt% ([Concawe, 2018](#)).

In the USA, concentrations of TBA and other oxygenates were measured in gasoline samples collected in states where the use of MTBE had been banned (from 2004 to 2020), as well as in samples collected before the bans (from 1993–2002 or 2004). In samples collected

before the bans, TBA was detected in three out of eight samples of oxyfuel or reformulated gasoline, at concentrations ranging from 7350 to 11 800 mg/kg, and in 15 out of 51 samples of conventional gasoline, at concentrations ranging from 723 to 7480 mg/kg. In samples collected after the ban, TBA was detected in three out of 76 gasoline samples, at concentrations ranging from 614 to 870 mg/kg. The authors attributed the pre-ban concentrations of TBA in gasoline to direct blending of TBA as an oxygenate rather than to its presence as an impurity of MTBE ([Stout and Healey, 2024](#)).

#### (c) Other uses

TBA is used in a variety of industrial processes as a solvent and dehydrating agent and in the manufacture of certain plastics, industrial cleaning products, and pharmaceuticals. It is also used in consumer products such as coatings on paperboard food containers, food flavouring, cosmetics, and perfume ([Chen, 2005](#); [US EPA, 2021a](#); [Api et al., 2023](#)). In 2022, flavour and fragrances represented an estimated 42% of the global market for TBA ([ChemAnalyst, 2023](#)). According to a leading manufacturer, TBA is primarily used as an intermediate for the manufacture of products such as resins, pharmaceuticals, and perfumes. Because of its unique chemical properties, TBA is also used as a solvent and stabilizer in water-based paints, to remove wax from lubricating oils, and to make ethanol undrinkable ([LyondellBasell, 2015](#)).

### 1.3 Analytical methods

For analytical methods for the detection and quantification of TBA, please see Section 1.3 of the monograph on MTBE, in the present volume.

## 1.4 Occurrence and exposure

### 1.4.1 Environmental occurrence

In addition to being a high-production-volume chemical and a degradation product of MTBE and ETBE, TBA can be produced naturally from the aerobic or anaerobic microbial metabolism of isobutane ([Quast et al., 2016](#)). [The Working Group noted a lack of data on the proportion of TBA in the environment that originates from the metabolism or degradation of MTBE and ETBE.]

Of the 524 toxic chemicals reported to the United States Environmental Protection Agency (US EPA) Toxic Release Inventory in 2022, TBA ranked number 80 in total releases (rank 1 being the highest). In the USA, 98 facilities reported releasing a total of [287.8 tonnes] of TBA on-site, including in air ([148.4 tonnes]), water ([4.6 tonnes]), or land ([134.8 tonnes]). An additional [273.6 tonnes] were disposed of off-site. In this database, off-site disposal includes the transfer of waste containing the chemical for disposal or other release. Of the total released, the majority came from the chemical industry sector ([73.7%]), followed by the petroleum industry sector (21.0%) ([US EPA, 2022](#)). In Canada, 2.3 tonnes of released TBA were transferred for recycling, and 1.2 tonnes were transferred for treatment in 2018 ([ECCC, 2023](#)). Comparable data were not available on environmental releases of TBA from other countries.

#### (a) Air

TBA is a vapour that degrades in the atmosphere in reaction with photochemically produced hydroxyl radicals; its half-life is 14 days ([US EPA, 2021a](#)). Empirical data on ambient air measurements of TBA were limited. In three studies on indoor air monitoring of TBA in Canada, the highest concentration was found in a study from the national 2009–2011 Canadian Health Measures Survey. In passive air samples

collected from 3857 homes of survey participants, TBA was detected in nearly 90% of samples. The arithmetic mean was  $0.66 \mu\text{g}/\text{m}^3$  (95% CI,  $0.45\text{--}0.86 \mu\text{g}/\text{m}^3$ ), the geometric mean was  $0.20 \mu\text{g}/\text{m}^3$  (95% CI,  $0.16\text{--}0.26 \mu\text{g}/\text{m}^3$ ), and the 99th percentile was  $9.40 \mu\text{g}/\text{m}^3$  ([Zhu et al., 2013](#)). [The Working Group noted that no information on the source of TBA was available in the publication.]

#### (b) Water

In groundwater samples collected between 2000 and 2001 at sites with leaking underground fuel tanks near community drinking-water sources in the Los Angeles region, California, USA, TBA was detected at 61.1% of sites tested (530 sites). The minimum limit of detection (LOD) was  $10 \mu\text{g}/\text{L}$ , and the geometric mean was  $1730 \mu\text{g}/\text{L}$  (range,  $6 \mu\text{g}/\text{L}$  to  $4.4 \times 10^6 \mu\text{g}/\text{L}$ ). On the basis of measures of groundwater levels and plume length, the authors concluded that TBA contamination of groundwater was on a scale comparable to that of MTBE ([Shih et al., 2004](#)). In an assessment of 263 closed, leaking underground storage tank sites in southern California from 2016 to 2019, a median TBA concentration of  $1200 \mu\text{g}/\text{L}$  was measured in groundwater, and there was an increase in maximum TBA plume lengths over time ([Toumari and Suffet, 2023](#)).

In an area of the USA spanning parts of Colorado and New Mexico where there were significant coal resources, TBA levels were measured in samples collected between 2010 and 2014 from 44 domestic water and groundwater monitoring wells and four surface water sites. At the water wells, the maximum TBA concentration was  $395 \mu\text{g}/\text{L}$ , and the average was  $75.1 \mu\text{g}/\text{L}$ . None of the surface water sites contained TBA at concentrations above the LOD of  $5 \mu\text{g}/\text{L}$ . The authors concluded that TBA contamination in the area probably resulted from a combination of widespread commercial use and natural processes rather than from a single point source ([Quast et al., 2016](#)).

In Düsseldorf, Germany, 96 samples were collected from 14 groundwater wells contaminated by a gasoline spill between 2001 and 2003. TBA was one of 12 target chemicals monitored. Out of 96 samples, 93 had TBA concentrations above the LOD ( $0.11 \mu\text{g}/\text{L}$ ). TBA concentrations ranged from  $0.18 \mu\text{g}/\text{L}$  to  $443 \mu\text{g}/\text{L}$ ; the average was  $35.1 \mu\text{g}/\text{L}$ , and the median was  $3.38 \mu\text{g}/\text{L}$ . The authors found similarities between TBA and MTBE measurements and concluded that TBA may be as persistent as MTBE in the aquifer ([Rosell et al., 2005](#)).

TBA has also been detected in drinking-water from sources unrelated to the contamination of underground water. In Finland, TBA was detected in drinking-water after the installation of plastic pipes made with cross-linked polyethylene. TBA levels were as high as  $140 \mu\text{g}/\text{L}$  in water that had remained stagnant for 24 hours ([Pelto-Huikko et al., 2021](#)). A wildfire in Santa Rosa, California, USA, resulted in widespread chemical contamination, and nearly 2 years after the fire, TBA was detected in drinking-water at concentrations as high as  $29 \mu\text{g}/\text{L}$  ([Proctor et al., 2020](#)).

#### (c) Soil

TBA is expected to move easily with water in the soil and potentially lead to groundwater contamination. Biodegradation of TBA in soil occurs more slowly than for ethanol or isopropanol; the half-life of TBA in anoxic soils is estimated to be about 200 days ([US EPA, 2021a](#)). Few empirical measurement data were available regarding TBA levels in soil. In the Republic of Korea, among 56 soil samples taken from various regions near underground fuel storage tanks the median and mean TBA concentrations were  $40.8$  and  $89.6 \mu\text{g}/\text{kg}$ , respectively (range,  $0.6\text{--}2237.7 \mu\text{g}/\text{kg}$ ) ([Shin, 2012](#)).

*(d) Food*

TBA is reported to naturally occur in food ([Api et al., 2023](#)). It can also be present in food as a result of its use as an incidental additive during commercial food production ([ECCC, 2023](#)). It can also come into contact with food via its use on surfaces of food utensils and in coatings of food containers ([Chen, 2005](#); [US EPA, 2021a](#)). No empirical measurement data were available regarding TBA levels in food.

*(e) Consumer products*

In 1998, manufacturers in the USA voluntarily reported the use of TBA in 32 different types of cosmetic product, such as makeup, colognes, hair sprays, and skin lotions, to the United States Food and Drug Administration (US FDA). At that time, TBA concentrations ranged from 0.00001% to 0.5% ([Chen, 2005](#)). According to the US FDA Voluntary Cosmetic Registration Program survey data for 2023, TBA was reported to be used in 136 cosmetic product formulations, 115 of which were in leave-on formulations. The highest reported maximum concentration of use for TBA was 0.91% in aftershave lotions ([Expert Panel for Cosmetic Ingredient Safety, 2024](#)).

The TBA content of gasoline is described in Section 1.2. As previously noted, TBA may be present in gasoline as an impurity or as a degradation product of the additives MTBE or ETBE, and as a stabilizer for bioethanol ([Concawe, 2012](#); [Toumari and Suffet, 2021](#)).

*1.4.2 Occupational exposure*

No studies on direct measurement of TBA exposure among workers were available. In several studies, TBA has been measured in workers as a metabolite of MTBE to assess MTBE exposure (see Section 1.4.2 of the monograph on MTBE in the present volume). [The Working Group noted that TBA has a boiling-point between those of ETBE and *tert*-amyl methyl ether (TAME). Occupational exposure

to TBA can therefore occur through inhalation, skin contact, or ingestion, particularly in industries in which TBA is produced or used. Exposure scenarios identified for gasoline – such as manufacturing, blending, distribution, and storage, and refuelling activities – will also be relevant for TBA, if gasoline contains this additive.] Workers can be assumed to be exposed to TBA in fuel production and petroleum refineries or in chemical plants during TBA production, blending, or transport ([Mahler, 1997](#)).

[TBA vapours can escape during blending, tank filling, or leaks. Service station attendants or maintenance workers may encounter TBA vapour during fuel dispensing or servicing of equipment. Exposure would depend on the use of vapour recovery systems (stage II), proper training, and availability and use of personal protective equipment. Workers involved in cleaning up TBA spills or leaks from storage tanks, pipelines, or contaminated groundwater are also likely to be exposed via inhalation of TBA vapour emissions during soil excavation or water treatment. Laboratory technicians analysing TBA concentrations in fuel or environmental samples could potentially be exposed to TBA vapour from open containers or during sample preparation.]

*1.4.3 Exposure of the general population*

Data on the environmental occurrence of TBA are described in Section 1.4.1. No empirical measurement data were available regarding exposures of the general population to TBA. [The Working Group noted that, on the basis of the presence of TBA in air, food, drinking-water, and cosmetics, exposure to TBA by inhalation, orally or dermally would be expected in some circumstances.]



**Table 1.1 Occupational exposure limits for *tert*-butyl alcohol**

| Country or region | 8-hour TWA<br>(ppm; mg/m <sup>3</sup> ) | STEL<br>(ppm; mg/m <sup>3</sup> ) |
|-------------------|-----------------------------------------|-----------------------------------|
| Australia         | 100; 303                                | 150; 455                          |
| Austria           | 20; 62                                  | 80; 248                           |
| Belgium           | 100; 307                                |                                   |
| Canada (Ontario)  | 100 ppm                                 |                                   |
| Finland           | 50; 150                                 | 75; 230                           |
| Japan             | 50; 150                                 |                                   |
| New Zealand       | 20; 62                                  | 80; 248                           |
| Norway            | 25;75                                   |                                   |
| South Africa      | 200 ppm                                 |                                   |
| Switzerland       | 20; 60                                  | 80; 240                           |
| United Kingdom    | 100; 308                                | 150; 462                          |
| USA (NIOSH)       | 100; 300                                | 150; 450                          |
| USA (OSHA)        | 100; 300                                |                                   |

NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million; STEL, short-term exposure limit; TWA, time-weighted average; USA, United States of America.

Adapted from [IFA \(2025\)](#), [Norwegian Labour Inspection Authority \(2025\)](#).

## 1.5 Exposure limits, regulations, and guidelines

### 1.5.1 Exposure limits and guidelines

Several countries have established occupational exposure limit values (see [Table 1.1](#)). The permissible exposure limit of the United States Occupational Safety and Health Administration (OSHA) and the recommended occupational exposure limit for TBA from the National Institute for Occupational Safety and Health (NIOSH) are both 100 ppm (300 mg/m<sup>3</sup>) for a time-weighted average (TWA), and NIOSH additionally recommends a short-term exposure limit (STEL) of 150 ppm (450 mg/m<sup>3</sup>) ([OSHA, 2024](#)).

Several countries have the same exposure limit value of 100 ppm (303 mg/m<sup>3</sup>) and these include Belgium, Canada (Ontario and Québec provinces), France, Ireland, and Spain. Other countries have TWA and STEL limit values that match the NIOSH recommended limit values for both, and these include Australia; New Zealand; Poland; South Africa (mining), the Republic of Korea, and the United Kingdom ([IFA, 2024](#)).

Denmark, Finland, Japan, and Sweden have TWA limit values of 50 ppm (150 mg/m<sup>3</sup>). Austria, Germany, and Switzerland have TWA limit values of 20 ppm (62 mg/m<sup>3</sup>) and STEL values of 80 ppm (248 mg/m<sup>3</sup>). South Africa has a TWA limit of 200 ppm [620 mg/m<sup>3</sup>] for non-mining activities ([IFA, 2024](#)). In 1995, the American Conference of Governmental Industrial Hygienists (ACGIH) adopted its current recommended 8-hour TWA occupational exposure limit for TBA of 100 ppm (303 mg/m<sup>3</sup>), to minimize the risk of narcosis. Data were found insufficient to recommend a STEL ([ACGIH, 2025](#)).

In 2021, the US EPA issued an inhalation reference concentration of 5 mg/m<sup>3</sup> and an oral reference dose of 0.4 mg/kg day for chronic exposure to TBA, based on health outcomes other than cancer ([US EPA, 2021a](#)). The US FDA recommended a permitted daily exposure of 35 mg/day for TBA as an impurity in pharmaceuticals ([US FDA, 2021](#)). US FDA regulations permit TBA to be used in defoaming agents and surface lubricants that come into contact with food ([US FDA, 2025](#)).



[The Working Group noted that no reference values for biological monitoring were available.]

### 1.5.2 Regulations for use in gasoline

The European Union allows TBA at a maximum of 15% v/v in gasoline ([European Commission, 2009](#)).

## 2. Cancer in Humans

No data were available to the Working Group.

## 3. Cancer in Experimental Animals

See [Table 3.1](#).

### 3.1 Mouse

#### 3.1.1 Oral administration (drinking-water)

In a well-conducted study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 60 male and 60 female B6C3F<sub>1</sub> mice (age, 7 weeks) were exposed to *tert*-butyl alcohol (TBA) (purity, >99%) by drinking-water ad libitum at concentrations of 0, 5, 10, or 20 mg/mL in deionized water for up to 103 weeks. Based on water consumption, the average dose of TBA during the study was approximately 540, 1040, and 2070 mg/kg per day in males and approximately 510, 1020, and 2110 mg/kg per day in females in the respective dose groups ([NTP, 1995](#); also reported by [Cirvello et al., 1995](#)). At study termination, survival in the group of males at 20 mg/mL was significantly lower than that in the control group of males, with reduced survival beginning early in the study. As a result, a planned 15-month interim assessment of 10 males and 10 females per group was not conducted, and all 60 males and 60 females per group were retained as part

of the study cohort. Survival in other groups of male mice and all groups of female mice was similar to that of the controls. At study termination, survival was 27/60, 36/60, 34/60, and 17/60 in males and 36/60, 35/60, 41/60, and 42/60 in females in the groups at 0 (control), 5, 10, and 20 mg/mL, respectively. Body weights in male mice exposed to TBA were within 10% of those of controls throughout the study. Female mice in the group at 20 mg/mL had lower body weights (<90% of controls) beginning in week 13 and continuing throughout the remainder of the study. After euthanasia, a complete necropsy was performed, and all tissues were examined microscopically.

In males, there was a nonsignificant increase in the incidence of follicular cell adenoma of the thyroid gland in the group at 10 mg/mL – incidence being 1/60 (2%), 0/59, 4/59 (7%), and 1/57 (2%) in the groups at 0 (control), 5, 10, and 20 mg/mL, respectively. There was a slight but nonsignificant increase in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid gland – incidence being 1/60 (2%), 0/59, 4/59 (7%), and 2/57 (4%) in the groups at 0 (control), 5, 10, and 20 mg/mL, respectively. The incidence of follicular cell adenoma, and follicular cell adenoma or carcinoma (combined) of the thyroid gland in the groups at 10 mg/mL and 20 mg/mL exceeded the upper bound of the range observed in historical controls from this laboratory – 4/240 (1.7% ± 0.5%); range, 1–2%. [The Working Group noted that the higher incidence of follicular cell adenoma of the thyroid gland was not dose-related and not statistically significant, but it was above the upper bound observed in the historical controls for the group at the intermediate and highest dose for the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid gland.] The incidence of haemangioma (all organs) – 0/60 (0%), 6/60 (10%), 1/60 (2%), and 1/60 (2%) in the groups at 0 (control), 5, 10, and 20 mg/mL, respectively – was significantly increased in the 5 mg/mL

**Table 3.1 Studies of carcinogenicity in experimental animals exposed to *tert*-butyl alcohol**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                                                                                     | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                                                        | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                | Significance                                                                                                                                                                                                                                                                   | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Mouse, B6C3F <sub>1</sub> (M)<br>7 wk<br>103 wk<br><a href="#">NTP (1995)</a> ,<br>also reported by<br><a href="#">Cirvello et al. (1995)</a> | Oral administration<br>(drinking-water)<br>TBA, > 99%<br>Deionized water<br>0, 5, 10, 20 mg/mL<br>(approximately 0, 540, 1040, 2070 mg/kg bw) <i>ad libitum</i><br>for 103 wk<br>60, 60, 60, 60<br>27, 36, 34, 17 | <i>Thyroid gland</i><br>Follicular cell adenoma<br>1/60 (2%), 0/59, 4/59 (7%),<br>1/57 (2%)<br>Follicular cell adenoma or carcinoma (combined)<br>1/60 (2%), 0/59 (0%),<br>4/59 (7%), 2/57 (4%)<br><i>All organs</i><br>Haemangioma<br>0/60, 6/60, 1/60 (2%),<br>1/60 (2%)<br><br>Haemangiosarcoma<br>4/60 (7%), 7/60 (12%),<br>3/60 (5%), 2/60 (3%)<br>Haemangioma or haemangiosarcoma (combined)<br>4/60 (7%), 13/60 (22%)*,<br>4/60 (7%), 3/60 (5%) | NS<br><br>NS<br><br>* <i>P</i> = 0.014, Fisher exact test; <i>P</i> = 0.040, life-table test; <i>P</i> = 0.028, logistic regression test<br><br>NS<br><br>* <i>P</i> = 0.062, life-table test; <i>P</i> = 0.027, logistic regression test; <i>P</i> = 0.017, Fisher exact test | <i>Principal strengths:</i> Well-conducted study that appeared to have been conducted by GLP standards, the durations of exposure and observation were adequate, there were high numbers per group, male and female animals were used, and the quality of the descriptions of gross and microscopic examinations was high.<br><br><i>Other comments:</i> Reduced survival in males at the highest dose.<br><br><i>Historical controls:</i> Follicular cell adenoma of the thyroid gland, 4/240 (1.7%); range, 1–2%. Follicular cell adenoma or carcinoma (combined) of the thyroid gland, 4/240 (1.7%); range, 1–2%. |
| Full carcinogenicity<br>Mouse, B6C3F <sub>1</sub> (F)<br>7 wk<br>103 wk<br><a href="#">NTP (1995)</a>                                                                 | Oral administration<br>(drinking-water)<br>TBA, > 99%<br>Deionized water<br>0, 5, 10, 20 mg/mL<br>(approximately 0, 540, 1020, 2110 mg/kg bw) <i>ad libitum</i><br>for 103 wk<br>60, 60, 60, 60<br>36, 35, 41, 42 | <i>Thyroid gland</i><br>Follicular cell adenoma<br>2/58 (3%), 3/60 (5%),<br>2/59 (3%), 9/59 (15%)*                                                                                                                                                                                                                                                                                                                                                     | <i>P</i> = 0.007, Cochran–Armitage trend test; <i>P</i> = 0.011, logistic regression trend test; <i>P</i> = 0.016, life-table trend test<br><br>* <i>P</i> = 0.039, Fisher exact test                                                                                          | <i>Principal strengths:</i> Well-conducted study that appeared to have been conducted by GLP standards, the durations of exposure and observation were adequate, there were high numbers per group, male and female animals were used, and the quality of the descriptions of gross and microscopic examinations was high.<br><br><i>Historical controls:</i> Follicular cell adenoma of the thyroid gland, 8/238 (3.4%); range, 0–5%.                                                                                                                                                                               |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference          | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                        | Results                                                                                                                                                                                                                                                                                                                                                                                                                         | Significance                                                                                                                                                                                                                                                             | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, F344/N (M)<br>7 wk<br>103 wk<br><a href="#">NTP (1995)</a> | Oral administration<br>(drinking-water)<br>TBA, > 99%<br>Deionized water<br>0, 1.25, 2.5, 5 mg/mL<br>(approximately 0, 90, 200,<br>420 mg/kg bw)<br>50, 50, 50, 50<br>10, 6, 4, 1 | <i>Kidney</i><br>Renal tubule adenoma (single section):<br>1/50 (2%), 3/50 (6%),<br>4/50 (8%), 3/50 (6%)<br>Renal tubule carcinoma (single section) :<br>0/50 (0%), 0/50 (0%),<br>0/50 (0%), 1/50 (2%)<br>Renal tubule adenoma or carcinoma (single section):<br>1/50 (2%), 3/50 (6%),<br>4/50 (8%), 3/50 (6%)<br>Renal tubule adenoma (single and step section):<br>8/50 (16%), 11/50 (22%),<br>19/50 (38%)*,<br>13/50 (26%)** | NS<br>NS<br>NS<br>NS<br>$P < 0.001$ , life-table trend<br>test; $P = 0.011$ , logistic<br>regression trend test<br>* $P = 0.002$ , logistic<br>regression test; $P < 0.001$ ,<br>life-table test; $P = 0.012$ ,<br>Fisher exact test<br>** $P = 0.006$ , life-table test | <i>Principal strengths:</i> Well-conducted study that appeared to have been conducted by GLP standards, the durations of exposure and observation were adequate, there were high numbers per group, male and female animals were used, the quality of the descriptions of gross and microscopic examinations was high.<br><i>Historical controls:</i> Renal tubule adenoma or carcinoma (combined), 1/277 (0.4%); range, 0–2%. Follicular cell adenoma of the thyroid gland, 1/277 (0.4%); range, 0–2%. Follicular cell carcinoma of the thyroid gland, 0/277 (0.0%). Follicular cell adenoma or carcinoma (combined) of the thyroid gland, 1/277 (0.4%); range, 0–2%. Historical control data were not available for single and extended evaluations (combined).<br><i>Other comments:</i> A histological reanalysis by <a href="#">Hard et al. (2011)</a> showed additional significant differences between TBA-exposed and control male rats: 4/50, 13/50*, 18/50**, and 12/50* for the groups at 0 (control), 1.25, 2.5, and 5 mg/mL, respectively). Significant increases in all TBA-exposed groups (* $P < 0.05$ , chi-squared test, and ** $P < 0.01$ , chi-squared test) in the incidence of renal tubule adenoma or carcinoma (combined) according to the extended evaluation slide set. |

**Table 3.1 (continued)**

| Study design<br>Species, strain<br>(sex)<br>Age at start<br>Duration<br>Reference                     | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                                                         | Results                                                                                                                      | Significance                                                                                                                                                                                                                      | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full<br>carcinogenicity<br>Rat, F344/N (M)<br>7 wk<br>103 wk<br><a href="#">NTP (1995)</a><br>(cont.) |                                                                                                                                                                                    | Renal tubule adenoma or carcinoma (single and step section):<br>8/50 (16%), 13/50 (26%)**,<br>19/50 (38%)*,<br>13/50 (26%)** | $P < 0.001$ , life-table trend test; $P = 0.018$ , logistic regression trend test<br>* $P = 0.002$ , logistic regression test; $P < 0.001$ , life-table test; $P = 0.012$ , Fisher exact test<br>** $P = 0.006$ , life-table test |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Full<br>carcinogenicity<br>Rat, F344/N (F)<br>7 wk<br>103 wk<br><a href="#">NTP (1995)</a>            | Oral administration (drinking-water)<br>TBA, > 99%<br>Deionized water<br>0, 2.5, 5, and 10 mg/mL (approximately 0, 180, 330, and 650 mg/kg bw)<br>50, 50, 50, 50<br>28, 24, 22, 12 | Any tumour type: No significant increase in the incidence of tumours                                                         |                                                                                                                                                                                                                                   | <i>Principal strengths:</i> Well-conducted study that appeared to have been conducted by GLP standards, the durations of exposure and observation were adequate, there were high numbers per group, male and female animals were used, and the quality of the descriptions of gross and microscopic examinations was high.<br><br><i>Principal limitations:</i> Low survival in all groups of male rats at the end of the study, which may have had an impact on the dose–response analysis of neoplasms. |

bw, body weight; F, female(s); F344, Fischer 344; GLP, Good Laboratory Practice; M, male(s); NS, not significant; TBA, *tert*-butyl alcohol; wk, week(s).

( $P=0.040$ , life-table test;  $P=0.028$ , logistic regression test;  $P=0.014$ , Fisher exact test) compared with that in the control group. The incidence of haemangioma or haemangiosarcoma (combined) of all organs – 4/60 (7%), 13/60 (22%), 4/60 (7%), and 3/60 (5%) in the groups at 0 (control), 5, 10, and 20 mg/mL, respectively – was statistically increased at 5 mg/mL ( $P=0.062$ , life-table test;  $P=0.027$ , logistic regression test;  $P=0.017$ , Fisher exact test) compared with that in the control group. There was no statistically significant increase in the incidence of haemangiosarcoma in all organs. [The Working Group noted that the significant increase in the incidence of haemangioma of all organs, and of haemangioma or haemangiosarcoma (combined) of all organs, was not dose-related and was observed only in the group at the lowest dose.]

In females, there was a significant positive trend ( $P=0.011$ , logistic regression trend test;  $P=0.016$ , life-table trend test;  $P=0.007$ , Cochran–Armitage trend test) in the incidence of follicular cell adenoma of the thyroid gland – 2/58 (3%), 3/60 (5%), 2/59 (3%), and 9/59 (15%) for the groups at 0 (control), 5, 10, and 20 mg/mL, respectively – with the incidence being significantly increased ( $P=0.039$ , logistic regression test;  $P=0.028$ , Fisher exact test) in the group at 20 mg/mL compared with the control group. The incidence of follicular cell adenoma at 20 mg/mL exceeded the upper bound of the range observed in historical controls from this laboratory – 8/238 (3.4%); range, 0–5%.

Regarding non-neoplastic lesions, there was a significant increase in the incidence of follicular cell hyperplasia of the thyroid gland in all groups of male and female mice exposed to TBA. [The Working Group noted that only the incidence of follicular cell adenoma of the thyroid gland (a benign tumour) was significantly elevated in females. The Working Group considered that the statistically significant increase in thyroid follicular cell adenoma confined to females at the highest dose was modest evidence of

carcinogenicity.] There were also increases in the incidence of chronic inflammation and urothelial hyperplasia [namely transitional epithelial hyperplasia] of the urinary bladder in male mice at 20 mg/mL. To a lesser extent, these lesions of the urinary bladder were also seen in female mice, with chronic inflammation of the urinary bladder reaching statistical significance at 20 mg/mL.

[The Working Group noted that this was a well-conducted study that complied with GLP, males and females and multiple doses were used, the durations of exposure and observation were adequate, there was an adequate number of animals per group, and the descriptions of gross and microscopic examinations were of high quality.]

## 3.2 Rat

### 3.2.1 Oral administration (drinking-water)

In a well-conducted study of chronic toxicity and carcinogenicity that complied with GLP, groups of 60 male and 60 female Fischer 344 (F344/N) rats (age, 7 weeks) were exposed ad libitum to drinking-water (deionized water) containing TBA (purity, >99%) at concentrations of 0, 1.25, 2.5, or 5 mg/mL for males or 0, 2.5, 5, or 10 mg/mL for females. An interim assessment was conducted at 15 months in 10 males and females per group. The remaining 50 rats per sex per group were provided with drinking-water containing TBA for up to 103 weeks. Based on water consumption, the average dose of TBA during the study was approximately 90, 200, and 420 mg/kg per day in males and approximately 180, 330, and 650 mg/kg per day in females in the respective dose groups ([NTP, 1995](#); also reported by [Cirvello et al., 1995](#)). At study termination, survival was 10/50, 6/50, 4/50, and 1/50 for males in the groups at 0 (control), 1.25, 2.5, and 5 mg/mL, respectively, and 28/50, 24/50, 22/50, and 12/50 for females in the groups

at 0 (control), 2.5, 5, and 10 mg/mL, respectively. Survival of rats at the highest concentration was significantly lower than that of the respective controls for males (5 mg/mL) and females (10 mg/mL). For male rats, survival was poor in all groups, with survival in TBA-exposed males following a similar curve as that in control males. Only 10/50 males in the control group survived to the end of the study. The survival curve for females at the highest concentration was similar to that for other groups and controls until week 90 of the study, when survival in the group at 10 mg/mL began to decline more rapidly than that in other groups. In male rats, body weights in the groups at 1.25 and 2.5 mg/mL were similar to those of the controls until approximately week 90 of study, with terminal body weights of 85 and 83% of controls, respectively. Body weights in males at 5 mg/mL were < 90% of those of controls beginning in week 69 of the study, with terminal body weights being 76% of those of the controls. In females, body weights in the groups at 2.5 and 5 mg/mL were within 5% of those of controls throughout the study. The females at 10 mg/mL had body weights that were < 90% of those of controls beginning in week 81, with terminal body weights being 79% of those of controls. After euthanasia, a complete necropsy was performed, and all tissues were examined microscopically.

In males, according to a standard (single section) histological examination, there was a small number of renal tubule adenomas, carcinomas, and adenomas or carcinomas (combined) of the kidney. There was a slight but nonsignificant increase in the incidence of renal tubule adenoma – 1/50 (2%), 3/50 (6%), 4/50 (8%), and 3/50 (6%) – and renal tubule carcinoma – 0/50, 0/50, 0/50, and 1/50 (2%) in the groups at 0 (control), 1.25, 2.5, and 5 mg/mL, respectively. The incidence of renal tubule adenoma or carcinoma (combined) in all TBA-exposed groups – 1/50 (2%), 3/50 (6%), 4/50 (8%), and 3/50 (6%) in the groups at 0 (control), 1.25, 2.5, and 5 mg/mL,

respectively – exceeded the upper bound of the range for historical controls for the combination of adenoma and carcinoma for single section examination (1/277, 0.4%; range, 0–2%), although the incidence in TBA-exposed groups was not statistically significant. [The Working Group considered that these renal tumours are rare.] In addition, one male at 5 mg/mL at the 15-month interim evaluation was diagnosed with renal tubule adenoma.

For all male rats, an extended evaluation of the kidney was conducted using preparation and microscopic examination of additional histological sections. When combining the diagnoses from the standard and extended evaluations of the kidney, there was a significant positive trend ( $P < 0.001$ , life-table trend test;  $P = 0.011$ , logistic regression trend test) in the incidence of renal tubule adenoma – 8/50 (16%), 11/50 (22%), 19/50 (38%), and 13/50 (26%) – and the incidence was significantly increased at 2.5 mg/mL ( $P = 0.002$ , logistic regression test;  $P < 0.001$ , life-table test;  $P = 0.012$ , Fisher exact test) and 5 mg/mL ( $P = 0.006$ , life-table test). There was a significant positive trend ( $P < 0.001$ , life-table trend test;  $P = 0.018$ , logistic regression trend test) in the incidence of renal tubule adenoma or carcinoma (combined) – 8/50 (16%), 13/50 (22%), 19/50 (38%), and 13/50 (26%) for the groups at 0 (control), 1.25, 2.5, and 5 mg/mL, respectively – with the incidence being significantly increased at 2.5 mg/mL ( $P = 0.002$ , logistic regression test;  $P < 0.001$ , life-table test;  $P = 0.012$ , Fisher exact test) and 5 mg/mL ( $P = 0.006$ , life-table test) compared with the control groups. [The Working Group also noted that, although the study showed an increase in the incidence of renal tumours, it remained uncertain whether the low survival in all groups and the high incidence of renal tumours in the control group, which was above that for the historical control data from this laboratory, had compromised the outcomes of the study.]



In females, no significant increase in the incidence of any tumour was observed.

For non-neoplastic lesions, nephropathy of the kidney was diagnosed in nearly all animals in all groups of males and females, and there was a small but statistically significant increase in severity in the high-exposure group (the average severity score was 3.0 in controls versus 3.3 in the group at 5 mg/mL). There were dose-related significant increases in the incidence of mineralization in rats in the group at 5 mg/mL, and of linear mineralization and urothelial hyperplasia [namely transitional epithelial hyperplasia] in the groups at 2.5 and 5 mg/mL. The average severity scores for these lesions were also higher, although not significant, in the group at 5 mg/mL than in controls. During the extended evaluation, the diagnosis of renal tubule hyperplasia, considered rare by the authors, was also revealed to show a significant increase in the group at 5 mg/mL. [The Working Group could not determine whether the tubular hyperplasia observed by the pathology working group was atypical tubular hyperplasia, and indicative of a preneoplastic lesion. The Working Group noted that for neoplastic lesions the highest response was observed in the group at 2.5 mg/mL. It was also noted that reduced survival in males at 5 mg/mL may have had an impact on the development of neoplasms in that group.] At the 15-month interim evaluation, there was a significant increase in mineralization of the kidney in rats at 5 mg/mL. The incidence and severity of nephropathy did not change with TBA exposure after 15 months in male rats.

In a separate 13-week exposure study reported in the same publication ([NTP, 1995](#)), rats were provided with drinking-water containing TBA at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/mL. Male rats in the group at 40 mg/mL were removed from the study at an earlier stage. The incidence of nephropathy was slightly higher in all groups of TBA-exposed males (10/10) that survived to the scheduled termination than in

control males (7/10). There was a statistically significant increase in severity of nephropathy according to the Mann–Whitney U test in all groups of TBA-exposed males that survived to scheduled termination. In the 13-week study in males, there was also an increase in the incidence of mineralization and in the amount of observed hyaline droplet accumulation in all groups of rats exposed to drinking-water containing TBA.

In females, there was an increase in the incidence of inflammatory cell infiltrates in the kidney and of transitional epithelial hyperplasia (i.e. urothelial hyperplasia). There was also an increase in the average severity score for nephropathy of the kidney. At the 15-month interim evaluation, there was no change in the incidence or severity of mineralization or nephropathy of the kidney in female rats. In the separate 13-week study ([NTP, 1995](#)), there were significant increases in the incidence, but not the severity, of nephropathy in female rats. [The Working Group noted that this was a well-conducted study that complied with GLP, both males and females and multiple doses were used, the durations of exposure and observation were adequate, there was an adequate number of animals per group, and the descriptions of gross and microscopic examinations were of high quality. There was low survival in all groups of male rats at the end of the study, which may have had an impact on the dose–response analysis of neoplasms.]

After the publication of the report by the National Toxicology Program (NTP) ([NTP, 1995](#)), an independent pathology working group was convened to further evaluate the male rat kidney with the goal of better understanding mechanistic changes and the potential role of  $\alpha_{2u}$ -globulin in the development of kidney tumours after TBA exposure ([Hard et al., 2011](#)). The pathology working group reviewed all the rat kidney histology slides (single and extended evaluations) for neoplasms from the NTP study. The review resulted in minor changes to results

for the TBA-exposed groups and a reduction in the number of males in the control group diagnosed with renal tubule adenoma or carcinoma (combined). The lower incidence in the control group (4/50 in the review, compared with 8/50 in the publication by the [NTP, 1995](#)), led to additional significant differences between TBA-exposed and control male rats. According to the diagnoses after review ([Hard et al., 2011](#)), in male rats, there were significant pairwise increases in the incidence of renal tubule adenoma or carcinoma (combined), using the extended evaluation slide set, in all TBA-exposed groups ( $P < 0.05$ , chi-squared test, at 1.25 and 5 mg/mL;  $P < 0.01$ , chi-squared test at 2.5 mg/mL).

Regarding non-neoplastic lesions, the pathology working group reviewed male rat kidney slides from the control group and group at the highest dose (5 mg/mL) ([Hard et al., 2011](#)). After review, there were small changes in the severity score for nephropathy, with the average severity decreasing from 2.9 to 2.8 for the control group (0 mg/mL), and from 3.3 to 3.2 for the group at 5 mg/mL. The incidence of transitional epithelium hyperplasia ([NTP, 1995](#)), termed “hyperplasia of the papilla lining” by [Hard et al. \(2011\)](#), was changed from 40 to 41 in the group at 5 mg/mL and was unchanged in males in the control group. The incidence of linear mineralization ([NTP, 1995](#)), termed “linear papillary mineralization” by [Hard et al. \(2011\)](#), was reduced from 46 to 41 in males at 5 mg/mL and was unchanged in males in the control group. The pathology working group also summarized the severity of male rat nephropathy by differentiating animals with and without neoplasms, demonstrating that rats with neoplasms had consistently higher severity grades than did rats in the same dose group that did not have neoplasms. In a re-evaluation of slides from the 13-week study by the [NTP \(1995\)](#), [Hard et al. \(2019\)](#) were able to demonstrate cell proliferation in the renal cortex by performing a quantitative assessment of mitotic figures. This assessment

showed a dose-dependent increase in mitotic figures in males in the groups at 5 and 10 mg/mL compared with the concurrent controls, with no corresponding change in female rats.]

### 3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of TBA has been assessed in one well-conducted study that complied with GLP in male and female B6C3F<sub>1</sub> mice treated by oral administration (drinking-water) ([NTP, 1995](#); also reported by [Cirvello et al., 1995](#)), and in one well-conducted GLP study in male and female F344/N rats treated by oral administration (drinking-water) ([NTP, 1995](#); also reported by [Cirvello et al., 1995](#)) [histopathological re-evaluation of the kidney histology slides (single and extended evaluations) was carried out by [Hard et al. \(2011, 2019\)](#)].

In the well-conducted GLP study in male and female B6C3F<sub>1</sub> mice treated with TBA by oral administration (drinking-water) ([NTP, 1995](#); also reported by [Cirvello et al., 1995](#)), in males, there was a significant increase in the incidence of haemangioma (all organs) and of haemangioma or haemangiosarcoma (combined) of all organs. [The Working Group noted that this significant increase in the incidence of haemangioma of all organs and of haemangioma or haemangiosarcoma (combined) of all organs was observed only in the group at the lowest dose. Therefore, the Working Group concluded that these increases were not treatment-related.] In females, there was a significant positive trend in the incidence of follicular cell adenoma of the thyroid, with the incidence being significantly increased. [The Working Group noted that only the incidence of follicular cell adenoma of the thyroid gland (a benign tumour) was significantly increased in females. The Working Group considered that the statistically significant increase in thyroid follicular cell adenoma that was confined to females

at the high-dose female animals was limited evidence of carcinogenicity.]

In the well-conducted GLP study in male and female F344/N rats treated with TBA by oral administration (drinking-water) (NTP, 1995; also reported by Cirvello et al., 1995), in males, there was a significant positive trend in the incidence of renal tubule adenoma [by standard and extended evaluations] and of renal tubule adenoma or carcinoma (combined) [by standard and extended evaluations], and the incidence was significantly increased. In the histopathological re-analysis, Hard et al. (2011) reported a significant increase in the incidence of renal tubule adenoma or carcinoma (combined) in all groups of males exposed to TBA according to the extended evaluation slide set. [The Working Group considered that renal tumours are rare, as substantiated by the historical control data; however, this was contradicted by the reported incidence of renal tumours in the concurrent controls.]

## 4. Mechanistic Evidence

### 4.1 Absorption, distribution, metabolism, and excretion

[The Working Group noted that TBA is a small hydrophilic molecule. On the basis of its physical and chemical properties, it is expected to be absorbed via ingestion or inhalation more efficiently than through the skin (dermal), and to be rapidly distributed to various tissues throughout the body, as has been reported in a study in a healthy volunteer exposed orally, and in a few studies on oral and inhalation exposure in rodents.] This section focuses on the absorption, distribution, metabolism, and excretion of TBA after direct exposure. The toxicokinetics of TBA as an intermediate metabolite of MTBE and ETBE have been described in Section 4.1 of the

monographs on MTBE and ETBE in the present volume. The potential steps in the metabolism of TBA are described in Fig. 4.1.

#### 4.1.1 Humans

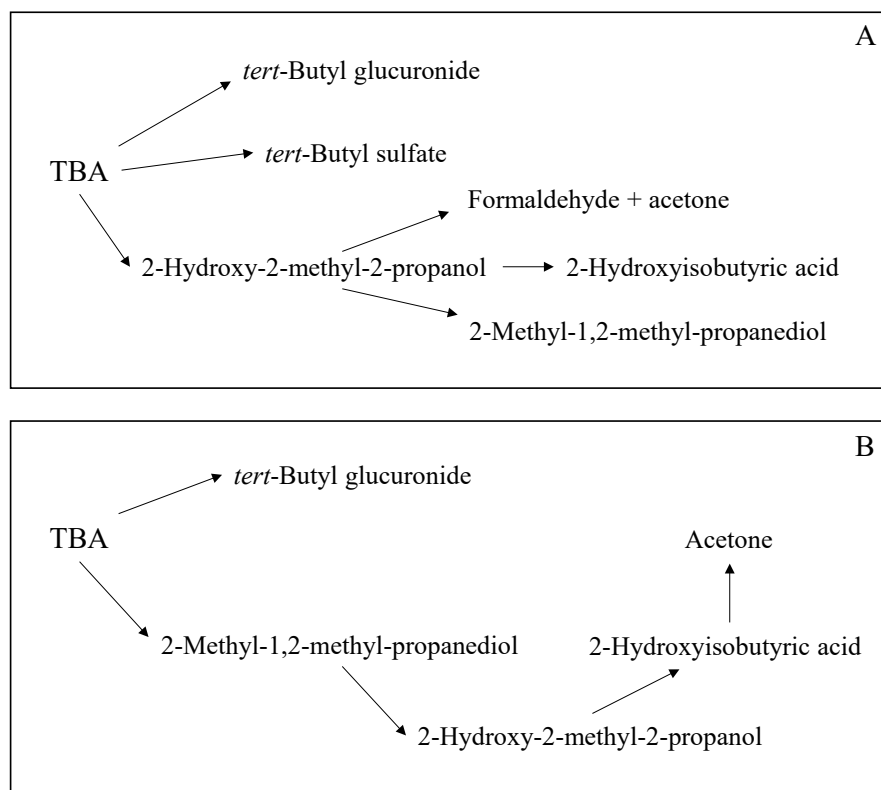
A male volunteer was treated orally with  $^{13}\text{C}$ -labelled TBA at a dose of 5 mg/kg bw in gel capsules. Urine samples were collected for 48 hours, at 12-hour intervals, and analysed by nuclear magnetic resonance (NMR) and by GC-MS. The analysis of urine samples showed the presence of TBA, TBA glucuronide, 2-hydroxyisobutyric acid (2-HIBA; also known as 2-hydroxyisobutyrate and alpha-hydroxyisobutyric acid), and 2-methyl-1,2 propanediol; with 2-HIBA as the main metabolite. Only traces of TBA sulfate were detected. In contrast, TBA sulfate was identified as the major product excreted in urine samples from rats (Bernauer et al., 1998).

#### 4.1.2 Experimental systems

##### (a) Non-human mammalian systems in vivo

Kamil et al. (1953) reported that in Chinchilla rabbits exposed orally to TBA at 4 mmol/kg bw, 24.4% of the administered dose was excreted as the glucuronide form.

In a study to investigate the effects of alcohol withdrawal, McComb and Goldstein (1979) measured TBA levels in air and in blood. Male Swiss-Webster mice were treated with a priming dose of TBA and then exposed continuously in a chamber for up to 9 days. During this time, the concentration of TBA in the chamber was increased gradually from 50  $\mu\text{mol/L}$  to 115  $\mu\text{mol/L}$  of air. Despite the increasing concentration of TBA in the air compartment, the concentration of TBA in the blood remained constant, with some fluctuation between 4 mM and 8 mM. In a second set of experiments, it was observed that TBA administered as an intraperitoneal injection at 8.1 mmol/kg was

**Fig. 4.1 Metabolism of *tert*-butyl alcohol**

TBA, *tert*-butyl alcohol.

A. According to [Dekant et al. \(2001\)](#). B. According to [US EPA \(2021b\)](#).

excreted in 8–9 hours. A similar dose of TBA administered by inhalation over 3 days, resulting in a concentration of 8 mM in the blood, was excreted in approximately 3 hours. [The Working Group noted that prolonged exposures caused an increase of two- to threefold in the metabolism of TBA.]

In rats exposed to a single oral dose of TBA of 25 mmol/kg bw, by gavage, the concentration of TBA in the blood decreased slowly from 13.2 mM at 2 hours, and 12.6 mM at 5 hours, to 11.4 mM at 20 hours ([Beaugé et al., 1981](#)).

In rats exposed to a single intraperitoneal injection of TBA at 1 g/kg bw, headspace GC analysis of blood samples detected a decrease in the concentration of TBA following close to first-order kinetics with a half-life of 9.1 hours.

In another experiment, acetone was detected, by GC-MS analysis, in the urine samples of rats exposed to TBA at serial doses of 0.75, 1.0, 1.5, and 2.0 g/kg bw. The amount of acetone excreted during the 24 hours after exposure to TBA varied between 0.5% and 9.5% of the administered dose, with no clear dose–recovery relation. Additional studies using  $^{14}\text{C}$ -labelled TBA confirmed the presence of  $^{14}\text{C}$ -labelled carbon dioxide in exhaled air and  $^{14}\text{C}$ -labelled acetone in the urine ([Baker et al., 1982](#)).

[Faulkner et al. \(1989\)](#) exposed two groups of six female C57BL/6J mice by oral gavage to TBA at a dose of 10.5 mmol/kg bw or to tap water, five times, at 12-hour intervals. Twenty-four hours after the fifth dose, the same dose of TBA was administered to both groups, and blood

was collected from the infraorbital sinus and analysed by headspace GC. The TBA concentration in the blood increased to 12–13 mM within 1 hour and then decreased following close to zero-order kinetics. The mean elimination rate was slightly higher in the TBA pre-treated mice (1.28 mmol/L per hour) than in the control mice dosed with tap water (1.19 mmol/L per hour) ([Faulkner et al., 1989](#)).

The same research team exposed groups of eight male C57BL/6J mice to TBA at oral doses of 5.0, 10.0, and 20.0 mmol/kg bw. Blood from the three groups was sampled at up to 6.3 hours, 9.7 hours, and 17 hours, and analysed by headspace GC. Since the decrease in blood concentration of TBA followed close to zero-order kinetics, the data were fitted to a one-compartment Michaelis–Menten toxicokinetic model. It was observed that the  $V_{\max}$  estimates increased with dose, and the volume of distribution decreased, suggesting either multiple elimination pathways or dose-related effects on distribution or elimination ([Faulkner and Hussain, 1989](#)).

[Poet et al. \(1997\)](#) investigated the toxicokinetics of TBA in F344 male and female rats after intravenous exposure. The doses were 37.5, 75, 150, and 300 mg/kg bw. The concentration of TBA in the blood was measured up to 24 hours after dosing. Half-lives of 3 minutes and 3.8 hours in males and females were assessed by a two-compartment model analysis. At the highest dose, the second half-life increased to 5.0 hours in males, and to 4.3 hours in females. At lower doses, a rapid distribution phase was followed by a slower elimination phase. The steady-state volume of distribution ( $V_{ss}$ ) for TBA was 4.5-fold that of total body water, indicating extensive tissue binding. The clearance from blood (around 3.5 mL/minute per kg) was lower than the estimated glomerular filtration rate (around 7 mL/minute per kg). At the highest dose, the clearance of TBA from the blood decreased, and the area under the curve (AUC) increased more than proportionally to the dose as the dose increased,

indicating saturated elimination. Acetone levels were highly variable.

[Bernauer et al. \(1998\)](#) exposed F344 male rats (three per dose group) by gavage to unlabelled TBA and to  $^{13}\text{C}$ -labelled TBA at a dose of 250 mg/kg bw. The urine was analysed by NMR and GC-MS. Free TBA, TBA glucuronide, TBA sulfate, 2-HIBA, and 2-methyl-1,2-propanediol were identified in the urine as having originated from exposure to TBA. Headspace GC-MS also detected small amounts of  $^{13}\text{C}$ -labelled acetone in urine from rats exposed to  $^{13}\text{C}$ -labelled TBA by gavage. TBA sulfate was the main metabolite, in contrast to exposure to MTBE via inhalation, where 2-HIBA was the major urinary metabolite (see Section 4.1.2(a) of the monograph on MTBE, in the present volume).

In a study of dermal absorption in rats conducted according to the OECD test guideline (TG 417), groups of four Sprague-Dawley male rats were exposed topically to  $^{14}\text{C}$ -labelled TBA under a semi-occlusive dressing, for 6 hours. The rats were separately placed in a metabolism cage to collect urine, faeces, and volatiles. After 6 hours, the application site was washed to remove any unabsorbed dose. The dermal absorption of TBA was 6–11%, some of which may have been a result of evaporation from the application site rather than being expired by the rats. [The Working Group noted that the study was cited in the European Chemicals Agency (ECHA) registration dossier on 2-methyl-2-propanol ([ECHA, 2025b](#)).]

In a study on sex-specific differences in TBA-induced  $\alpha_{2u}$ -globulin nephropathy and renal cell proliferation (see Sections 4.2.7 and 4.3), [Borghoff et al. \(2001\)](#) investigated sex-specific differences in dosimetry in the kidney of F344 rats. Groups of rats (five per sex and dose) were exposed to TBA vapour at 0, 250, 450, or 1750 ppm for 6 hours per day for 10 days. The TBA kidney:blood ratio was higher in male rats (ratios of between 2 and 3 at the lowest exposure level, and between 1 and 3 at the highest exposure



level) than in female rats (ratios below 1) at all exposure levels and time points tested. [The Working Group noted that the finding suggested that TBA was retained for a longer time in male rat kidney than in female rat kidney.]

In a study that aimed to improve the physiologically based pharmacokinetic (PBPK) model for MTBE (see Section 4.1.2(c) of the monograph on MTBE in the present volume), [Leavens and Borghoff \(2009\)](#) exposed male and female F344 rats to TBA vapour at 250, 450, or 1750 ppm, by inhalation for 6 hours, either once only or once per day for 8 days. The rats were euthanized at 2, 4, 6, and 8 hours post-exposure, and blood, liver, and kidney samples were analysed for the detection of TBA. After the first exposure at 1750 ppm, TBA concentrations in the kidney peaked at ~4 mM (estimated from Fig. 10 at 2 hours post-exposure) in both sexes. At day 8 of exposure, the concentration of TBA in the kidney peaked at slightly below 4 mM in male rats and at ~3 mM in females. [The Working Group noted that the decrease in concentrations indicated induction of the metabolism of TBA.]

(b) *Non-human mammalian systems in vitro*

(i) *Tissue partitioning*

Liquid:air, tissue:air, and tissue:blood partition coefficients for TBA along with those calculated for MTBE are described in Section 4.1.2(b) of the monograph on MTBE in the present volume.

(ii) *Metabolism*

[Cederbaum and Cohen \(1980\)](#) observed that in liver microsomes prepared from Sprague-Dawley rats, TBA is oxidatively demethylated, producing formaldehyde. Furthermore, the iron-chelating agent desferrioxamine, known to block the generation of hydroxyl radicals ( $\cdot\text{OH}$ ), was able to inhibit the production of formaldehyde induced by TBA in rat liver microsomes by > 90% ([Cederbaum and Dicker, 1983](#)). This suggested that hydroxyl radicals play an important role

in the metabolism of TBA to formaldehyde ([Cederbaum et al., 1978, 1983](#); [Cederbaum and Cohen, 1980](#); [Cederbaum and Dicker, 1983](#)).

[The Working Group noted that studies conducted with TBA, MTBE, and ETBE (see Section 4.1 of the monographs on MTBE and ETBE) showed that TBA is oxidatively metabolized, mainly in the liver, to 2-methyl-1,2-propanediol, and further to 2-HIBA. These metabolites are excreted in the urine along with minor amounts of TBA.]

(c) *Physiologically based pharmacokinetic models*

No PBPK models addressing direct exposure to TBA were available. Toxicokinetic and PBPK models for MTBE and ETBE, including TBA, are described in Section 4.1.2(c) of the monographs on MTBE and ETBE.

*Synopsis*

[Overall, the Working Group noted that most of the evidence regarding the absorption, distribution, excretion, and metabolism of TBA derived from studies investigating exposure to MTBE and ETBE, considering that TBA is a major intermediary metabolite of these ethers. Nevertheless, the few studies (in humans and in experimental systems) that investigated TBA exposure directly provided a similar overall kinetic profile. TBA is absorbed via inhalation and after ingestion. A small fraction of the absorbed dose is excreted unchanged in the urine. A major proportion of TBA is either conjugated or further metabolized to 2-HIBA, 2-methyl-1,2-propanediol, and formaldehyde plus acetone, before excretion in the urine. TBA and its metabolites are readily distributed throughout the body. The patterns of metabolism and metabolic rates of TBA are qualitatively and quantitatively similar in humans and in rats.]



## 4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens ([Smith et al., 2016](#)) encompassed by the agent TBA. Evidence was available for the key characteristics “is electrophilic or can be metabolically activated to an electrophile”, “is genotoxic”, “induces oxidative stress”, “induces chronic inflammation”, “is immunosuppressive”, “modulates receptor-mediated effects”, and “alters cell proliferation, cell death, or nutrient supply”. There were no data for the remaining key characteristics of carcinogens.

### 4.2.1 *Is electrophilic or can be metabolically activated to an electrophile*

#### *Experimental systems*

One study in vivo was available to the Working Group. [Yuan et al. \(2007\)](#) investigated the formation of TBA-derived DNA adducts in the liver, lung, and kidney of male Kunming mice exposed by oral gavage to <sup>14</sup>C-labelled TBA. Mice ( $n = 8$  per dose) were exposed to TBA at a dose of 0 (saline alone), 0.099, 0.990, 10, 101, or 997 µg/kg with a specific activity of 1.60, 0.181, 0.0688, 0.0138, and 0.00978 mCi/mol, respectively. Six hours after exposure, samples of liver, kidney, and lung tissue were collected for DNA isolation, and DNA analysis by ultra-sensitive accelerator mass spectrometry. The authors observed a dose-related increase in TBA-derived DNA adducts formed in the liver, lung, and kidney, reported to be in the range of 0.1 to >1000 adducts per 10<sup>11</sup> nucleotides. [The Working Group noted that individual data were not provided, but a dose-response relation was provided in various plots; there was a linear response up to 10 µg/kg bw, followed by saturation. The doses of TBA appeared to be very low. Data for the control group were not reported. Despite the above limitations, one strength of the

study was the exposure to labelled TBA, which facilitated the discrimination of endogenous (background) DNA adducts from exogenous (exposure-related) DNA adducts.]

### 4.2.2 *Is genotoxic*

#### *(a) Humans*

##### *Human cells in vitro*

One study in human cells in vitro was available to the Working Group. [Tang et al. \(1997\)](#) investigated the cytotoxicity and genotoxicity of TBA in human leukaemia cells (HL-60). Single cell gel electrophoresis or comet assay were used to measure DNA strand breaks in HL-60 cells after incubation with TBA at a concentration of 1, 5, 10, or 30 mmol/L for 1 hour. Although no cytotoxicity was observed, TBA was reported to induce DNA fragmentation in HL-60 cells. [The Working Group noted that the quality of the study was limited, and the information provided was insufficient to allow evaluation of the study conclusions.]

#### *(b) Experimental systems*

##### *(i) Non-human mammalian systems in vivo*

See [Table 4.1](#).

The genotoxicity of TBA was evaluated in CD-1 mice exposed orally to TBA at a dose of 1500 mg/kg per day for 2 days ([Thompson et al., 2024](#)). The dose was selected via an earlier dose-range finder study, as the maximum tolerated dose, relative to the 2-year rodent bioassay. No significant increase in DNA strand breaks or decrease in DNA crosslinks were observed by comet assay in the thyroid tissue of the exposed mice compared with controls. In another study, wildtype (WT) (*Aldh2*<sup>+/+</sup>) and *Aldh2* gene knockout (KO) (*Aldh2*<sup>-/-</sup>) mice were exposed to TBA in drinking-water for 6 weeks. Increased levels DNA strand breaks, as measured by alkaline comet assay, were observed in leukocytes of both WT and KO mice ([Lin et al., 2020](#)).

**Table 4.1 End-points relevant to genotoxicity in non-human mammalian systems in vivo exposed to *tert*-butyl alcohol**

| End-point (assay)                             | Species, strain, (sex)                                                                  | Tissue                              | Results <sup>a</sup> | Dose (LED or HID)                       | Route, duration, dosing regimen                                                         | Comments                                                                                                                                                                                            | Reference                                                  |
|-----------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------|----------------------|-----------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| DNA breakage and DNA crosslinks (comet assay) | Mouse, CD-1 (M)                                                                         | Thyroid gland                       | –                    | 1500 mg/kg per day                      | Oral gavage; once daily for 2 consecutive days                                          | Positive control (ethyl methanesulfonate) significantly increased the %tail DNA. TBA-induced thyroid toxicity was not observed by histopathological analysis or low-molecular-weight DNA diffusion. | <a href="#">Thompson et al. (2024)</a>                     |
| DNA strand breaks (alkaline comet assay)      | Mouse, C57BL/6 WT ( <i>Aldh2</i> <sup>+/+</sup> ) or KO ( <i>Aldh2</i> <sup>-/-</sup> ) | Blood, leukocytes                   | +                    | 5 mg/mL in KO mice; 20 mg/mL in WT mice | Drinking-water, for 6 wk                                                                |                                                                                                                                                                                                     | <a href="#">Lin et al. (2020)</a>                          |
| Micronucleus formation                        | Rat, Sprague-Dawley (M, F)                                                              | Bone marrow from femur              | –                    | 20 000 mg/m <sup>3</sup>                | Inhalation; 0, 2000, 10 000, and 20 000 mg/m <sup>3</sup> , 6 h/day, 5 days/wk for 4 wk | Gasoline exposure vs gasoline with TBA was assessed, not just TBA. Positive control was cyclophosphamide (40 mg/kg).                                                                                | <a href="#">Schreiner et al. (2014)</a>                    |
| Sister-chromatid exchange                     |                                                                                         | Blood, peripheral abdominal (aorta) | –                    | 20 000 mg/m <sup>3</sup>                |                                                                                         |                                                                                                                                                                                                     |                                                            |
| Micronucleus formation                        | Rat, F344/N (M)                                                                         | Bone marrow                         | –                    | 1250 µg/mL                              | Intraperitoneal injection; 3×, at intervals of 24 h                                     |                                                                                                                                                                                                     | <a href="#">Mahler (1997)</a> ; <a href="#">NTP (1995)</a> |
| Micronucleus formation                        | Mouse, B6C3F <sub>1</sub> (M, F)                                                        | Peripheral abdominal (aorta) blood  | –                    | 40 000 ppm                              | Oral; drinking-water; 3000, 5000, 10 000, 20 000, or 40 000 ppm, for 13 wk              | The findings overlapped with those reported in <a href="#">NTP (1995)</a> .                                                                                                                         |                                                            |

*Aldh2*, aldehyde dehydrogenase 2; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; KO, knockout; LED, lowest effective dose; M, male(s); ppm, parts per million; TBA, *tert*-butyl alcohol; wk, week(s); WT, wildtype.

<sup>a</sup> +, positive; –, negative; +/-, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative findings in a study of limited quality.

[Schreiner et al. \(2014\)](#) performed a micronucleus assay in tissue from rats exposed by inhalation to gasoline blended with TBA at a dose of 0, 2000, 10 000, or 20 000 mg/m<sup>3</sup> for 4 weeks (6 hours per day, 5 days per week). Bone marrow was collected from both femurs, 24 hours after the final exposure, and no consistent increase in the frequency of micronuclei was observed in exposed rats compared with controls. In the same study, sister-chromatid exchange was assessed in peripheral lymphocytes of blood collected from the abdominal aorta. No statistical increase in sister-chromatid exchange or a dose-response trend was observed in either sex compared with controls. [The Working Group noted that this study assessed gasoline blended with TBA, but not TBA alone. The Working Group noted that this study was part of a broader research effort to test various vapours of gasoline from different generation methods, and relative test materials, as reported by [Henley et al. \(2014\)](#). Seven different materials were tested, including base gasoline alone, gasoline plus several ethers (MTBE, ETBE, TAME, or DIPE), or gasoline plus an alcohol (ethanol or TBA) (see also the monographs on MTBE and ETBE in the present volume).]

In an NTP report ([NTP, 1995](#); [Mahler, 1997](#)) on the carcinogenicity of TBA in F344/N rats and B6C3F<sub>1</sub> mice, a micronucleus assay was also conducted. No induction of micronuclei was observed in the bone marrow of F344/N rats treated by intraperitoneal injection with TBA (at doses of 0, 3000, 5000, 10 000, 20 000, or 40 000 ppm), or in peripheral blood erythrocytes from B6C3F<sub>1</sub> mice exposed to TBA in drinking-water for 13 weeks.

## (ii) Non-human mammalian systems in vitro

See [Table 4.2](#).

[McGregor et al. \(1988\)](#) did not observe any induction of mutagenesis in a gene mutation assay performed in L5178Y mouse lymphoma cells exposed to TBA in the presence or absence

of metabolic activation. Similarly, [Mahler \(1997\)](#) reported no increase in sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary (CHO) cells exposed to TBA at concentrations up to 5000 µg/mL, in the presence or absence of metabolic activation.

[Sgambato et al. \(2009\)](#) investigated the effects of exposure to TBA (at 0–100 mM, for 48 hours) on DNA damage in Rat-1 diploid immortalized fibroblasts. The median inhibitory concentration (IC<sub>50</sub>) of TBA in this test system was 0.44 mM. Cells were treated with TBA at the IC<sub>50</sub> for 24 or 48 hours, then DNA content was assessed by flow cytometry. Oxidative damage to DNA was assessed by measuring levels of 8-hydroxydeoxyguanosine (8-hydroxy-2'-deoxyguanosine, 8-OHdG) by immunohistochemistry (see also [Section 4.2.3](#)). [The Working Group noted that the reduction in cell numbers was ascribed to a cytostatic rather than to a cytotoxic effect; however, the data sustaining the hypothesis were not shown.] Also, DNA strand breaks were assessed by comet assay in cells exposed to TBA at the IC<sub>50</sub> for 30 minutes, 4 hours, or 12 hours. Exposure to TBA induced fragmentation of the DNA double strand, as confirmed by the lengthening of the tail moment, after 30 minutes. However, the authors observed a drastic reduction in DNA damage (% tail moment) after 4 hours, and disappearance after 12 hours. [The Working Group noted that it was not possible to determine whether this reduction in DNA damage was caused by cell death or other factors.]

## (iii) Bacterial systems

See [Table 4.3](#).

[Williams-Hill et al. \(1999\)](#) evaluated the mutagenicity of TBA in *Salmonella typhimurium* strain TA102 in the presence of 9000 × g supernatant (S9) of microsomes from Aroclor-induced rats. Under the conditions used, the frequency of mutants per plate increased in a dose-related manner, reaching a level of about twofold that in the control strain, with TBA at a concentration

**Table 4.2 End-points relevant to genotoxicity in non-human mammalian systems in vitro exposed to *tert*-butyl alcohol**

| End-point                 | Cell line                                    | Results <sup>a</sup>               |                                 | Concentration<br>(LEC or HIC) | Comments                                                                                                                     | Reference                                 |
|---------------------------|----------------------------------------------|------------------------------------|---------------------------------|-------------------------------|------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
|                           |                                              | Without<br>metabolic<br>activation | With<br>metabolic<br>activation |                               |                                                                                                                              |                                           |
| Gene mutation             | Mouse lymphoma cell (L5178Y)                 | (+)                                | –                               | 5000 µg/mL                    | Small increase in the mutant fraction in only one experiment without S9, but this was not reproduced in a second experiment. | <a href="#">McGregor et al. (1988)</a>    |
| Gene mutation             | Mouse lymphoma cell (L5178Y)                 | –                                  | –                               | 5000 µg/mL                    |                                                                                                                              | <a href="#">Mahler (1997); NTP (1995)</a> |
| Sister-chromatid exchange | Chinese hamster ovary cells (CHO)            | –                                  | –                               | 5000 µg/mL                    |                                                                                                                              | <a href="#">Mahler (1997); NTP (1995)</a> |
| Chromosomal aberrations   | Chinese hamster ovary cells (CHO)            | –                                  | –                               | 5000 µg/mL                    |                                                                                                                              | <a href="#">Mahler (1997); NTP (1995)</a> |
| DNA strand breaks         | Rat diploid immortalized fibroblasts (Rat-1) | +                                  | NT                              | 0.44 mM (IC <sub>50</sub> )   | TBA at 0–100 mM, for 48 h comet assay. Effect transitory at 4 h (negative at 12 h).                                          | <a href="#">Sgambato et al. (2009)</a>    |

h, hour(s); HIC, highest ineffective concentration; IC<sub>50</sub>, median inhibitory concentration; LEC, lowest effective concentration, NT, not tested; S9, 9000 × g supernatant; TBA, *tert*-butyl alcohol.

<sup>a</sup> +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality.

**Table 4.3 End-points relevant to genotoxicity in non-mammalian experimental systems exposed to *tert*-butyl alcohol**

| End-point                                      | Test system<br>(species, strain)              | Results <sup>a</sup>               |                                 | Concentration<br>(LEC or HIC) | Comments                                                                                                                                                                                                                                                                                                                        | Reference                                                  |
|------------------------------------------------|-----------------------------------------------|------------------------------------|---------------------------------|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
|                                                |                                               | Without<br>metabolic<br>activation | With<br>metabolic<br>activation |                               |                                                                                                                                                                                                                                                                                                                                 |                                                            |
| Gene mutation                                  | <i>Salmonella typhimurium</i> , TA102         | NR                                 | (+)                             | 700 µg/plate                  | Not many details were provided for TBA experiments and the study mostly focused on MTBE. The concentrations were not reported, therefore the concentrations were estimated by the Working Group using the published figure. The criteria for a significant response were not described. TBA was only tested with S9 activation. | <a href="#">Williams-Hill et al. (1999)</a>                |
| Gene mutation<br>(base substitution,<br>at AT) | <i>Salmonella typhimurium</i><br>TA102        | –                                  | –                               | 5000 µg/plate                 | TBA was tested with and without S9 activation.                                                                                                                                                                                                                                                                                  | <a href="#">McGregor et al. (2005)</a>                     |
| Gene mutation<br>(base substitution,<br>at GC) | <i>Salmonella typhimurium</i><br>TA135, TA100 | –                                  | –                               | 10 000 µg/plate               | Each test consisted of triplicate plates of concurrent positive and negative controls and at least five concentrations of TBA. In the absence of toxicity, 10 000 µg/plate was selected as the highest dose. All assays were repeated with or without rat or hamster liver S9.                                                  | <a href="#">Mahler (1997)</a> ; <a href="#">NTP (1995)</a> |
| (Frameshift +1)                                | TA98                                          | –                                  | –                               | 10 000 µg/plate               |                                                                                                                                                                                                                                                                                                                                 |                                                            |
| (Frameshift –1)                                | TA1537                                        | –                                  | –                               | 10 000 µg/plate               |                                                                                                                                                                                                                                                                                                                                 |                                                            |

AT and GC, nucleotide bases adenine and thymine, guanine and cytosine; HIC, highest ineffective concentration; LEC, lowest effective concentration; MTBE, methyl *tert*-butyl ether; NR, not reported; S9, 9000 × *g* supernatant; TBA, *tert*-butyl alcohol.

<sup>a</sup> +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality.

of 2000 µg/plate. [The Working Group noted that the criteria to define a significant response were not described, and the agent was not tested in the absence of metabolic activation.] In another study ([McGregor et al., 2005](#)), TBA (dissolved in water or dimethyl sulfoxide), at concentrations of up to 5000 µg/plate, was tested in *S. typhimurium* strain TA102, with or without metabolic activation (S9). No significant mutagenic response was observed in any of the experiments.

In an NTP study ([Mahler, 1997](#); [NTP 1995](#)), exposure to TBA, at concentrations in the range of 100–10 000 µg/plate, gave negative responses in four different *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537), either with or without metabolic activation with S9 microsomes from the liver of Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters.

#### (iv) *Acellular systems*

[Siddiqi and Bothe \(1987\)](#) investigated the formation in vitro of DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) in irradiated calf thymus DNA exposed to TBA, considered as a hydroxyl radical (·OH) scavenger. The rate of formation of SSBs was reported to be higher than that of DSBs but was not concentration-related. With increasing TBA concentrations (0–0.03 M), DSB formation was reported to decrease, as assessed through the coefficients of the chemical reaction.

[Epe et al. \(1996\)](#) investigated the potential inhibitory effect of TBA on the formation of SSBs or formamidopyrimidine/8-oxoguanine DNA N-glycosylase (Fpg)-sensitive base modifications caused by pre-incubation with peroxy-nitrite, 3-morpholinostyrylamine (SIN-1), or hydroxyl radicals. The authors observed that TBA (~20 mM) inhibited 50% of the DNA damage induced by hydroxyl radicals and singlet oxygen. In contrast, TBA at a higher concentration (200 mM), was reported to induce an increase of ~40% of peroxy-nitrite-mediated oxidation of dihydrorhodamine 123,

and iron(III)–ethylenediaminetetraacetic acid (Fe(III)–EDTA)-dependent nitration of 4-hydroxyphenylacetate (4-HPA). [The Working Group noted that these findings indicated that TBA did not act as a scavenger.]

[Udovicić et al. \(1996\)](#) investigated the potential role of TBA as a scavenger of primary water radicals, or as a sensitizer, thus increasing SSB formation in DNA irradiated (<sup>60</sup>Co or electron pulse irradiation) in the absence of oxygen. Based on the rate of SSBs formation, and based on the rate of reaction with DSBs, it was reported that SSBs were induced mainly by TBA-OH· radical, and to a minor extent by H· and ·OH radicals. [The Working Group noted that the secondary radicals of TBA played a significant role in the formation of SSBs and the scavenger properties of TBA should be evaluated carefully.] The formation of SSBs in plasmid DNA pre-exposed to <sup>137</sup>Cs gamma irradiation in the presence of scavenger-derived radicals was investigated by [Milligan and Ward \(1994\)](#). Although the formation of SSBs was reported to increase with scavenger TBA, it was unclear which radical was responsible for the increase.

[Distel and Schuessler \(1997\)](#) investigated the effects of irradiation and the addition of hydroxyl (·OH) radicals such as TBA in the formation or reduction of DSBs in calf thymus DNA. The authors investigated the hypothesis that addition of hydroxyl radicals (TBA and others) to DNA solutions would increase DSBs. In the presence of TBA (1.5 × 10<sup>-3</sup> mol dm<sup>-3</sup>), there was a decreased yield of DSBs because of the presence of oxygen. It was indicated that, under nitrogen, TBA provided some protection against DSB formation (see also Section 4.2.3).

#### *Synopsis*

[The Working Group noted that few data were available and these were mostly negative for genotoxicity.]



### 4.2.3 Induces oxidative stress

#### (a) Humans

##### *Human cells in vitro*

[Ma et al. \(2018\)](#) investigated oxidative stress in murine hippocampal neuronal HT22 cells after exposure to TBA (0–2.0 mM) for 6 hours. It was reported that TBA enhanced oxidative stress by increasing the intracellular content of reactive oxygen species (ROS) and malondialdehyde (MDA), and at the same time decreasing the content of superoxide dismutase (SOD) (only in 1.0 and 2.0 mM) and the ratio). In addition, the authors observed that Sirtuin 1 (SIRT1) was able to mitigate the oxidative stress induced by TBA.

#### (b) Experimental systems

##### (i) *Non-human mammalian systems in vivo*

[Acharya et al. \(1995\)](#) investigated oxidative stress in Wistar rats exposed to TBA at 0.5% (5 mg/mL, v/v in water). There was no significant alteration in GSH levels in the liver, but there was a decrease in levels in the kidney in the exposed animals compared with the controls. Similarly, no increase in lipid peroxidation, as measured by diene conjugation, was observed in the exposed group compared with the control group ([Acharya et al., 1995](#)).

In another study, increased levels of hepatic 8-OHdG were observed in WT and *Aldh2* KO (*Aldh2*<sup>-/-</sup>) mice exposed to TBA in drinking-water for 6 weeks (the LED was 20 mg/mL in WT mice and 5 mg/mL in KO mice) ([Lin et al., 2020](#)). In addition, the serum levels of total GSH, the oxidized form GSSG, the reduced form GSH, and the ratio of GSH:GSSG were all significantly decreased in the groups of KO mice at the lower and higher doses compared with the controls. Inversely, increased levels of total serum levels of GSH, GSH concentrations and ratio of GSH/GSSG were observed in WT mice in the groups at the higher dose compared with the controls ([Lin et al., 2020](#)).

##### (ii) *Non-human mammalian systems in vitro*

In the study of [Sgambato et al. \(2009\)](#), conducted in Rat-1 diploid immortalized fibroblasts (see also Section 4.2.2), exposure to TBA at 0.44 mM for 4 hours increased levels of 8-OHdG, assessed by immunohistochemistry, immediately after 30 minutes of treatment. However, it was reported that the DNA damage was rapidly repaired and disappeared within 12 hours of treatment. [The Working Group noted that this transitory effect could be caused by various factors not discussed in the study, for example, culture conditions or cell death, thus limiting the reliability of the finding.]

##### (iii) *Other experimental systems*

[The Working Group noted that, because of the high reaction rate constant of TBA with hydroxyl radicals ( $\cdot\text{OH}$ ) compared with that for other molecules, TBA is assumed to fully scavenge hydroxyl radicals ([Buxton et al., 1988](#)); therefore, further redox reactions could be inhibited by the addition of scavengers such as TBA ([Kozmér et al., 2016](#)).]

[Distel and Schuessler \(1997\)](#) investigated radiation-induced DNA strand breaks in calf thymus DNA and the effects of addition of hydroxyl radical ( $\cdot\text{OH}$ ) scavengers, such as TBA, in the reduction of formation of DNA strand breaks. They investigated the hypothesis that the addition of  $\cdot\text{OH}$  radicals (TBA and others) to DNA solutions would increase DNA strand breaks. They reported, in the presence of TBA ( $1.5 \times 10^{-3} \text{ mol dm}^{-3}$ ), decreased yield of DNA strand breaks, which was linked to the presence of oxygen and its potential protective effect. It was indicated that, under nitrogen, TBA provided some protection against DNA strand breaks under the test conditions.

The elimination of hydroxyl radical ( $\cdot\text{OH}$ ) activity and the transformation of hydroxyl radicals to oxidatively inert products via the addition of a scavenger, TBA, was also investigated by [Gao et al. \(2021\)](#). The authors investigated the effect of

TBA (at concentrations of 1, 10, or 100 mM) on manganese ( $Mn^{2+}$ ) oxidation induced by nitrate photolysis. [The Working Group noted that, under the experimental conditions, TBA did not behave as an inert agent in scavenging  $\cdot OH$  by terminating the redox reaction or by eliminating the role of  $\cdot OH$ . The mechanism through which the secondary peroxy radical ( $ROO\cdot$ ) was formed facilitated the oxidation of  $Mn^{2+}$  to manganese oxide ( $MnO_2$ ), thus indicating the potential for further oxidative reactions.]

### Synopsis

[The Working Group noted that there was a paucity of data regarding the key characteristic “induces oxidative stress”.]

## 4.2.4 Induces chronic inflammation

### Experimental systems

[Kolls et al. \(1995\)](#) investigated the lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and tumour necrosis factor alpha (TNF $\alpha$ ) in the lungs of male Sprague-Dawley rats exposed intraperitoneally to TBA at 3.6 g/kg, or to phosphate buffered saline (PBS) at 1 mL/kg, or to ethanol at 5.5 g/kg (20%). At 30 minutes after the initial dosing, rats were treated intratracheally with either PBS (0.5 mL) or *Escherichia coli* endotoxin (1 mg/kg, *E. coli* LPS 026:B6). At 2–4 hours after intratracheal administration, the rats were euthanized, and lavage was performed on the lungs. Bronchoalveolar lavage fluid was analysed for TNF $\alpha$  by enzyme-linked immunosorbent assay (ELISA). Alveolar macrophages or polymorphonuclear neutrophils were also isolated to determine cell counts, and measurements of cell-associated TNF $\alpha$ , and TNF $\alpha$  messenger RNA (mRNA). Compared with the administration of PBS, the administration of TBA 30 minutes before intratracheal LPS resulted in a 45% reduction in TNF $\alpha$  bioactivity; however, there was no effect on TNF $\alpha$  mRNA levels.

Transitional epithelial hyperplasia (see Section 4.2.7 and [Table 4.3](#)) accompanied by inflammation of the urinary bladder (at 20 and 40 mg/mL in males, and at 40 mg/mL in females) was identified through histopathology analysis in F344/N rats (10 animals per sex per dose) exposed to TBA (0, 2.5, 5, 10, 20, or 40 mg/mL) in drinking-water for 13 weeks ([NTP, 1995](#)). Inflammation of the urinary bladder consisted of increased numbers of macrophages, lymphocytes, and plasma cells in the lamina propria. In addition, a significant increase in the incidence of mineralization of the kidney was observed in male rats exposed to TBA at 10, 20, or 40 mg/mL. Furthermore, the accumulation of hyaline droplets in the kidney of males (at doses of 5, 10, and 20 mg/mL) and the “severity” of nephropathy in males (at doses of 2.5, 5, 10, and 20 mg/mL) and females (at doses of 10, 20, and 40 mg/mL) were significantly higher than those in the controls. An increase in the incidence of transitional epithelial hyperplasia (see Section 4.2.7) and inflammation of the urinary bladder was also observed in male mice exposed to TBA at 20 and 40 mg/mL and female mice at 40 mg/mL for 13 weeks. For both rats and mice, the incidence and severity of the urinary bladder lesions were higher in males than in females ([NTP, 1995](#)). In F344/N rats exposed to TBA in drinking-water for 2 years, the incidence of mineralization in the kidney increased with dose and was significantly higher in male rats treated with TBA at 5 mg/mL than in the controls ([NTP, 1995](#)). Inflammation of the kidney was increased in female rats exposed to TBA at 5 or 10 mg/mL, as observed at the end of the 2-year exposure. Although the severity of nephropathy was increased in both male and female exposed rats, linear foci of mineralization were present only in the renal papilla of exposed males. In the same study, in B6C3F<sub>1</sub> mice treated with TBA for 2 years, the incidence of chronic inflammation of the urinary bladder was increased in males at 20 mg/mL and to a lesser extent in females at 20 mg/mL. [The Working Group noted that

exposure to TBA was associated with increased nephropathy of the kidney in male and female rats, chronic inflammation of the urinary bladder (see Section 4.2.7) in male and, to a lesser extent, in female mice.]

In another study, [Acharya et al. \(1997\)](#) observed lymphocytic infiltration in the periportal area of the liver of rats exposed to TBA at 5 mg/mL in drinking-water for 10 weeks.

### Synopsis

[The Working Group noted that there was a paucity of data regarding the key characteristic “induces chronic inflammation”.]

## 4.2.5 Is immunosuppressive

### Experimental systems

#### (i) Non-human mammalian systems in vivo

[White et al. \(2014\)](#) investigated the immunotoxicity of gasoline or gasoline combined with various fuel oxygenates (including TBA) in female Sprague-Dawley rats after inhalation of vapour condensates (0, 2000, 10 000, or 20 000 mg/mg<sup>3</sup> for 6 hours/day, 5 days per week, for 4 weeks). The generation and characterization of the test materials were described by [Henley et al. \(2014\)](#). The antibody-forming cell (AFC) response to the T-dependent antigen, sheep erythrocytes (SRBCs), was used to determine the effects of the vapour condensates on humoral components of the immune system. There were no statistically significant effects on spleen weight, or spleen cell number. As for gasoline vapour condensate, exposure to vapour condensate of gasoline combined with TBA did not affect rat humoral immunity since no significant changes in the immunoglobulin M (IgM) AFC response to SRBCs was observed, when evaluated either as specific activity (AFC/10<sup>6</sup> spleen cells) or as total spleen activity (AFC/spleen). [The Working Group noted that the effects of

gasoline containing TBA were assessed and not those of TBA alone.]

#### (ii) Non-human mammalian systems in vitro

Only one in vitro study using rat mast cell line RBL2H3 was available to the Working Group ([Yamaki and Yoshino, 2009](#)). The potential immunosuppressive effects of TBA were assessed by evaluating IgE-dependent degranulation (measured by release of  $\beta$ -hexosaminidase), calcium ion ( $\text{Ca}^{2+}$ ) mobilization (using cytometry), phosphorylation of Akt, and global tyrosine phosphorylation (monoclonal antibodies for phosphorylated tyrosine) of RBL2H3 mast cells. RBL2H3 cells were exposed to TBA in solution at 0.1%, 0.3%, or 1.0%. TBA inhibited IgE-induced release of  $\beta$ -hexosaminidase and IgE-mediated increase in intracellular  $\text{Ca}^{2+}$ , phosphorylation of Akt, and global tyrosine phosphorylation. TBA inhibited Fc $\epsilon$ RI-mediated mast cell degranulation through the activation of protein tyrosine kinase and phosphatidylinositol 3-kinase (PI3K) and calcium mobilization (by inhibition of intracellular calcium ion concentration increase) in RBL2H3 cells in vitro.

### Synopsis

[The Working Group noted that there was a paucity of data regarding the key characteristic “is immunosuppressive”.]

## 4.2.6 Modulates receptor-mediated effects

### Experimental systems

#### (i) Non-human mammalian systems in vivo

[Blanck et al. \(2010\)](#) evaluated the effects of exposure to TBA in drinking-water in female B6C3F<sub>1</sub> mice exposed to TBA at a dose of 0, 2.0, or 20 mg/mL for 3 or 14 days (equal to 0, 344, or 818 mg/kg bw for 3 days, or 0, 418, or 1616 mg/kg bw for 14 days). Several end-points were investigated, including gross and histological alterations of the thyroid gland and of the liver, serum levels of the thyroid hormones triiodothyronine (T3),

thyroxine (T4), thyroid-stimulating hormone (TSH), total hepatic cytochrome P450 (CYP) content, specific CYP activities, and quantitative PCR analysis of gene transcripts of specific CYP enzymes (CYP1A1, CYP2B9, CYP2B10, CYP3A11), sulfuryl transferases (SULT1A1, SULT2A2, and SULTN), and glucuronyltransferases (UGT1A1, UGT2B1, and UGT2B5). Although there were no pathological findings in the thyroid gland tissue, a reduction in T3 and T4 levels was observed. Induction of CYP2B10 and SULT1a1 was significant at both doses, and a slight induction of CYP2B9 in mice at 20 mg/mL was observed by quantitative polymerase chain reaction (qPCR) analysis of gene transcripts. [The Working Group noted that in experimental systems, the induction of specific hepatic and phase II enzymes could result in a decrease in the levels of circulating thyroid hormones in female mice at high doses.]

(iii) *Non-human mammalian systems in vitro*

[de Peyster et al. \(2009\)](#) evaluated the effects of TBA at 50 and 100 mM on testosterone production in a human chorionic gonadotropin (hCG)-stimulated isolated rat Leydig cell model. There was inhibition of testosterone production in rats at both concentrations compared with untreated controls. [de Peyster et al. \(2014\)](#) also investigated the effects of TBA (0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 µM) on endocrine system components involved in steroidogenesis. For the androgen receptor-binding assay, androgen receptor was isolated from the prostate gland of adult male Sprague-Dawley rats. The H295R cell line was used to assess steroidogenesis. No statistically significant changes in estradiol or testosterone concentrations were observed at any of the concentrations tested in the H295R steroidogenesis assay. In addition, TBA was classified as a non-inhibitor of aromatase binding, based on a mean aromatase activity of 102.3% ( $\pm$  1.7% standard deviation), at the highest tested concentration. [The Working Group noted that

TBA did not exert any significant effects on the steroidogenesis pathway, or on aromatase activity in vitro; it also did not bind to the androgen receptor in vitro when tested at low concentrations. Furthermore, the Working Group noted that the agent was not tested in the high-throughput screening assays generated by the Toxicology Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA.]

*Synopsis*

[The Working Group noted that there was a paucity of data regarding the key characteristic “modulates receptor-mediated effects”.]

**4.2.7 Alters cell proliferation, cell death, or nutrient supply**

(a) *Humans*

*Human cells in vitro*

Only one in vitro study in human arterial endothelial cells (HAECs) was available to the Working Group. [Spyridopoulos et al. \(2001\)](#) investigated the potential cellular effects on atherogenesis of exposure to alcohols in conjunction with oxysterols (7 $\beta$ -hydroxycholesterol, 7-ketocholesterol, and cholesterol-5,6-epoxides). HAEC cells were isolated from human iliac and renal arteries, and co-exposed to cholesterol 5 $\beta$ ,6 $\beta$ -epoxide ( $\beta$ -epoxide) at 10 mg/mL and TBA (0, 0.005, 0.01, or 0.05%). Addition of 0.01% TBA amplified oxysterol-induced cell death by about fourfold in endothelial cells, whereas TBA alone at up to 1% did not cause cell death. [The Working Group noted that the study mainly focused on ethanol.]



(b) *Experimental systems*(i) *Non-human mammalian systems in vivo*

See [Table 4.4](#).

In one study it was reported that exposure to TBA (0.5% v/v) in drinking-water caused periportal proliferation together with other histological alterations in the liver, such as centrilobular necrosis, vacuolation in hepatocytes, and loss of hepatic architecture, in male Wistar rats ([Acharya et al., 1997](#)).

[Borghoff et al. \(2001\)](#) reported the occurrence of  $\alpha_{2u}$ -globulin-mediated nephropathy in rodents after exposure to TBA. The induction of  $\alpha_{2u}$ -globulin nephropathy and of sex-specific differences in renal cell proliferation in F344 rats was evaluated after exposure to TBA at 0, 250, 450, or 1750 ppm via inhalation for 10 days. Accumulation of proteins, specifically  $\alpha_{2u}$ -globulin, and an increase in tubule cell proliferation were observed in the kidney of male rats exposed to TBA at the highest dose. Renal  $\alpha_{2u}$ -globulin concentration was correlated with cell proliferation in male rats, but no increase in renal cell proliferation was observed in female rats.

In an NTP study ([NTP, 1995](#); also described in Sections 3 and 4.2.4), male and female F344/N rats and B6C3F<sub>1</sub> mice were exposed to TBA in drinking-water for 13 weeks or 2 years. In the 13-week studies, transitional cell hyperplasia of the bladder mucosa was reported in groups of male rats and mice at 20 and 40 mg/mL. Protein droplets in the kidney and renal tubule epithelial regeneration were observed only in male rats. In the 2-year studies, follicular cell hyperplasia of the thyroid gland and hyperplasia of the urinary bladder were reported in mice, and an increased incidence of renal tubule hyperplasia of the kidney was reported in male rats. [The Working Group noted that the same results reported in the NTP study (1995) were also published by [Cirvello et al. \(1995\)](#) and by [Lindamood et al. \(1992\)](#).]

In several other articles, re-analysis of the histopathological slides of the NTP study was

reported ([Takahashi et al., 1993](#); [Hard et al., 2011, 2019](#)). In a revaluation of slides from the [NTP \(1995\)](#) 13-week study, [Hard et al. \(2019\)](#) were able to demonstrate cell proliferation in the renal cortex by performing a quantitative assessment of mitotic figures. This evaluation showed a dose-dependent increase in mitosis in male rats in the groups at 5 and 10 mg/mL compared with the concurrent controls, and no changes in treated female rats.

[Takahashi et al. \(1993\)](#) re-investigated  $\alpha_{2u}$ -globulin-mediated nephropathy and associated cell proliferation in male F344 rats ( $n = 10$ ) after the administration of TBA (0, 0.25, 0.5, 1, 2, and 4% w/v, equivalent to 0, 2.5, 5, 10, 20, and 40 mg/mL) in drinking-water for 13 weeks. Treatment-induced alterations in S-phase cells were evaluated in the kidney by measuring levels of proliferating cell nuclear antigen (PCNA) via immunohistochemistry. Treatment-related increases in hyaline droplets and abnormal intracytoplasmic deposits were reported in the proximal epithelial cells of the cortex and outer medulla in rats exposed to TBA at 0.25–2%. Although the number of PCNA-stained S-phase nuclei was significantly increased in rats in the group at 2%, there was a significant decrease in PCNA-labelled renal tubular epithelial cells in rats in the group at 4% compared with the control group. A re-evaluation of the NTP findings regarding alterations in the kidney, conducted by [Hard et al. \(2011\)](#), confirmed that, in the 2-year bioassay, the occurrence of chronic progressive nephropathy (CPN) was more pronounced in males and females at the highest dose (5 mg/mL), with hyperplasia of the papilla lining and no evidence of urothelial (transitional) cell hyperplasia. [The Working Group noted that the above three studies ([Takahashi et al., 1993](#); [Hard et al., 2011, 2019](#)) re-analysed the same samples from the study by the [NTP \(1995\)](#); and for this reason the overall data supporting increased cell proliferation after TBA exposure remained of limited relevance to the present evaluation.]

**Table 4.4 End-points relevant to cell proliferation, cell death, or nutrient supply in non-human mammalian systems in vivo exposed to *tert*-butyl alcohol**

| End-point                           | Assay                                 | Species, strain (sex)                                | Tissue             | Results <sup>a</sup>                | Dose (LED or HID)                       | Route, duration, dosing regimen                            | Comments                                                                                                                     | Reference                               |
|-------------------------------------|---------------------------------------|------------------------------------------------------|--------------------|-------------------------------------|-----------------------------------------|------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| Cell proliferation                  | Immunohistochemical staining for BrdU | Rat, F344 (M, F)                                     | Kidney             | ↑ In males<br>No changes in females | 250 ppm in males<br>1750 ppm in females | Inhalation; 6 h/day for 10 days (0, 250, 450, or 1750 ppm) | Dose-dependent effect. Renal $\alpha_{2u}$ -globulin was positively correlated with renal cell proliferation in males.       | <a href="#">Borghoff et al. (2001)</a>  |
| Periportal proliferation            | Histopathology                        | Rat, Wistar (M)                                      | Liver<br>Kidney    | ↑<br>No change                      | 0.5% (v/v) [5 mg/mL]                    | Drinking-water, 10 wk                                      | Degeneration of renal tubules, with syncytial arrangement of the nucleus in the renal tubular epithelial cells was reported. | <a href="#">Acharya et al. (1997)</a>   |
| Transitional cell hyperplasia       | Histopathology                        | Rat, F344 (M, F)<br>Mouse, B6C3F <sub>1</sub> (M, F) | Bladder<br>Bladder | ↑ In males<br>↑ In males            | 40 mg/mL in males<br>20 mg/mL in males  | Drinking-water, 13 wk<br>Drinking-water, 13 wk             |                                                                                                                              | <a href="#">NTP (1995)</a>              |
| Transitional epithelial hyperplasia | Histopathology                        | Rat, F344 (M, F)                                     | Kidney             | ↑                                   | 2.5 mg/mL in males; 10 mg/mL in females | Drinking-water, 2 yr                                       |                                                                                                                              |                                         |
| Renal tubule hyperplasia            | Histopathology                        | Rat, F344 (M, F)                                     | Kidney             | ↑ In males                          | 5 mg/mL in males                        | Drinking-water, 2 yr                                       |                                                                                                                              |                                         |
| Follicular cell hyperplasia         | Histopathology                        | Mouse, B6C3F <sub>1</sub> (M, F)                     | Thyroid gland      | ↑                                   | 5 mg/mL in males; 10 mg/mL in females   | Drinking-water, 2 yr                                       |                                                                                                                              |                                         |
| Transitional epithelial hyperplasia | Histopathology                        | Mouse, B6C3F <sub>1</sub> (M, F)                     | Bladder            | ↑ In males                          | 20 mg/mL in males                       | Drinking-water, 2 yr                                       |                                                                                                                              |                                         |
| <b>Re-evaluation studies</b>        |                                       |                                                      |                    |                                     |                                         |                                                            |                                                                                                                              |                                         |
| Replicative DNA synthesis           | Immunohistochemical staining for PCNA | Rat, F344 (M)                                        | Kidney             | ↑                                   | 2% (20 mg/mL) TBA                       | Drinking-water, 13 wk                                      | This study was a re-examination of existing kidney slides from a 90-day toxicity study ( <a href="#">NTP, 1995</a> ).        | <a href="#">Takahashi et al. (1993)</a> |



**Table 4.4 (continued)**

| End-point                         | Assay          | Species, strain (sex) | Tissue  | Results <sup>a</sup> | Dose (LED or HID)                        | Route, duration, dosing regimen | Comments                                                                                                              | Reference                          |
|-----------------------------------|----------------|-----------------------|---------|----------------------|------------------------------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Hyperplasia of the papilla lining | Histopathology | Rat, F344 (M, F)      | Kidney  | +                    | 5 mg/mL in males and 10 mg/mL in females | Drinking-water, 2 yr            | This study was a re-examination of existing kidney slides from a 90-day toxicity study ( <a href="#">NTP, 1995</a> ). | <a href="#">Hard et al. (2011)</a> |
| Transitional cell hyperplasia     |                |                       | Bladder | –                    |                                          |                                 |                                                                                                                       |                                    |

BrDU, bromodeoxyuridine; F, female(s); F344, Fischer 344; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; M, male(s); PCNA, proliferating cell nuclear antigen; ppm, parts per million; TBA, *tert*-butyl alcohol; v/v, volume per volume; wk, week(s); yr, year(s).

<sup>a</sup> +, positive; –, negative; ↑, increase; ↓, decrease; or no change.

(iii) *Non-human mammalian systems in vitro*

[Sgambato et al. \(2009\)](#) investigated the toxicity of TBA and its effects on cell growth and cell cycle progression in Rat-1 diploid immortalized fibroblasts. Cells were exposed to TBA (0–100 mM) for 48 hours. The  $IC_{50}$  for TBA was reported to be 0.44 mM. TBA inhibited cell growth by ~11.4% and 31.5% after 24 hours and by 31.7% and 53.7% after 48 hours, respectively, at  $IC_{50}$  and at one tenth of the  $IC_{50}$ . TBA exposure at the  $IC_{50}$  altered the cell cycle, causing accumulation in G0/G1 phase and reduction of cells in S phase in 48 hours, with an indication of apoptosis, observed as a comparable progressive increase in the subdiploid peak. In addition, cell cycle progression was also investigated with TBA at a concentration of 0.44 mM, by protein expression analysis of p53, cyclin D1, the cyclin-dependent kinase (CDK) inhibitor p27<sup>Kip1</sup>, and retinoblastoma protein (pRb). The expression of p53 protein increased between 6 hours and 12 hours after treatment, then declined thereafter. A progressive increase in pRb (indicating cell cycle arrest), rapid upregulation of p27<sup>Kip1</sup>, and a reduction in expression levels of cyclin D1 were also observed.

[Guizzetti et al. \(2004\)](#) investigated phospholipase D activation and downstream signalling targets in carbachol-induced proliferation of astrocytoma cells after exposure to TBA (0–25 mM) and other alcohols. Whereas other alcohols induced concentration-dependent inhibition of carbachol-induced DNA synthesis, TBA was reported to be less effective. TBA did not affect phospholipase D-mediated formation of protein kinase C zeta (PKC  $\zeta$ ) activator phosphatidic acid, or phosphatidyl-*tert*-butanol, or the activation of PKC  $\zeta$  and p70S6K (an important downstream target for PKC) in astrocytoma cells stimulated with carbachol.

In a study by [Kötter and Klein \(1999\)](#), alcohol-induced inhibition of cell proliferation by disruption of phospholipase D-mediated

signalling was investigated in rat cortical primary astrocytes. Although the formation of phosphatidic acid and astroglial cell proliferation were unaffected by TBA, platelet-derived growth factor (PDGF)-induced cell proliferation was inhibited by all the butanols tested, with TBA (0.3% v/v) being the least potent.

*Synopsis*

[The Working Group noted that there was some evidence that exposure to TBA can increase cell proliferation and induce hyperplasia in the kidney and urinary bladder in male and female rats and mice.]

**4.3 Other relevant evidence***Studies reporting effects related to  $\alpha_{2u}$ -globulin in the kidney of male rats*

As for automotive gasoline, MTBE, and ETBE (see the monographs on these agents in the present volume), several studies have reported on the potential of TBA to induce tumours in the kidney of male rats through a mechanism mediated by the protein  $\alpha_{2u}$ -globulin. Exposure to TBA for 2 years was associated with mineralization and renal tubule hyperplasia in male rats, transitional epithelial hyperplasia (see Section 4.2.7), and increased severity of nephropathy of the kidney in male and female rats, follicular cell hyperplasia of the thyroid gland in male and female mice (see Section 4.2.7), and chronic inflammation (see Section 4.2.4) and hyperplasia of the urinary bladder (see Section 4.2.7) in male mice and, to a lesser extent, in female mice.

The incidence of mineralization in the kidney increased with dose and was significantly greater in male rats exposed to TBA than in the controls. [The Working Group noted that agencies in the USA had concluded that the spectrum of renal neoplastic and non-neoplastic lesions and responses observed in male and female rats exposed to TBA indicated that multiple

mechanisms, either  $\alpha_{2u}$ -globulin-mediated or not  $\alpha_{2u}$ -globulin-mediated, could be responsible ([NTP, 1995](#); [US EPA, 2020](#)).]

Alterations in kidney tissue were identified by [Acharya et al. \(1997\)](#) in male Wistar rats after TBA exposure at subtoxic doses in drinking-water. Histopathology examination confirmed degeneration of the kidney tissue, with degeneration of the Bowman capsule membrane and renal tubular cell proliferation in certain areas. Nevertheless, other studies have linked alterations in the kidney to chronic nephropathy that seems to be mediated by the accumulation of the protein  $\alpha_{2u}$ -globulin. [Borghoff et al. \(1992\)](#) described how  $\alpha_{2u}$ -globulin protein released by the liver can accumulate in the kidney, passing through the glomerular filter in the nephron. The authors measured the amount of globulin in kidney homogenates and protein droplets after exposure to unleaded gasoline and 2,2,4-trimethylpentane.

[Takahashi et al. \(1993\)](#) reported treatment-related increases in hyaline droplets and abnormal intracytoplasmic deposits in the proximal epithelial cells of the cortex and outer medulla, and  $\alpha_{2u}$ -globulin nephropathy and associated cell proliferation in a subchronic study in male F344 rats exposed to TBA (0.25%, 0.5%, 1%, 2%, and 4% w/v) in drinking-water. The severity of nephropathy increased with hyaline droplet accumulation and deposition of abnormal crystals. Although the number of PCNA-stained S-phase nuclei was significantly increased in the 2% dose group, there was a significant decrease in PCNA-labelled renal tubular epithelial cells in the 4% dose group, when compared with the controls.

In another study, a reversible, non-covalent binding of TBA to  $\alpha_{2u}$ -globulin was reported in F344 rats treated with TBA by gavage as a single dose of 500 mg/kg ([Williams and Borghoff, 2001](#)). The renal concentration of  $\alpha_{2u}$ -globulin, as measured in the kidney cell cytosol by ELISA, was found to be significantly higher in treated

male rats than in controls. The kidney cell cytosol was further analysed by gel filtration, and the presence of droplets was confirmed in male rats only, and results indicated that TBA was binding to  $\alpha_{2u}$ -globulin. Similar results were reported in a study in rats treated by inhalation ([Borghoff et al., 2001](#)), in which it was also found that the renal concentration of  $\alpha_{2u}$ -globulin was positively correlated with cell proliferation in the kidney of male rats and with exposure levels (0, 250, 450, and 1750 ppm for up to 10 days, 6 hours per day). A series of PBPK model studies have been refined to include various parameters and to describe the binding to globulin ([Leavens and Borghoff, 2009](#); [Salazar et al., 2015](#); [Borghoff et al., 2017](#)).

More recent re-evaluation of the NTP data by [Hard et al. \(2011, 2019, 2022\)](#) confirmed the accumulation of droplets in the proximal convoluted tubules and precursors of granular casts in the outer medulla indicating  $\alpha_{2u}$ -globulin nephropathy in males in the 13-week study. In this study, the normal pattern of round hyaline droplets in proximal convoluted tubules was replaced by a pattern of angular droplet accumulation and identified precursors of granular casts in the outer medulla that are typical of  $\alpha_{2u}$ -globulin nephropathy ([Hard et al., 2011](#)).

In the 2-year study, CPN was confirmed to be more pronounced in males and females at the highest dose (5 mg/mL) with hyperplasia of the papilla lining and no evidence of urothelial (transitional) cell hyperplasia. Linear papillary mineralization, another hallmark of the  $\alpha_{2u}$ -globulin pathway, was seen only in treated male rats. CPN was exacerbated in males and females at the highest dose, with a relationship between advanced grades of CPN and renal tumour occurrence. Hyperplasia of the papilla lining was a component of CPN in both sexes, but there was no pelvic urothelial hyperplasia. Females at the highest dose showed no TBA-related nephrotoxicity ([Hard et al., 2011](#)).

[Hard et al. \(2019\)](#) further reviewed the histology and confirmed the TBA-related development of renal tumours. The non-neoplastic and neoplastic histopathological changes in the kidney associated with TBA exposure ([NTP, 1995](#)) were explained by  $\alpha_{2u}$ -globulin nephropathy or enhanced CPN with no additional modes of action identified.

In a study of reproductive toxicity, the effects of vapour condensate of gasoline or of gasoline blended with various fuel oxygenates (such as TBA, 16.5% of total mass) were evaluated in rats after inhalation exposure at 0, 2000, 10 000, or 20 000 mg/m<sup>3</sup>, for 6 hours per day, 7 days per week, over one or two generations ([Gray et al., 2014](#)). There were no remarkable clinical observations, pathological changes to reproductive organs, or adverse reproductive findings observed. A statistically significant reduction in body-weight gain during the premating period was observed in females exposed to gasoline with TBA at 20 000 mg/m<sup>3</sup>. Although no data were shown, it was reported that a statistically significant increase was observed in male kidney weights in the groups at 10 000 and 20 000 mg/m<sup>3</sup>, consistent with light hydrocarbon nephropathy. In addition, the presence of hyaline droplets in the kidney of males at 20 000 mg/m<sup>3</sup> was observed.

As described in the monographs on automotive gasoline and the other oxygenate additives (see Section 4.3 in the respective monographs), [Clark et al. \(2014\)](#) exposed Sprague-Dawley rats to vapour condensates of either gasoline alone or gasoline combined with TBA for 13 weeks via inhalation (6 hours per day, 5 days per week). Target concentrations were 0, 2000, 10 000, or 20 000 mg/m<sup>3</sup>. Increased kidney weight and light hydrocarbon nephropathy were observed in treated male rats in all studies and were reversible, or nearly reversible (to almost baseline levels), after 4 weeks of no exposure. The no-observed-adverse-effect concentration (NOAEC) for gasoline plus TBA was 2000 mg/m<sup>3</sup>. [The

Working Group noted that in these two studies the observed effects in the kidney might reflect the effects of gasoline plus TBA, and not of TBA alone. The Working Group further noted that the available literature indicated that in male rats the  $\alpha_{2u}$ -globulin-mediated response could be one mechanism explaining the kidney tumours in the NTP study ([NTP, 1995](#)).]

## 5. Summary of Data Reported

### 5.1 Exposure characterization

*tert*-Butyl alcohol (TBA) is a volatile alcohol and a high-production-volume chemical, which is manufactured as a major co-product in the synthesis of propylene oxide. TBA is used in the production of isobutylene for synthetic rubber manufacturing and in the production of the gasoline oxygenates methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE). It is present in gasoline in low amounts, either as part of the formulation to increase combustion efficiency or as an impurity from MTBE or ETBE. TBA is used as a solvent and dehydrating agent, and in the manufacture of certain plastics, industrial cleaning products, pharmaceuticals, coatings on paperboard food containers, food flavourings, cosmetics, and fragrances. Contemporary information on environmental occurrence and exposure is limited, and no information was available from low- and middle-income countries (LMICs). In addition to being released into the environment by industrial activities, TBA can result naturally from the microbial metabolism of isobutane. TBA has been detected in ground-water contaminated via leaking underground storage tanks and in drinking-water after the installation of plastic pipes. Occupational exposure is expected to occur mainly in the production of chemicals and various products, as well as through gasoline vapour. Several countries have

established standards for occupational exposure to TBA.

## 5.2 Cancer in humans

No data were available to the Working Group.

## 5.3 Cancer in experimental animals

Treatment with TBA caused an increase in the incidence of malignant neoplasms or a combination of benign and malignant neoplasms in one sex (male) of a single species (rat) in one study that complied with Good Laboratory Practice (GLP).

In the well-conducted GLP study in male and female F344/N rats exposed by oral administration (drinking-water), TBA caused renal tubule adenoma, and renal tubule adenoma or carcinoma (combined) in males.

## 5.4 Mechanistic evidence

Regarding the absorption, distribution, metabolism, and excretion of TBA, data were available from studies in humans and from experimental systems. Most of these data on TBA were obtained from studies with MTBE and ETBE, because TBA is a major intermediate metabolite of these ethers. A single study of oral administration in one human volunteer showed that TBA is absorbed orally.

In rodents, TBA is absorbed via inhalation and after ingestion. Studies showed that TBA is oxidatively metabolized, mainly in the liver, to 2-methyl-1,2-propanediol and further to 2-hydroxyisobutyric acid (2-HIBA). These metabolites are excreted in the urine together with minor amounts of TBA and formaldehyde plus acetone. TBA and its metabolites are readily distributed in the body. The metabolism patterns of TBA are qualitatively similar in humans and rats.

There is suggestive evidence from experimental systems that TBA exhibits one of the key characteristics of carcinogens, namely, that TBA alters cell proliferation, cell death, or nutrient supply. No data were available in exposed humans and in human primary cells. In a few studies, cell proliferation and hyperplasia were reported in the kidney and the bladder in male and female rats and mice.

For other key characteristics of carcinogens, there is a paucity of available data.

# 6. Evaluation and Rationale

## 6.1 Cancer in humans

There is *inadequate* evidence in humans regarding the carcinogenicity of *tert*-butyl alcohol.

## 6.2 Cancer in experimental animals

There is *limited* evidence in experimental animals for the carcinogenicity of *tert*-butyl alcohol.

## 6.3 Mechanistic evidence

There is *limited* mechanistic evidence.

## 6.4 Overall evaluation

*tert*-Butyl alcohol is *not classifiable as to its carcinogenicity to humans* (Group 3).

## 6.5 Rationale

The Group 3 evaluation for *tert*-butyl alcohol (TBA) is based on *limited* evidence for cancer in experimental animals, *limited* mechanistic evidence, and *inadequate* evidence regarding cancer in humans. The *limited* evidence for cancer



in experimental animals is based on an increase in the incidence of malignant neoplasms and a combination of benign or malignant neoplasm in one sex (male) of one species (rat), in one study conducted under Good Laboratory Practice. For TBA, the mechanistic evidence was *limited* on the basis of suggestive evidence of altered cell proliferation, cell death, or nutrient supply. The evidence regarding cancer in humans was *inadequate* because no studies were available.

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# DIISOPROPYL ETHER

## 1. Exposure Characterization

### 1.1 Identification of the agent

#### 1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 108-20-3 ([NCBI, 2025](#))

EC/List No.: 203-560-6 ([ECHA, 2025a](#))

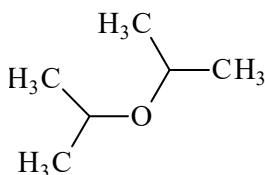
IUPAC systematic name: 2-propan-2-yloxypropane ([NCBI, 2025](#))

Synonyms: isopropyl ether, diisopropyl ether, 2-isopropoxypropane, 2-isopropoxyisopropane, 2-propan-2-yloxypropane, 2,2'-oxy-bispropane, 2,2'-oxydipropene ([ECHA, 2025a](#)); 1,1'-dimethyldiethyl ether, 2,2'-oxybispropane ([NCBI, 2025](#)).

#### 1.1.2 Structural and molecular information

Molecular formula: C<sub>6</sub>H<sub>14</sub>O ([ECHA, 2025a](#))

Molecular structure: ([ECHA, 2025a](#))



Relative molecular mass: 102.17 ([NCBI, 2025](#)).

#### 1.1.3 Chemical and physical properties

*Description:* clear, colourless, highly flammable liquid with a characteristic ethereal odour ([NCBI, 2025](#)). The odour threshold is of 0.071 mg/m<sup>3</sup> ([Ruth, 1986](#)) or [0.72 mg/m<sup>3</sup>] ([IFA, 2025](#)).

*Boiling-point:* 68.2–68.27 °C at 101.325 kPa ([ECHA, 2025a](#))

*Melting point:* –85.89 °C at 101.325 kPa ([ECHA, 2025a](#))

*Relative density* (water = 1): 0.723 at 20 °C ([ECHA, 2025a](#))

*Vapour pressure:* 19.865 kPa at 25 °C ([ECHA, 2025a](#))

*Water solubility:* 3.11 g/L at 20.2 °C ([ECHA, 2025a](#))

*Octanol/water partition coefficient (P):* log K<sub>ow</sub> = 2.4 at 20 °C ([ECHA, 2025a](#))

*Henry's law constant:* 2.57 at 25 °C ([ChemicalBook, 2025](#))

*Autoignition temperature:* 415 °C at 101.9 kPa ([ECHA, 2025a](#))

*Explosive limits:* 1.4–7.9% vol. in air ([NCBI, 2025](#))

*Relative vapour density* (air = 1): 3.5 ([NCBI, 2025](#))

*Relative density of the vapour/air mixture at 20 °C* (air = 1): 1.5 ([ILO, 2021](#)).

### 1.1.4 Technical products and impurities

Historically, commercial diisopropyl ether (DIPE) may have contained approximately 3% isopropyl alcohol, <0.01% sulfur, and <0.04% peroxide ([Browning, 1965](#)). A current product with a purity of  $\geq 98.5\%$  may contain  $\leq 0.001\%$  peroxides (as  $\text{H}_2\text{O}_2$ ),  $\leq 0.005\%$  free acid (as  $\text{C}_2\text{H}_5\text{COOH}$ ),  $\leq 0.1\%$  water, and  $\leq 0.5\%$  isopropanol as impurities ([Merck, 2025](#)).

## 1.2 Production and uses

### 1.2.1 Production

DIPE is produced as a by-product of the production of isopropyl alcohol from propylene ([NCBI, 2024](#)).

The Organisation for Economic Co-operation and Development has classified DIPE as a high-production-volume chemical ([OECD, 2024](#)). The market size for DIPE in 2022 was estimated to be 460 thousand tonnes ([ChemAnalyst, 2023](#)). Manufacturers or importers of DIPE can be found in many countries throughout the world, including Belgium, China, Germany, Japan, Sweden, and the United States of America (USA) ([ChemAnalyst, 2023](#); [ECHA, 2025b](#)). Production of DIPE in 2019 in the USA was reported to range from [22 680 to 45 359 tonnes] ([NCBI, 2024](#)). Data on total production volumes were not available for other countries.

### 1.2.2 Uses

DIPE is used as a solvent for paints, oils, waxes, resins, and dyes ([NCBI, 2024](#)). Other uses include the manufacture of active pharmaceutical ingredients ([Romanelli and Evandri, 2018](#)) and as a chemical in scanning electron microscopy laboratories ([Bance et al., 1981](#)).

DIPE also is used as a gasoline blending component to increase the octane levels of gasoline ([NCBI, 2024](#); [Stout and Healey, 2024](#)). Data on the prevalence of DIPE in blended gasoline

sources were limited from publicly available sources. In Europe, a survey by the fuel industry found DIPE in seven out of 650 gasoline samples tested from 1999 to 2009, in one or two samples from Bulgaria, Greece, Malta, Italy, and Sweden. The mean and median concentration of DIPE for all samples was below 0.01 m/m% (wt%), the maximum concentration detected was 0.88 wt% in premium gasoline from Greece in winter 2002/2003 ([Concawe, 2012](#)). [The Working Group noted that few data were publicly available on the composition and production volumes of gasoline and its additives. Because independently verifiable data were lacking, data published by Concawe, the science division of a trade organization, are presented.] In more recent data from the fuel industry covering 2010–2015 and a much smaller number of gasoline samples, the average DIPE concentrations remained below 0.01 wt% and the maximum concentration was 0.01 wt% in a sample from the United Kingdom, but DIPE was not analysed in two of the three additional data sets ([Concawe, 2018](#)).

In the USA, the concentrations of DIPE and other oxygenates were measured in gasoline samples collected in states that had banned the use of methyl *tert*-butyl ether (MTBE) (from 2004 to 2020) and in gasoline samples collected before the bans (from 1993–2002 or 2004). In samples collected before the bans, DIPE was not detected in 18 samples of oxyfuel or reformulated gasoline or in 57 samples of conventional gasoline. In samples collected after the ban, DIPE was detected in 11 out of 76 gasoline samples, at concentrations ranging from 43 to 3480 mg/kg ([Stout and Healey, 2024](#)).

## 1.3 Analytical methods

For analytical methods of detection and quantification of DIPE, see Section 1.3 of the monograph on MTBE in the present volume.

## 1.4 Occurrence and exposure

### 1.4.1 Environmental occurrence

#### (a) Air

According to the physical properties of DIPE (see Section 1.1), some evaporation would be expected. However, no information on concentrations of DIPE in air was found in the literature.

#### (b) Water

Fuel oxygenates including DIPE were analysed in groundwater at sites with leaking underground fuel tanks near community drinking-water sources in the greater Los Angeles region, California, USA in 2000–2001. With a minimum limit of detection (LOD) of 2 µg/L, DIPE was detected at 23.7% of sites tested (206 sites). The geometric mean was 31 µg/L (range, 0.36–4700 µg/L) ([Shih et al., 2004](#)).

In a study focused on MTBE levels in samples collected between 1993 and 2002 in the USA in groundwater wells used for drinking-water, an incidental finding was the detection of DIPE in 0.19% of samples (4 of 2077 samples) ([Moran et al., 2005](#)). Although the DIPE values were not reported, a related report indicated detection frequencies of 0.58% and 0.13% for the assessment levels of 0.02 µg/L and 0.2 µg/L, respectively, in 1547 environmental water samples ([Zogorski et al., 2006](#)).

In Düsseldorf, Germany, 96 samples were collected from 14 groundwater wells contaminated by a gasoline spill between 2001 and 2003. DIPE was one of 12 target chemicals monitored. Out of 96 samples, 37 contained DIPE at concentrations above the LOD (0.008 µg/L). DIPE concentrations ranged from 0.01 µg/L to 0.29 µg/L; the average was 0.07 µg/L, and the median was 0.05 µg/L ([Rosell et al., 2005](#)). In 2012, an industry trade association issued a report that provided limited data on measures of DIPE in groundwater in Europe. In Switzerland, DIPE levels in 2006 from 19 groundwater

sampling locations were all below the limit of quantification (0.05 µg/L). In groundwater and surface water monitoring data from England and Wales in 1997–1998, DIPE levels were consistently below the LOD (50 µg/L) in 96 samples from 14 locations (for groundwater) ([Concawe, 2012](#)). [The Working Group noted the higher LOD for DIPE in some older studies.] Surface water of the river Rhine, used for the production of drinking-water, has been monitored for DIPE in the Kingdom of the Netherlands since 2003. DIPE concentrations were mainly below or close to the LOD (0.01–0.1 µg/L) ([RIWA-Rijn, 2025](#)). The maximum concentration measured was 0.199 µg/L in 2017 ([RIWA-Rijn, 2017](#)).

#### (c) Soil

No empirical measurement data were available regarding DIPE in soil.

#### (d) Food and consumer products

No empirical measurement data were available regarding DIPE in food. Other than in gasoline (described in Section 1.2.2), no empirical measurement data were available regarding DIPE in consumer products.

### 1.4.2 Occupational exposure

DIPE is still used today primarily as a solvent in various industrial applications (as an extraction solvent, reaction medium, and intermediate in the synthesis of fine chemicals, and a degreasing and cleaning agent). [The Working Group could not identify any occupational exposure studies for DIPE.]

[DIPE exposures among workers are expected to follow similar patterns as those for other ethers such as MTBE, with inhalation and skin contact being the primary routes of exposure. Workers in fuel production, transport, and storage are expected to have the highest potential for exposure. According to their respective boiling points, DIPE is less volatile than MTBE but more volatile

than ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME). Volatility influences factors like evaporation rates and fuel blending characteristics, and thus DIPE would be considered similar to MTBE.]

#### 1.4.3 Exposure of the general population

Limited data were available regarding exposure of the general population to DIPE. Potential routes of exposure include inhalation during gasoline pumping or from air pollution near sources of DIPE releases, and ingestion from contaminated water supplies. A literature search identified two studies that examined personal exposures to DIPE, unrelated to occupational exposures, that used biomonitoring approaches.

In central Italy, urinary levels of DIPE and three other gasoline oxygenates (MTBE, ETBE, and TAME) were measured in a sample of Caucasian [White] schoolchildren aged 5–11 years. Samples were collected from children during summer 2017 ( $n = 313$ ) and winter 2018 ( $n = 263$ ) in two areas where population and traffic density were characterized as low (rural) or medium (urban). Of the oxygenates measured, only urinary DIPE was below its quantification limit (6.1 ng/L) in all samples. The authors interpreted these findings as indicating that there was minimal or no use of DIPE as a gasoline additive in Italy ([Antonucci et al., 2021](#)).

In the USA, a new method for detecting low levels of DIPE and other oxygenate ethers was applied to blood samples previously collected from three volunteers. The volunteers were described only as healthy with no documented exposure to DIPE, MTBE, ETBE, or TAME. Two of the three volunteers were found to have trace levels of DIPE in their blood; average values with standard deviations over three samples per person were 43.9 (12.9) ng/L and 5.65 (0.39) ng/L ([Silva et al., 2008](#)).

## 1.5 Exposure limits, regulations, and guidelines

In the USA, the recommended occupational exposure limit for DIPE, first established in 1972 by the National Institute for Occupational Safety and Health (NIOSH), is 500 ppm (2100 mg/m<sup>3</sup>) ([NIOSH, 2019](#)). This value was subsequently adopted as the Occupational Safety and Health Administration permissible exposure limit in 1989.

Only the USA and South Africa were identified as having a time-weighted average (TWA) limit value for DIPE of 500 ppm [2100 mg/m<sup>3</sup>]. Many countries have a TWA limit value of half that amount, 250 ppm (1040 mg/m<sup>3</sup>), including Australia, Austria, Belgium, Canada (Ontario and Quebec provinces), Denmark, Finland, France, Ireland, New Zealand, Singapore, South Africa (mining), Republic of Korea, Spain, and the United Kingdom. Germany and Switzerland have a TWA limit value of 200 ppm (850 mg/m<sup>3</sup>), and Norway has a TWA limit value of 125 ppm (525 mg/m<sup>3</sup>) ([IFA, 2024](#)). Some countries, namely Australia, Belgium, Canada, Ireland, New Zealand, Singapore, South Africa, Spain, and the United Kingdom, also have a short-term exposure limit (STEL) of 310 ppm (1300 mg/m<sup>3</sup>) ([IFA, 2024](#)). Other countries have higher values for the STEL, including Denmark (500 ppm; 2100 mg/m<sup>3</sup>), and Germany and Switzerland (400 ppm; 1700 mg/m<sup>3</sup>).

In the USA in 2024, the American Conference of Governmental Industrial Hygienists (ACGIH) adopted a new recommended 8-hour TWA occupational exposure limit for DIPE of 20 ppm (84 mg/m<sup>3</sup>), to minimize the risk of adverse fetal effects and of systemic toxicity in adults ([ACGIH, 2024](#)). The previous STEL of 310 ppm, first adopted in 1976, was withdrawn.

In 2011, the United States Environmental Protection Agency (US EPA) issued a provisional reference concentration (inhalation) for DIPE for the general population, for both subchronic and

chronic exposure, of 0.7 mg/m<sup>3</sup>, based on health outcomes other than cancer ([US EPA, 2011](#)).

[The Working Group noted that no reference values related to biological monitoring of DIPE were available.]

## 2. Cancer in Humans

No data were available to the Working Group.

## 3. Cancer in Experimental Animals

### 3.1 Rat

See [Table 3.1](#).

#### *Oral administration (gavage)*

In a study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 100 male and 100 female Sprague-Dawley rats, (age, 8 weeks at start) were exposed orally by gavage to DIPE (purity, >98%) in 1 mL of extra virgin olive oil at target doses of 0 (control), 250, or 1000 mg/kg body weight (bw) for 4 days per week for up to 78 weeks ([Belpoggi et al., 2002](#)). Control animals received 1 mL of extra virgin olive oil without DIPE. After this treatment, all rats were maintained under observation until spontaneous death, without receiving any additional treatment. A decrease in survival was observed in treated males compared with controls during the period between age 56 and 88 weeks, and it was not reported whether one or both treated groups were affected. [Survival data were not reported and/or tabulated.] The authors reported that the experiment ended after 163 weeks with the death of the last rat at age 171 weeks. The authors stated that there were no significant differences in daily water and feed consumption and body weight across groups. [No data on body weight were

reported.] All rats underwent complete necropsy and histopathological evaluation.

In males, there was a significant positive trend ( $P < 0.05$ , Cochran–Armitage trend test) in the incidence of haemolymphoreticular tumours (including lymphoblastic lymphoma, lymphoblastic leukaemia, lymphocytic lymphoma, lymphoimmunoblastic lymphoma, and histiocytic sarcoma/monocytic leukaemia and myeloid leukaemia), with the incidence – 17/100, 27/100, and 31/100 in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively – being significantly increased ( $P < 0.05$ ,  $\chi^2$  test; [ $P < 0.0154$ , one-tailed Fisher exact test]) at the highest dose compared with the vehicle control group.

In males, the incidence of carcinoma of the ear duct – 1/100, 9/100, and 4/100 in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively – was significantly increased in the group at 250 mg/kg bw compared with the control group ( $P < 0.05$ ,  $\chi^2$  test; [ $P = 0.0092$ , Fisher exact test]). There was also a significant increase in the incidence of total malignant tumours in the group at 250 mg/kg bw compared with the control group ( $P < 0.05$ ,  $\chi^2$  test; [ $P = 0.0143$ , one-tailed Fisher exact test]), incidence being 30/100, 46/100, and 38/100 in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively.

There was a slight but nonsignificant increase in the incidence of testicular interstitial cell adenoma – 0/100, 2/100, and 2/100 in the groups at 0, 250, and 1000 mg/kg bw, respectively – and in the incidence of glioma (oligodendroglioma) – 0/100, 4/100, and 3/100 in the groups at 0, 250, and 1000 mg/kg bw, respectively. [The Working Group noted that gliomas were rare tumours (incidence, approximately 0.5–1%) among historical controls in this colony of Sprague-Dawley rats.]



**Table 3.1 Studies of carcinogenicity in experimental animals exposed to diisopropyl ether**

| Study design<br>Species, strain (sex)<br>Age at start<br>Duration<br>Reference                                              | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                   | Tumour incidence                                                                                                                                                                                                                                                                                                                                                                                   | Significance                                                                                                                                                                                                                                                                                                                                                 | Comments                                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Rat, Sprague-Dawley (CRC/RF colony) (M)<br>8 wk<br>135 wk<br><a href="#">Belpoggi et al. (2002)</a> | Oral administration (gavage)<br>DIPE, > 98%<br>Extra virgin olive oil<br>0, 250, 1000 mg/kg bw<br>4 days/wk for 78 wk<br>100, 100, 100<br>NR | <i>Haemolymphoreticular system</i><br>Lymphoma/leukaemia<br>17/100, 27/100, 31/100*<br><br><i>Ear duct</i><br>Carcinoma<br>1/100, 9/100*, 4/100<br><br><i>Testis</i><br>Interstitial cell adenoma<br>0/100, 2/100, 2/100<br><br><i>Brain</i><br>Glioma (oligodendroglioma)<br>0/100, 4/100, 3/100<br><br><i>All organs</i><br>Total malignant tumours<br>Tumour incidence: 30/100, 46/100*, 38/100 | $P < 0.05$ , Cochran–Armitage trend test<br>* $P < 0.05$ , $\chi^2$ test; [ $P = 0.0154$ , one-tailed Fisher exact test]<br><br><br><br><br><br><br><br><br><br>$P < 0.05$ , $\chi^2$ test; [ $P = 0.0092$ , one-tailed Fisher exact test]<br><br><br><br><br><br><br><br><br><br>$P < 0.05$ , $\chi^2$ test; [ $P = 0.0143$ , one-tailed Fisher exact test] | <i>Principal strengths:</i> GLP study, complete histopathology, male and female animals used, lifetime observation, high number of animals per group.<br><br><i>Principal limitations:</i> Contains little experimental detail on exact design, clinical observations, body-weight gain or survival, lack of historical control data, lack of mortality-adjusted tumour incidence analysis. |
| Full carcinogenicity<br>Rat, Sprague-Dawley (CRC/RF colony) (F)<br>8 wk<br>135 wk<br><a href="#">Belpoggi et al. (2002)</a> | Oral gavage (gavage)<br>DIPE, > 98%<br>Extra virgin olive oil<br>0, 250, 1000 mg/kg bw<br>4 days/wk for 78 wk<br>100, 100, 100<br>NR         | <i>Haemolymphoreticular system</i><br>Lymphoma/leukaemia<br>7/100, 28/100*, 41/100*<br><br><i>Ear duct</i><br>Carcinoma<br>2/100, 3/100, 4/100                                                                                                                                                                                                                                                     | $P < 0.01$ , Cochran–Armitage trend test<br>* $P < 0.01$ , $\chi^2$ test; [ $P < 0.0001$ , one-tailed Fisher exact test]<br><br><br><br><br><br><br><br><br><br>$P < 0.01$ , Cochran–Armitage trend test<br>* $P < 0.01$ , $\chi^2$ test; [ $P < 0.0001$ , one-tailed Fisher exact test]                                                                     | <i>Principal strengths:</i> GLP study, complete histopathology, male and female animals used, lifetime observation, high number of animals per group.<br><br><i>Principal limitations:</i> Contains little experimental detail on exact design, clinical observations, body-weight gain or survival, lack of historical control data, lack of mortality-adjusted tumour incidence analysis. |



**Table 3.1 (continued)**

| Study design<br>Species, strain (sex)<br>Age at start<br>Duration<br>Reference                                                         | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals | Tumour incidence                                                                                                                                                                                                                                                                 | Significance                                                                                                                                                                                                                            | Comments |
|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Full carcinogenicity<br>Rat, Sprague-Dawley (CRC/RF colony) (F)<br>8 wk<br>135 wk<br><a href="#">Belpoggi et al. (2002)</a><br>(cont.) |                                                                                                            | <i>Uterus and vagina</i><br>Schwannoma<br>3/100, 8/100, 5/100<br><i>Brain</i><br>Glioma (oligodendroglioma)<br>2/100, 3/100, 2/100<br><i>Brain</i><br>Multiform glioblastoma<br>0/100, 1/100, 0/100<br><i>All organs</i><br>Total malignant tumours<br>37/100, 55/100*, 57/100** | NS<br><br>NS<br><br>NS<br><br>$P < 0.01$ , Cochran–Armitage trend test<br>* $P < 0.05$ , $\chi^2$ test; [ $P = 0.0078$ , one-tailed Fisher exact test]<br>** $P < 0.01$ , $\chi^2$ test; [ $P = 0.0035$ , one-tailed Fisher exact test] |          |

bw, body weight; DIPE, diisopropyl ether; F, female(s); GLP, Good Laboratory Practice; M, male(s); NR, not reported; NS, not significant; wk, week(s).

In females, there was a significant positive trend ( $P < 0.01$ , Cochran–Armitage trend test) in the incidence of haemolymphoreticular tumours, with the incidence – 7/100, 28/100, and 41/100 in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively – being significantly increased in the groups at 250 mg/kg bw ( $P < 0.01$ ,  $\chi^2$  test; [ $P < 0.0001$ , one-tailed Fisher exact test]) and 1000 mg/kg bw ( $P < 0.01$ ,  $\chi^2$  test; [ $P < 0.0001$ , one-tailed Fisher exact test]) compared with the control group. There was also a significant positive trend ( $P < 0.01$ , Cochran–Armitage trend test) in the incidence of total malignant tumours – 37/100 (37%), 55/100 (55%), and 57/100 (57%) in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively – and there was a significant increase in the groups at 250 mg/kg bw ( $P < 0.05$ ,  $\chi^2$  test; [ $P = 0.0078$ , one-tailed Fisher exact test]) and 1000 mg/kg bw ( $P < 0.01$ ,  $\chi^2$  test; [ $P = 0.0035$ , one-tailed Fisher exact test]) compared with the control group.

Also in females, there was a slight, nonsignificant increase in the incidence of carcinoma of the ear duct (2/100, 3/100, 4/100), schwannoma of the uterus-vagina (3/100, 8/100, 5/100), glioma (oligodendroglioma) (2/100, 3/100, 2/100), and multiform glioblastoma (0/100, 1/100, 0/100) in the groups at 0 (control), 250, and 1000 mg/kg bw, respectively.

[The Working Group noted that the increase in the total number of haemolymphoreticular tumours (in males and females) was statistically significant only when the haemolymphoreticular tumours were merged as lymphoma/leukaemia, but there was no evidence of any statistically significant effect when the analysis was conducted for the specific cellular type. The Working Group also noted that the diagnostic criteria did not follow the current standards for the haemolymphopoietic system, including tumour diagnostic dispositions. The study pathologist classified the haemolymphoreticular tumours as several types using standard haematoxylin and eosin stain; confirmation by

immunohistochemistry would have resolved uncertainty in cellular subtype recognition. In addition, the study authors reported increases in the incidence of several neoplastic lesions in the treatment groups compared with the control group; however, these findings did not reach statistical significance (i.e. carcinoma of the ear duct in females, interstitial cell adenoma of the testis, a slight increase in malignant schwannoma of the uterus-vagina, and an increased incidence of malignant glial tumours in the brain) and/or a clear dose–response relation (i.e. carcinoma of the ear duct in males). Although the Working Group noted that gliomas are rare tumours, overall, it concluded that the slight increases in the incidence of these tumours in females were not treatment-related.]

[Although not reported, the authors mentioned in the text that there were no treatment-related increases in non-neoplastic changes. Overall, the Working Group concluded that there was no evidence of a relationship with DIPE treatment.]

[The Working Group noted that this was a GLP study that was strengthened by the use of males and females, a high number of animals per group ( $n = 100$ ), an adequate duration of exposure (up to 78 weeks), complete histopathology, and that the study covered the full lifespan of the animals. The Working Group also noted that the study had several limitations, including that the treatment schedule was 4 days per week for 78 weeks, the poor reporting, the lack of data on body weight, the lack of adequate histopathological description of tumours, the lack of information on non-neoplastic lesions, the lack of historical control data, and the use of two doses only, which limited the statistical power of the study.]

### 3.2 Evidence synthesis for cancer in experimental animals

The carcinogenicity of DIPE has been assessed in one GLP study in male and female Sprague-Dawley rats treated by oral administration (gavage) ([Belpoggi et al., 2002](#)).

In this study ([Belpoggi et al., 2002](#)), in males, there was a significant positive trend in the incidence of haemolymphoreticular tumours, with the incidence being significantly increased compared with that in the vehicle control group. There was a significant increase in the incidence of carcinoma of the ear duct in treated rats compared with the vehicle controls. There was also a significant increase in the incidence of total malignant tumours in treated rats compared with the vehicle control. In females, there was a significant positive trend in the incidence of haemolymphoreticular tumours, with the incidence being significantly increased in all treated groups compared with the vehicle control group. There was also a significant positive trend in the incidence of total malignant tumours, with the incidence being significantly increased in all treated groups compared with the vehicle control group.

[The Working Group noted that the increase in the total number of haemolymphoreticular tumours (in males and females) was statistically significant only when the haemolymphoreticular tumours were merged as lymphoma/leukaemia, but there is no evidence of any statistically significant effect when the analysis was conducted on the specific cellular type. The Working Group also noted that diagnostic criteria did not follow the current standards for the haemolymphopoietic system, including tumour diagnostic dispositions. The Working Group also noted that gliomas are rare tumours and concluded these neoplasms were not treatment-related because of the lack of a dose–response relation.]

## 4. Mechanistic Evidence

### 4.1 Absorption, distribution, metabolism, and excretion

No studies were available to the Working Group.

[The Working Group noted that DIPE and MTBE share similar molecular structures, in that they are both small molecules and are uncharged slightly polar ethers with six and five carbons, respectively. Therefore, it is reasonable to assume that the absorption, distribution, and excretion of unchanged DIPE is similar to that of MTBE (see Section 4.1 of the monograph on MTBE and Annex 4, Supplementary material for Section 4, Mechanistic evidence, online only, available from: <https://publications.iarc.who.int/652>).]

### 4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens ([Smith et al., 2016](#)) encompassed by the agent DIPE. Evidence was available for the key characteristics “is genotoxic” and “is immunosuppressive”. There were no data for the remaining key characteristics of carcinogens.

#### 4.2.1 Experimental systems

See [Table 4.1](#).

In a study on the potential genotoxicity of DIPE that was available to the Working Group, [Schreiner et al. \(2014\)](#) performed assays for micronucleus formation and sister-chromatid exchange in peripheral blood cells of Sprague-Dawley male and female rats that were exposed to gasoline plus DIPE (approximately 18%) by inhalation for 4 weeks (5 days per week) at three doses (2, 10, and 20 g/m<sup>3</sup>). No alterations in either genotoxicity end-point were observed.

**Table 4.1 End-points relevant to genotoxicity and immunosuppression in non-human mammalian systems in vivo exposed to diisopropyl ether**

| Assay                              | Species, strain (sex)      | Tissue | Results <sup>a</sup>                                                              | Dose (LED or HID)   | Route, duration, dosing regimen                                                         | Comments                                                                                                   | Reference                               |
|------------------------------------|----------------------------|--------|-----------------------------------------------------------------------------------|---------------------|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| Micronucleus formation             | Rat, Sprague-Dawley (M, F) | Blood  | –                                                                                 | 20 g/m <sup>3</sup> | Gasoline + 18% DIPE at 2, 10, or 20 g/m <sup>3</sup> by inhalation for 4 wk (5 days/wk) | Did not assess the effects of DIPE alone, only in combination with gasoline. Relevant to KC2. <sup>b</sup> | <a href="#">Schreiner et al. (2014)</a> |
| Sister-chromatid exchange          |                            |        | –                                                                                 |                     |                                                                                         |                                                                                                            |                                         |
| IgM antibody-forming cell response | Rat, Sprague-Dawley (F)    | Spleen | ↓ IgM antibody-forming cell response (at highest dose)                            | 20 g/m <sup>3</sup> | Gasoline + 18% DIPE at 2, 10 or 20 g/m <sup>3</sup> by inhalation for 4 wk (5 days/wk)  | Did not evaluate the effects of DIPE alone, only in combination with gasoline; only evaluated females.     | <a href="#">White et al. (2014)</a>     |
| Spleen weight                      |                            |        | ↑ Relative spleen weight, (only at lowest dose)<br>No change, total spleen weight | 2 g/m <sup>3</sup>  |                                                                                         | Relevant to KC7. <sup>c</sup>                                                                              |                                         |
| Spleen cell viability              |                            |        | No change                                                                         |                     |                                                                                         |                                                                                                            |                                         |

DIPE, diisopropyl ether; F, female(s); HID, highest ineffective dose; IgM, immunoglobulin M; LED, lowest effective dose; M, male(s); wk, week(s).

<sup>a</sup> +, positive; –, negative; ↑, increase; ↓, decrease; or no change.

<sup>b</sup> KC2, key characteristic of carcinogens 2, “is genotoxic”.

<sup>c</sup> KC7, key characteristic of carcinogens 7, “is immunosuppressive”.

[The Working Group noted that in this study DIPE was tested only in combination with gasoline.]

In a study using the same experimental design as above, [White et al. \(2014\)](#) assessed the immunotoxic effects of exposure vapour condensate of gasoline combined with DIPE in Sprague-Dawley female rats, showing that only the highest dose decreased the immunoglobulin M (IgM) antibody-forming cell response. No alterations in total weight, absolute spleen weight, or viability of spleen cells were observed. However, after exposure to gasoline plus DIPE at the lowest dose, a statistically significant increase in relative spleen weight was reported. [The Working Group noted as limitations that the effects of DIPE alone were not assessed, as it was assessed only in combination with gasoline, and only females were evaluated.]

### Synopsis

[Overall, the Working Group noted the paucity of data on the mechanistic evidence associated with DIPE. In addition, the available studies did not assess the effects of DIPE alone, but only in combination with gasoline. Sprague-Dawley rats were the only species and strain used as an experimental model, with sparse types of end-point evaluated in the different studies, thus limiting conclusions.]

#### 4.2.2 Evaluation of high-throughput in vitro screening data

The mechanistic characteristics common to carcinogens (the 10 key characteristics of carcinogens) can be investigated through biochemical and cell-based assays run by the US EPA and the United States National Institutes of Health Toxicity Forecaster/Toxicology in the 21st Century (ToxCast/Tox21) high-throughput screening programmes ([Chiu et al., 2018](#); [Guyton et al., 2018](#)) (see Section 4.2.10 of the monograph on MTBE in the present volume and

Annex 4, Supplementary material for Section 4, Mechanistic evidence, online only, available from: <https://publications.iarc.who.int/652>).

DIPE (CAS No. 108-20-3, DTXSID4021890) has been evaluated in the US EPA and United States National Institutes of Health high-throughput screening assays.

Results were available for 111 assay end-points mapped to the key characteristics for DIPE.

DIPE was considered active in one out of 60 assay end-points mapped to the key characteristic: “alters cell proliferation, cell death, or nutrient supply” (KC10). The assay identified as active was the TOX21\_RT\_HEK293\_FLO\_08hr\_viability. The assay used a dead cell DNA stain for which an increase in measured fluorescence indicated a loss of cell viability. The “8hr” indicates the time at which the viability was measured, post exposure. These assays use HEK293T, a human embryonic kidney cell line, with chemical dosing in a 1536-well plate. The active call was noted with the flags “Hit-call potentially confounded by overfitting”, “Less than 50% efficacy” and “Cell viability assay fit with gnls winning model”. [The Working Group noted that this assay is evaluating cytotoxicity, which is not a relevant end-point for the key characteristics of carcinogens.]

## 5. Summary of Data Reported

### 5.1 Exposure characterization

Diisopropyl ether (DIPE) is a volatile ether that is produced as a by-product of the production of isopropyl alcohol from propylene. It is used as a solvent for paints, dyes, and oils; in the manufacture of pharmaceuticals; and as an additive in gasoline to increase combustion efficiency. Contemporary information on environmental occurrence and exposure was very limited and no information was available from low- and middle-income countries (LMICs). Based on the physical properties of DIPE, some evaporation

could be expected, but no information on concentrations of DIPE in air was available to the Working Group. DIPE has been detected in groundwater at sites with leaking underground fuel tanks. No studies on occupational exposure to DIPE were available; however, exposure may occur in the production and use of this chemical. Several countries have established standards for occupational exposure to DIPE.

## 5.2 Cancer in humans

No data were available to the Working Group.

## 5.3 Cancer in experimental animals

Treatment with DIPE caused an increase in the incidence of malignant neoplasms in one sex (male) of a single species (rat) in one well-conducted study that complied with Good Laboratory Practice (GLP).

In the well-conducted study by oral administration (gavage) study that complied with GLP in male and female Sprague-Dawley rats, DIPE caused carcinoma of the ear duct in males.

## 5.4 Mechanistic evidence

No direct data were available on the absorption, distribution, metabolism, or excretion of DIPE. On the basis of molecular structure, the absorption, distribution, and excretion of unchanged parent compound is expected to be similar for DIPE as for MTBE.

There was a paucity of data for the key characteristics of carcinogens.

DIPE was essentially without effects in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

## 6. Evaluation and Rationale

### 6.1 Cancer in humans

There is *inadequate* evidence in humans regarding the carcinogenicity of diisopropyl ether.

### 6.2 Cancer in experimental animals

There is *limited* evidence in experimental animals for the carcinogenicity of diisopropyl ether.

### 6.3 Mechanistic evidence

The mechanistic evidence is *inadequate*.

### 6.4 Overall evaluation

Diisopropyl ether is *not classifiable as to its carcinogenicity to humans* (Group 3).

### 6.5 Rationale

The Group 3 evaluation of diisopropyl ether is based on *limited* evidence for cancer in experimental animals, *inadequate* evidence regarding cancer in humans, and *inadequate* mechanistic evidence. The *limited* evidence for cancer in experimental animals is based on an increase in the incidence of malignant neoplasms in one sex (male) of a single species (rat) in one well-conducted study carried out under Good Laboratory Practice. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence is *inadequate* because there was a paucity of data.



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# TERT-AMYL METHYL ETHER

## 1. Exposure Characterization

### 1.1 Identification of the agent

#### 1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 994-05-8 ([ECHA, 2025a](#))

EC/List No.: 213-611-4 ([ECHA, 2025a](#))

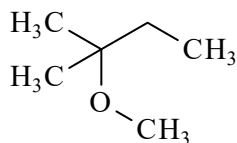
IUPAC systematic name: 2-methoxy-2-methylbutane ([NCBI, 2025](#))

Synonyms: methyl tert-pentyl ether; 1,1-dimethylpropyl methyl ether ([NCBI, 2025](#)).

#### 1.1.2 Structural and molecular information

Molecular formula: C<sub>6</sub>H<sub>14</sub>O ([NCBI, 2025](#))

Molecular structure: ([ECHA, 2025a](#))



Relative molecular mass: 102.17 ([NCBI, 2025](#)).

#### 1.1.3 Chemical and physical properties of the pure substance

Description: highly flammable colourless liquid and vapour with a sweetish odour ([ECHA, 2025a](#); [NCBI, 2025](#))

Boiling-point: 87.3 °C at 101.3 kPa ([ECHA, 2025a](#))

Melting point: -20 °C at 101.3 kPa ([ECHA, 2025a](#))

Density: 0.77 g/mL at 20 °C ([ECHA, 2025a](#))

Vapour pressure: 91 hPa at 25 °C ([ECHA, 2025a](#))

Water solubility: 10.4 g/L at 20 °C ([ECHA, 2025a](#))

Octanol/water partition coefficient (P): log K<sub>ow</sub> = 1.55 at 20 °C ([ECHA, 2025a](#))

Henry's law constant: 1.32 × 10<sup>-3</sup> atm·m<sup>3</sup>/mol at 25 °C ([NCBI, 2025](#))

Autoignition temperature: 430 °C at 101.3 kPa ([ECHA, 2025a](#))

Flash point: -18 °C at 101.3 kPa ([ECHA, 2025a](#))

Relative vapour density (air = 1): 3.6 ([NCBI, 2025](#)).

### 1.1.4 Impurities

Commercial products have a purity of  $\geq 96\%$  or  $\geq 99\%$ . Potential impurities include benzene,  $\leq 0.01\%$ ; water,  $\leq 0.02\%$ ; sum of C5 hydrocarbons,  $\leq 0.50\%$ ; sum of C7 ethers,  $\leq 0.50\%$ ; and *tert*-amyl alcohol (TAA),  $\leq 0.60\%$  ([Merck, 2025](#); [Santa Cruz Biotechnology Inc., 2025](#)).

## 1.2 Production and uses

### 1.2.1 Production

*tert*-Amyl methyl ether (TAME) is produced from methanol and isoamylenes (2-methyl-2-butene and 2-methyl-1-butene) using an acid catalyst. In Europe, nearly all TAME produced by petroleum refineries (97%) exists in a mixed stream of hydrocarbons composed of 10–30% TAME ([European Commission, 2007](#)). TAME is typically produced together with methyl *tert*-butyl ether (MTBE), but production volume is small compared with MTBE ([Bertau et al., 2021](#)).

The Organisation for Economic Co-operation and Development (OECD) classifies TAME as a high-production-volume chemical ([OECD, 2024](#)). In Europe, five companies had active registrations to import or manufacture 1 tonne or more of TAME in 2025 ([ECHA, 2025b](#)). TAME production in Europe was estimated to be between 170 000 and 250 000 tonnes in 2008 ([Bertau et al., 2021](#)). Production of TAME in the United States of America (USA) in 2019 was reported to range from [226 796 to 340 194 tonnes] ([NCBI, 2024](#)). Production and use of TAME are reported to be increasing in Asian countries such as China and India ([Persistence Market Research, c2017](#)). Data on total global production volume were not available from public sources.

### 1.2.2 Uses

TAME is added to fuel to increase the octane rating and is used as an oxygenate to increase the oxygen content and the efficiency of fuel

combustion ([Bertau et al., 2021](#)). No uses unrelated to fuels and fuel additives were identified ([NCBI, 2024](#)).

In Europe, a survey by the fuel industry found TAME in 650 premium and super premium gasoline samples collected in 1999–2009 from Cyprus, Finland, Greece, Italy, Malta, Romania, and Slovenia. The average TAME concentration in European premium unleaded gasoline from 2006–2009 was low ( $< 1\%$ ), and samples from Finland in winter 2007/2008 had the highest concentration at 11.22 m/m% (wt%) ([Concawe, 2012](#)). [The Working Group noted that few data were publicly available on the composition and production volumes of gasoline and its additives. Because independently verifiable data were lacking, data published by Concawe, the science division of a trade organization, are presented.] In more recent data from the fuel industry, the average TAME concentration from 100 samples in 2015 was 0.38 wt%, the median concentration was below 0.01 wt%, and the maximum concentration was 7.53 wt% in a sample from Finland in 2015 ([Concawe, 2018](#)).

In the USA, concentrations of TAME and other oxygenates were measured in gasoline samples collected in states that had banned the use of MTBE (from 2004 to 2020) and in gasoline samples collected before the bans (from 1993–2002 or 2004). In samples collected before the bans, TAME was detected in five out of 18 samples (28%) of oxyfuel or reformulated gasoline, at concentrations ranging from 3040 to 10 100 mg/kg, and in eight out of 57 samples (14%) of conventional gasoline, at concentrations ranging from 228 to 57 600 mg/kg. In samples collected after the ban, TAME was detected in 13 out of 76 gasoline samples (17%) at concentrations ranging from 28 to 727 mg/kg ([Stout and Healey, 2024](#)).



### 1.3 Analytical methods

For the analytical methods for the detection and quantification of TAME, see Section 1.3 of the monograph on MTBE in the present volume.

### 1.4 Occurrence and exposure

#### 1.4.1 Environmental occurrence

The available environmental measurement data on TAME in ambient air, water, and soil were limited. No empirical measurement data were available on TAME in food or consumer products. Measures of TAME in gasoline are described in Section 1.2.2.

##### (a) Air

In the European Union, a report on a risk assessment for TAME included limited air monitoring data from three production sites in Finland. Sampling details were not provided. Ambient air concentrations of TAME at the three sites were reported as  $<400 \mu\text{g}/\text{m}^3$ , well below  $0.1 \text{ mg}/\text{m}^3$ , and below  $1 \mu\text{g}/\text{m}^3$  ([European Commission, 2006](#)).

##### (b) Water

In an analysis of groundwater at sites where leaking underground fuel tanks were near community drinking-water sources in the greater Los Angeles region, California, USA, in 2000–2001, TAME was detected at 18.3% of sites tested (159 sites). The geometric mean was  $24 \mu\text{g}/\text{L}$  (range,  $0.38$ – $12\,000 \mu\text{g}/\text{L}$ ) ([Shih et al., 2004](#)). In a study focused on MTBE levels in groundwater in the USA, an incidental finding was the detection of TAME in 0.25% of samples tested (six of 2382 samples); the TAME values were not reported ([Moran et al., 2005](#)).

In Finland, groundwater monitoring data on TAME measurements at multiple locations from 1995 to 2003 showed a wide range of TAME con-

centrations, up to  $24\,000 \mu\text{g}/\text{L}$  ([European Commission, 2006](#)).

In Germany, TAME was one of 12 target chemicals monitored between 2001 and 2003 in 14 groundwater wells contaminated by a gasoline spill. Out of 96 samples, 27 had TAME concentrations above the limit of detection (LOD) ( $0.013 \mu\text{g}/\text{L}$ ). TAME concentrations ranged from  $0.02$  to  $0.15 \mu\text{g}/\text{L}$ ; the average was  $0.07 \mu\text{g}/\text{L}$ , and the median was  $0.05 \mu\text{g}/\text{L}$  ([Rosell et al., 2005](#)). In 2012, an industry trade association issued a report that summarized data on the occurrence of TAME and other gasoline oxygenates in groundwater in Europe from 2000 to 2010. In the United Kingdom, the TAME was detected in 10 groundwater samples, nine of them at concentrations of  $11$ – $115\,486 \mu\text{g}/\text{L}$ . In England and Wales, routine monitoring of groundwater detected a maximum value of  $0.184 \mu\text{g}/\text{L}$ . At sites targeted for investigations of contamination, the maximum value was  $117 \mu\text{g}/\text{L}$ . In Finland in 2009, baseline groundwater monitoring did not detect TAME. However, 23% of investigations at 287 sites assumed to be in the proximity of point sources detected TAME at concentrations above  $15 \mu\text{g}/\text{L}$ , including nine sites with concentrations above  $1000 \mu\text{g}/\text{L}$  ([Concawe, 2012](#)).

In the Islamic Republic of Iran, monitoring of fuel oxygenates in 30 groundwater wells in eight cities in Sistan and Baluchestan Province failed to detect TAME at concentrations above the LOD of  $66 \text{ ng}/\text{L}$  ([Kaykhaii and Mirbaloochzahi, 2008](#)). Surface water of the river Rhine, used for the production of drinking-water, has been monitored for diisopropyl ether (DIPE) in the Kingdom of the Netherlands since 2006. The highest maximum annual values were above the LOD ( $0.02$ – $0.05 \mu\text{g}/\text{L}$ ) only in 2006 ( $0.06 \mu\text{g}/\text{L}$ ) and 2015 ( $0.04 \mu\text{g}/\text{L}$ ) ([RIWA-Rijn, 2025](#)).

##### (c) Soil

In the United Kingdom, TAME was detected in 19 out of an unknown number of soil samples near service stations, at concentrations ranging

from 0.011 to 49 mg/kg ([Concawe, 2012](#)). In Ilomantsi, Finland, the TAME concentration in soil at a service station in 1997 was 1100 µg/L ([European Commission, 2006](#)). No other measurement data on TAME in soil were available.

#### 1.4.2 Occupational exposure

TAME exposures might occur in jobs and during tasks involving its production, blending, and distribution, particularly among process operators, chemical plant workers, fuel blending technicians, quality control laboratory personnel, and maintenance workers performing tasks such as equipment cleaning, sampling, loading and unloading of fuels, and system repairs where TAME-containing materials may be present. TAME exposures are expected to be absent in industries in which it has been phased out as a fuel additive (such as in the USA and parts of Europe) and to be reduced where engineering controls and protective measures are in place to minimize worker exposure. [Only two older studies quantifying TAME exposures were identified from the literature, thus providing the Working Group with only very limited data. The Working Group expected that TAME exposures would be similar to those for other fuel oxygenates in similar settings.]

Exposure of workers to TAME and MTBE from gasoline with different octane ratings (95, 98, and 99, containing 5.2–11.8% MTBE and 0–6% TAME) during tank lorry loading in Finland (October 1994 and August 1995) was quantified using both air and biological monitoring methods ([Vainiotalo et al., 1998](#)). The TAME personal air geometric mean was 0.98 mg/m<sup>3</sup> for the loading and sampling task (35 minutes) ([Vainiotalo et al., 1998](#)). Blood samples collected 20 minutes after gasoline loading showed that levels of TAME and its metabolite TAA were below the limit of quantification (LOQ) (< 7 and

< 100 nmol/L, respectively) in most blood and urine samples ([Vainiotalo et al., 1998](#)).

TAME exposures were quantified in urine from seven traffic policemen exposed to automotive vehicular traffic in Italy; however, all urinary TAME concentrations were below the LOD (6 ng/L) ([Scibetta et al., 2007](#)). [The seven policemen were sampled to test a newly developed method for quantification of several oxygenated additives. However, tasks carried out by the policeman during the sampling period were not described further in the published article.]

A study was conducted in the summer of 1996 at two self-service stations located in the Helsinki metropolitan area, Finland. Tanker truck drivers' exposure to gasoline vapours was studied by collecting breathing-zone samples during loading and unloading of gasoline during the summers of 1995 and 1996. The geometric mean for TAME ranged from 0.30 to 1.1 mg/m<sup>3</sup> ([Vainiotalo et al., 1999](#)).

TAME exposures in gasoline pump repairers and inspectors at service stations and repair shops in Finland in April to June 2004 were measured both with breathing air samples (passive charcoal sampler) and with biomonitoring (urine collected pre- and post-work shift, and pre-next-day shift) ([Vainiotalo et al., 2006](#)). Workers were exposed to gasoline blends containing mixtures of MTBE and TAME, and the total content of oxygenates was 11–12% (benzene, < 1%). The mean sampling period was 4.5 hours, and the mean time-weighted average (TWA) 8-hour concentrations for TAME were 1.3 mg/m<sup>3</sup> ([Vainiotalo et al., 2006](#)). The mean TAME concentration in air was 4.7 mg/m<sup>3</sup> over 4.5 hours of work, and the exposure range depended mostly on the job tasks being performed indoors or outdoors. Gauging repairs indoors resulted in the highest air concentration (29.3 mg/m<sup>3</sup>) and replacing the hose outdoors the lowest (below the LOD of 0.01 mg/m<sup>3</sup>). The TWA (8 hours) for pump repair workers working outdoors (*n* = 3) was 0.26 mg/m<sup>3</sup>, and that for working indoors



( $n = 2$ ) was  $4.0 \text{ mg/m}^3$ , and for gauge calibration outdoors ( $n = 5$ ) was  $0.98 \text{ mg/m}^3$ . Gasoline pump repairers belong to the most exposed population, particularly when this task was performed indoors. However, the range of exposures to TAME was not wide ( $2.5\text{--}5.5 \text{ mg/m}^3$ ) among the different occupational groups ([Vainiotalo et al., 2006](#)).

Urinary TAME was detected in only four out of nine samples. For the individual samples, there was a significant positive correlation between the exposure indicators MTBE plus TAME and TBA (an MTBE metabolite) plus TAA (Pearson coefficient of correlation,  $r = 0.86$ ), but there was no linear correlation between airborne concentrations of TAME and the corresponding urinary levels ([Vainiotalo et al., 2006](#)). The individual TAA concentrations in urine ranged from the LOQ ( $5 \text{ nmol/L}$ ) to  $147 \text{ nmol/L}$  (post-shift samples) where inspectors and pump repairer had the highest values ([Vainiotalo et al., 2006](#)).

#### 1.4.3 Exposure of the general population

The available data on exposure to TAME among populations that are not occupationally exposed to TAME were limited. Exposure via inhalation may occur for people who refuel their vehicles with gasoline that contains TAME or in areas near sources of TAME releases, and exposure via ingestion or dermal routes may occur from TAME-contaminated water ([European Commission, 2006](#)).

##### (a) Air monitoring

At two service stations in Finland during summer 1996, 167 personal breathing-zone samples were collected from customers while they were refuelling with 95-RON gasoline for 1 minute, using air pumped through charcoal tubes at a rate of 1 L per minute. TAME was present in gasoline on five of the seven sampling days, at concentrations ranging from 7.7% to 9.3% w/w. The adjusted geometric mean exposure

to TAME was  $1.9 \text{ mg/m}^3$ , and the maximum was  $29.1 \text{ mg/m}^3$ . Both stations had vapour recovery systems ([Vainiotalo et al., 1999](#)).

In Milan, Italy, personal air exposure (collected using a passive sampler with a 35–50 mesh charcoal cartridge) to TAME and other VOCs was measured among schoolchildren aged 7–11 years during spring 2018 ( $n = 82$ ) and winter 2019 ( $n = 89$ ) for a 16-hour period beginning in the late afternoon. All measurements for TAME fell below the LOQ ( $0.2 \text{ } \mu\text{g/m}^3$ ) ([Boniardi et al., 2023](#)).

##### (b) Biomonitoring

In central Italy, urinary levels of TAME and three other gasoline oxygenates (ETBE, MTBE, and DIPE) were measured in Caucasian [White] schoolchildren aged 5–11 years. Samples were collected as the child's last urine sample of the day before bed during summer 2017 ( $n = 313$ ) and winter 2018 ( $n = 263$ ) in two areas where population and traffic density were characterized as low (rural) or medium (urban). The mean values for urinary TAME were above the LOQ ( $2.9 \text{ ng/L}$ ) and were similar during the summer ( $18.7 \text{ ng/L}$ ) and winter ( $18.9 \text{ ng/L}$ ) seasons ([Antonucci et al., 2021](#)).

In the USA, an exploratory analysis for a new method to detect low levels of TAME and other ethers was conducted on a total of 11 blood samples previously collected from three volunteers. The volunteers were described only as healthy, with no documented exposure to TAME or other fuel oxygenates. TAME levels were below the LOD ( $0.6 \text{ ng/L}$ ) for all samples tested ([Silva et al., 2008](#)).

## 1.5 Exposure limits, regulations, and guidelines

Belgium, Canada (Ontario and Quebec provinces), and Finland have an occupational exposure limit for TAME of 20 ppm ( $84 \text{ mg/m}^3$ ) ([IFA,](#)

[2024](#)). In 2002, the American Conference of Governmental Industrial Hygienists adopted its current recommended 8-hour TWA occupational exposure limit for TAME of 20 ppm (84 mg/m<sup>3</sup>), based on limited human and animal data on health risks and a “preferred value approach” ([ACGIH, 2024](#)).

In the USA, TAME in drinking-water is not regulated at the federal level, but states may set limits. For example, New York has a public drinking-water standard for TAME of 50 µg/L ([New York State Department of Health, 2023](#)), and in Massachusetts, the guideline for TAME is 0.09 mg/L ([Massachusetts Department of Environmental Protection, 2020](#)).

[No reference values were found for biological monitoring of exposure of the general population and workers.]

## 2. Cancer in Humans

No data were available to the Working Group.

## 3. Cancer in Experimental Animals

### 3.1 Rat

See [Table 3.1](#).

#### *Oral administration (gavage)*

In a study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 100 male and 100 female Sprague-Dawley rats, (age, 8 weeks) were exposed orally (by gavage) to TAME (purity, >97%) in extra virgin olive oil (1 mL solution) at target doses of 0 (control), 250, or 750 mg/kg body weight (bw), 4 days per week for up to 78 weeks ([Belpoggi et al., 2002](#)). Rats in the control group received 1 mL of extra virgin olive oil without TAME. After treatment, all animals were main-

tained under observation until spontaneous death, without receiving any further treatment. A decrease in survival was observed in treated males compared with controls during the period between age 40 and 104 weeks, and the affected dose group was not specified in the text. [Survival data were not reported and/or tabulated.] The authors reported that the experiment ended after 135 weeks of treatment, with the death of the last animal at age 143 weeks. The authors stated that there were no significant differences in daily water and feed consumption and body weight across groups. [No data on body weight were reported.] All rats underwent complete necropsy with histopathological evaluation.

In males, there was a slight, nonsignificant increase in the incidence of lymphoma or leukaemia (17/100, 7/100, and 21/100), carcinoma of the ear duct (1/100, 4/100, and 5/100), testicular interstitial cell adenoma (1/100, 4/100, and 5/100), and the number of animals bearing malignant tumours (30/100, 24/100, and 37/100) among the respective TAME-treated groups (0, 250, and 750 mg/kg bw).

In females, there was a significant positive trend ( $P < 0.05$ , Cochran–Armitage trend test) in the incidence of haemolymphoreticular tumours (including lymphoblastic lymphoma, lymphoblastic leukaemia, lymphocytic lymphoma, lymphoimmunoblastic lymphoma, histiocytic sarcoma/monocytic leukaemia and myeloid leukaemia), with the incidence – 7/100, 14/100, and 27/100 in the groups at 0 (control), 250, and 750 mg/kg bw, respectively – being significantly increased ( $P < 0.01$ ,  $\chi^2$  test; [ $P < 0.0001$ , one-tailed Fisher exact test]) in the groups at 750 mg/kg bw compared with the control group.

**Table 3.1 Studies of carcinogenicity in experimental animals exposed to tert-amyl methyl ether**

| Study design<br>Species, strain (sex)<br>Age at start<br>Duration<br>Reference                                                 | Route<br>Agent tested, purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals                                     | Tumour incidence                                                                                                                                                                                                                                                                         | Significance                                                                                                                                                                                                               | Comments                                                                                                                                                                                                                                                                                                                                                                                      |
|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full carcinogenicity<br>Rat, Sprague-Dawley<br>(CRC/RF colony) (M)<br>8 wk<br>135 wk<br><a href="#">Belpoggi et al. (2002)</a> | Oral administration<br>(gavage)<br>TAME, > 97%<br>Extra virgin olive oil<br>0, 250, 750 mg/kg bw<br>4 days/wk for 78 wk<br>100, 100, 100<br>NR | <i>Haemolymphoreticular system</i><br>Lymphoma/leukaemia<br>17/100, 7/100, 21/100<br><i>Ear duct</i><br>Carcinoma<br>1/100, 4/100, 5/100<br><i>Testis</i><br>Interstitial cell adenoma<br>1/100, 4/100, 5/100<br><i>All organs</i><br>Total malignant tumours<br>30/100, 24/100, 37/100  | NS<br><br><br>NS<br><br>NS                                                                                                                                                                                                 | <i>Principal strengths:</i> Male and female animals used, lifetime observation, high number of animals per group, GLP study.<br><br><i>Principal limitations:</i> Contained little experimental detail on exact design, clinical observations, body-weight gain, or survival; lack of historical control data; lack of mortality-adjusted tumour incidence analysis.                          |
| Full carcinogenicity<br>Rat, Sprague-Dawley<br>(CRC/RF colony) (F)<br>8 wk<br>135 wk<br><a href="#">Belpoggi et al. (2002)</a> | Oral administration<br>(gavage)<br>TAME, > 97%<br>Extra virgin olive oil<br>0, 250, 750 mg/kg bw<br>4 days/wk for 78 wk<br>100, 100, 100<br>NR | <i>Haemolymphoreticular system</i><br>Lymphoma/leukaemia<br>7/100, 14/100, 27/100*<br><br><i>Ear duct</i><br>Carcinoma<br>2/100, 4/100, 4/100<br><i>Brain</i><br>Multiform glioblastoma<br>0/100, 0/100, 1/100<br><i>All organs</i><br>Total malignant tumours<br>37/100, 46/100, 44/100 | <br><br><br><br>P < 0.05, Cochran–Armitage trend test<br>*P < 0.01, $\chi^2$ test; [*P < 0.0001, Fisher exact test]<br>One female at 750 mg/kg bw had a lymphoblastic lymphoma and histiocytic sarcoma<br><br>NS<br><br>NS | <i>Principal strengths:</i> Complete histopathology, male and female animals used, lifetime observation, high number of animals per group, GLP study.<br><br><i>Principal limitations:</i> Contained little experimental detail on exact design, clinical observations, body-weight gain, or survival; lack of historical control data; lack of mortality-adjusted tumour incidence analysis. |

bw, body weight; F, female(s); GLP, Good Laboratory Practice; M, male(s); NR, not reported; NS, not significant; TAME, tert-amyl-methyl ether; wk, week(s).

[The Working Group noted that these increases were statistically significant in females (in the group at the higher dose) only when the haemolymphoreticular tumours were merged as lymphoma or leukaemia, but there was no evidence of any statistically significant effect when the analysis was conducted on the specific cellular type. The Working Group also noted that diagnostic criteria did not follow current standards for the haemolymphopoietic system, including tumour diagnostic dispositions. The study pathologist classified the haemolymphoreticular tumours as several types using the standard haematoxylin and eosin stain; a confirmation by immunohistochemistry would have resolved uncertainty in cellular subtype recognition. In addition, the authors reported increases in the incidence of several neoplastic lesions in the treatment groups compared with controls; however, these findings did not reach statistical significance and/or a clear dose–response relation. These included malignant tumours of the gastrointestinal tract, increased incidence of carcinoma of the ear duct, increased incidence of testicular interstitial cell adenoma, and increased incidence of malignant glial tumours). Overall, the Working Group concluded that none of these neoplasms were treatment-related.]

[The Working Group noted that this was a GLP study that was strengthened by the use of males and females, a high number of animals per group ( $n = 100$ ), adequate duration of exposure (up to 78 weeks), complete histopathology, and that the study covered the full lifespan of the animals. However, the Working Group noted that the treatment schedule was 4 days per week for 78 weeks. The Working Group also noted that the study had several additional limitations, e.g. poor reporting, lack of data on body weight, lack of adequate histopathological description of tumours, lack of information on non-neoplastic lesions, and lack of historical control data, and that there was no mention of blind examination, no pathology peer review was conducted, and

the use of two doses only limited the statistical power of the study.]

### 3.2 Evidence synthesis for cancer in experimental animals

The carcinogenicity of TAME has been assessed in one GLP study in male and female Sprague-Dawley rats treated by oral administration (gavage) ([Belpoggi et al., 2002](#)).

In this study ([Belpoggi et al., 2002](#)), there was a significant positive trend in the incidence of haemolymphoreticular tumours, and the incidence was significantly increased in females in the group at the highest dose.

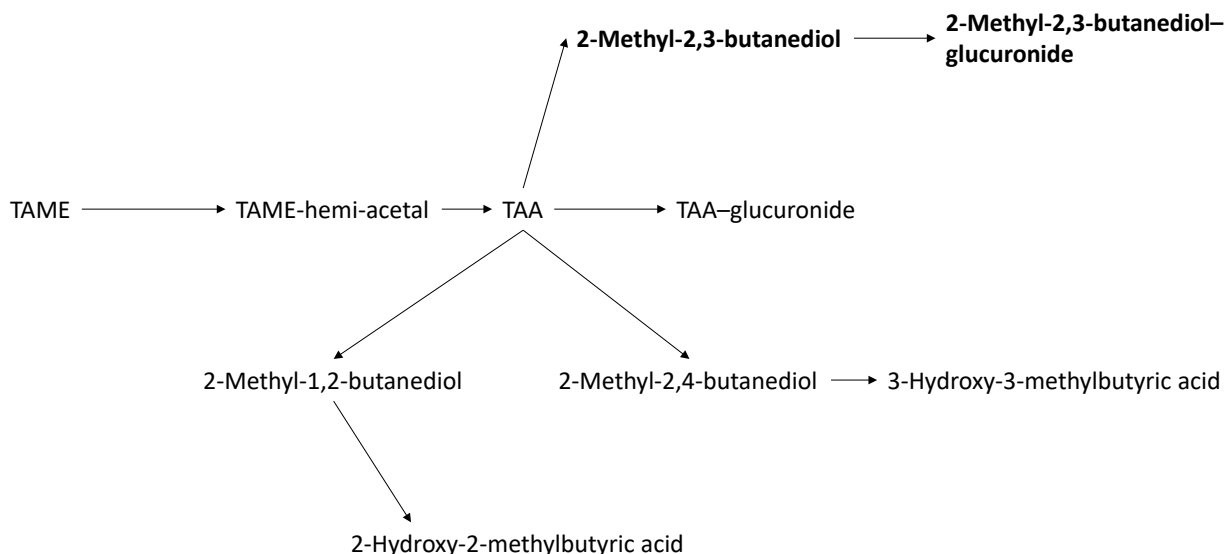
[The Working Group noted that the increase in the total number (female) of haemolymphoreticular tumours was statistically significant only when the haemolymphoreticular tumours were merged as lymphoma or leukaemia, but there was no evidence of any statistically significant effect when the analysis was conducted on the specific cellular type. The Working Group also noted that diagnostic criteria did not follow the current standards for the haemolymphopoietic system, including tumour diagnostic dispositions.]

## 4. Mechanistic Evidence

### 4.1 Absorption, distribution, metabolism, and excretion

[The Working Group noted that the absorption, distribution, metabolism, and excretion of TAME is similar that of other ether oxygenate additives to gasoline that are described in the present volume, specifically, MTBE and ETBE.]

The first step in the metabolism of TAME is the oxidation of its methyl group to form an unstable hemi-acetal that decomposes to TAA ([Fig. 4.1](#)). TAA is conjugated with glucuronic acid or further oxidized to different diols, which are

**Fig. 4.1 Metabolism of tert-amyl methyl ether**

TAA, *tert*-amyl alcohol; TAME, *tert*-amyl methyl ether.

Major metabolites in bold.

Adapted from [Dekant et al. \(2001\)](#).

then conjugated or, in the case of 2-methyl-1,2-butanediol, oxidized to the corresponding acid.

#### 4.1.1 Humans

[Amberg et al. \(1999\)](#) exposed one human volunteer, for 4 minutes, to  $^{13}\text{C}$ -labelled TAME at 27 000 ppm by inhalation from a 2 L gas sampling bag. Metabolites in the urine were analysed by  $^{13}\text{C}$  nuclear magnetic resonance (NMR). All metabolites of TAME identified in the urine samples were also detected in experimental systems *in vivo* (see Section 4.1.2). Thus, free and glucuronidated 2-methyl-2,3-butanediol, and a glucuronide of TAA, were identified as major urinary metabolites. Minor metabolites were TAA, 2-hydroxy-2-methylbutyric acid, and 3-hydroxy-3-methylbutyric acid. [The Working Group noted that TAME is extensively metabolized by rats and humans to TAA, which may be further oxidized to diols and carboxylic acids.

These reactions are likely to be mediated by cytochrome P450 (CYP)-dependent oxidation.]

Six healthy volunteers (three men and three women) were exposed for 4 hours to TAME at 4 ppm and 40 ppm in an exposure chamber. Blood was sampled up to 48 hours, and TAME and TAA were determined. Urine was sampled at 6-hour intervals for 72 hours, and analysed for TAA, 2-methyl-2,3-butane diol, 2-hydroxy-2-methylbutyric acid, and 3-hydroxy-3-methylbutyric acid. At the end of exposure at 4 or 40 ppm, TAME concentrations in the blood were 0.6  $\mu\text{M}$  and 4.4  $\mu\text{M}$ , respectively. The corresponding concentrations of TAA were 1.0  $\mu\text{M}$  and 9.0  $\mu\text{M}$ . The elimination from blood was rapid and similar at the two exposure levels, as assessed by the half-lives, which were in the range of 0.9–1.3 hours for TAME, and 3.2–5.8 hours for TAA. In the urine, 2-methyl-2,3-butane diol, 2-hydroxy-2-methylbutyric acid, and 3-hydroxy-3-methylbutyric acid were recovered as major products of excretion, when compared with TAA



and its glucuronide metabolite. Elimination half-lives of these metabolites in the urine ranged from 6 hours for TAA to 40 hours for 2-methyl-2,3-butane diol ([Amberg et al., 2000](#)).

[Vainiotalo et al. \(2007\)](#) studied the toxicokinetics of TAME in six healthy male volunteers exposed via inhalation. The men were exposed to TAME at 15 ppm or 50 ppm for 4 hours. The average relative respiratory uptake was 51%; approximately 30% of the absorbed dose was exhaled within 44 hours. TAME in the blood and in exhaled air reached the highest concentration at the end of exposure. Two post-exposure half-lives were observed, with average values of 1.2 and 4.9 hours. In the urine, TAME concentrations peaked towards the end of, or shortly after, exposure, with an average half-life of about 8 hours. TAA concentrations in the blood and breath peaked at 0.5–1 hour after exposure, then declined slowly, with a single half-life of about 6 hours. In the urine, maximum concentrations of TAA were observed towards the end of exposure, or during the first hour after exposure, the post-exposure half-life being about 8 hours. Approximately 0.1% and 0.3% of the retained dose of TAME was excreted as TAME and free TAA, respectively. It was estimated that 8 hours of exposure to TAME at 20 ppm resulted in TAME concentrations of 6  $\mu\text{M}$  in post-shift urine and 0.2  $\mu\text{M}$  in post-shift exhaled air, and a TAA concentration of 3  $\mu\text{M}$  in next-morning urine ([Vainiotalo et al., 2007](#)).

[The Working Group noted that overall, and in line with the metabolic pathway depicted in [Fig. 4.1](#), the three studies in humans showed a consistent toxicokinetic profile of efficient absorption by inhalation, excretion of unchanged TAME via exhalation, rapid metabolic elimination of both TAME and TAA from the blood (with half-lives of approximately 1 hour and 2–6 hours), and a slower decline in concentrations of TAME, TAA, and 2-methoxy-2,3-butanediol in the urine (with half-lives of 8, 6, and 40 hours, respectively). The inhalation toxicokinetic profile

of TAME in humans was similar to that of MTBE, for which more literature was available (see Section 4.1 of the monograph on MTBE in the present volume). Furthermore, the Working Group noted that no studies were available on TAME, or its metabolites, in exposed humans for exposure routes other than inhalation, or in tissues other than blood.]

#### 4.1.2 Experimental systems

##### (a) Non-human mammalian systems in vivo

[Amberg et al. \(1999\)](#) exposed Fischer 344 (F344) rats (two males, two females) to  $^{13}\text{C}$ -labelled TAME at 2000 ppm for 6 hours. Urine samples were collected for 48 hours, and analysed by  $^{13}\text{C}$ -NMR, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS/MS). TAA and free and glucuronidated 2-methyl-2,3-butanediol were the major urinary metabolites. Other metabolites, at low concentrations, were detected using NMR, and identified as 2-hydroxy-2-methylbutyric acid and 3-hydroxy-3-methylbutyric acid. In a separate experiment, three male rats were exposed to TAA at a dose of 250 mg/kg bw by gavage, and urine was collected for 48 hours. The metabolites were identical to those in the urine of rats that had inhaled  $^{13}\text{C}$ -labelled TAME.

[Amberg et al. \(2000\)](#) exposed F344 HN rats (five males, five females) to TAME at 4 and 40 ppm for 4 hours in an exposure chamber. At the end of the exposure, concentrations of TAME in the blood were 1.4  $\mu\text{M}$  and 9.6  $\mu\text{M}$ , and those of TAA were 1.8  $\mu\text{M}$  and 1.8  $\mu\text{M}$ , respectively. TAA was rapidly cleared from the blood. 2-Methyl-2,3-butanediol and its glucuronide were major TAME metabolites in the urine, whereas TAA and its glucuronide were present at lower concentrations. The recovery of TAME metabolites in the urine samples was higher in humans than in rats, suggesting more intensive biotransformation in humans.



[Sumner et al. \(2003a\)](#) investigated the metabolism in blood and the distribution of TAME, TAA, and acetone in male and female F344 rats and CD-1 mice (mostly four animals per time point) after nose-only inhalation exposure to TAME at 100, 500, and 2500 ppm for 6 hours. In rats, the area under the curve (AUC) of concentration-time in the blood for TAME was approximately proportional to the exposure concentration, thus suggesting a linear kinetic profile (non-saturated metabolism) up to 2500 ppm. In mice, after exposure to TAME at 2500 ppm or 500 ppm, the concentrations of TAA in blood were two- to threefold those observed in rats. In mice, exposure to TAME at 500 ppm resulted in increases of 10-fold (males) and 8-fold (females) in the blood AUC of TAME and 25-fold (males) and 17-fold (females) in the blood AUC of TAA compared with exposure to TAME at a lower dose of 100 ppm. [The Working Group noted that, according to the authors, these differences could result from saturation of a process involved in the further metabolism of TAA. In addition, at all exposure conditions, acetone levels increased during exposure and returned to control values within 16 hours. The source of acetone could be either a metabolite of TAA or an effect on endogenous metabolism caused by exposure to TAME.]

[Sumner et al. \(2003c\)](#) studied the tissue distribution of TAME (a mix of  $^{13}\text{C}$ -labelled and  $^{14}\text{C}$ -labelled) in male rats after 6-hour nose-only exposure at 2500 ppm. More than 95% of the total recovered radiolabel was excreted within 48 hours. Of this, approximately 44% was expired as organic volatiles and 3% as  $\text{CO}_2$ ; 51% was excreted in the urine and 1% in the faeces. TAME and TAA accounted for 90% or more of the radiolabel in expired air during the first 8 hours after exposure. Three major urinary metabolites were identified: TAA glucuronide, and 2,3-dihydroxy-2-methylbutane and its glucuronide conjugate. The percentage of TAA glucuronide was the highest during the first

8 hours, whereas the percentage of the other two metabolites increased subsequently.

The same group studied the exposure level of TAME, the effects of different routes of exposure (inhalation at 100–2500 ppm for 1 or 5 days; gavage at 10 (mice), 20 (rats), and 100 mg/kg bw), and species and sex-specific differences (male and female F344 rats and CD-1 mice) in the disposition of administered TAME ([Sumner et al., 2003b](#)). The disposition of TAME in male rats exposed via inhalation was similar to that reported in the previous study ([Sumner et al., 2003c](#)). However, the relative uptake of inhaled  $^{14}\text{C}$ -TAME in mice was 2–3-fold that in rats. Decreased relative uptake of TAME and decreased urinary excretion of 2,3-dihydroxy-2-methylbutane and its glucuronide conjugate were observed at high exposure levels, thus indicating saturated metabolism.

[Elovaara et al. \(2007\)](#) investigated the interactive effects of MTBE and TAME on the induction of CYP2E1, CYP2B1, and phase II enzymes, and triglyceridaemia and liver toxicity in female Wistar rats ( $n=4$  per test group). Enzyme induction was assessed by enzyme activity, and immune-inhibition assays. Exposure to TAME and/or MTBE (dose range, 10–20 mmol/day per kg bw for 3 days), resulted in hepatomegaly and induction of CYP activity. Both TAME and MTBE caused an increase of twofold in CYP2E1 activity; whereas TAME caused an increase in CYP2B1 activity of up to 260-fold, and MTBE caused an increase of only 20-fold. The authors observed slight or no alterations in phase II enzyme activities – NADPH:quinone oxidoreductase (NQO1), glutathione *S*-transferase (GST), and UDP-glucuronosyltransferase (UGT). TAME alone, but not MTBE, induced hypertriglyceridaemia. [The Working Group noted that the findings indicated some differences between MTBE or TAME in effects on lipid metabolism.]

[The Working Group noted that the toxicokinetic profile and the absorption, distribution, metabolism, and excretion of TAME in rodents

were similar to those observed in humans, as depicted in [Fig. 4.1](#), with efficient uptake via inhalation, and formation of free and glucuronidated 2-methyl-2,3-butanediol metabolites via TAA. The results of one study suggested that metabolism beyond TAA saturates at 500 ppm in mice but remains linear (proportional to exposure level) at up to 2500 ppm in rats. In one study it was reported that the recovery of TAME metabolites in urine was higher in humans than in rats, suggesting more intensive biotransformation in humans.]

(b) *In vitro*

(i) *Tissue partitioning*

[Nihlen et al. \(1995\)](#) determined liquid:air partition coefficients in vitro by head space-gas chromatography for three ethers in gasoline (MTBE, ETBE, and TAME). They reported the following average values for TAME: in human blood, 17.9; physiological saline, 11.9; and olive oil, 337. No significant sex-specific difference was observed with respect to the blood:air partition coefficient.

[Kaneko et al. \(2000\)](#) determined partition coefficients for the same three ethers along with the main metabolites (TBA and TAA). Similar results to those of [Nihlen et al. \(1995\)](#) were obtained for TAME: in fresh blood from rats, 15.5; physiological saline, 12.7; and olive oil, 357. In addition, Kaneko et al. reported several rat tissue:air partition coefficients for TAME: muscle, 24.0; brain, 24.5; kidney, 164; liver, 31.8; testes, 20.4; and fat, 328. The following partition coefficients were obtained for TAA: blood, 392; physiological saline, 487; olive oil, 451; muscle, 390; brain, 454; kidney, 475; liver, 481; testes, 336; and fat, 460.

(ii) *Metabolism in vitro*

[Hong et al. \(1997\)](#) compared the metabolic activity of ethers added to gasoline (MTBE, ETBE, and TAME) in microsomes prepared from various tissues of male Sprague-Dawley

rats. Of the tissues examined, olfactory mucosa had the highest microsomal activity for the metabolism of TAME, and metabolism of TAME was higher by 25-fold that of the other ethers. No detectable activity was found in microsomes prepared from the lungs, kidneys, and olfactory bulbs of the brain. The apparent  $K_m$  and  $V_{max}$  values for the metabolism of TAME in rat olfactory microsomes were 87 mM and 9.8 nmol/minute per mg, respectively. Coumarin significantly inhibited the metabolism of the three ethers. The addition of TAME also caused concentration-dependent inhibition of the microsomal metabolism of MTBE and ETBE (see Section 4.1 of the monograph on ETBE in the present volume).

[Hong et al. \(1999b\)](#) used liver microsomes from *CYP2E1* knockout (2E1<sup>-/-</sup>) female mice (to investigate the contribution of *CYP2E1* to the metabolism of MTBE, ETBE, and TAME). The microsomes were active in metabolizing all three ethers. The microsomal metabolizing activity (nmol/minute per mg) in *CYP2E1* knockout mice was similar to that in sex- and age-matched wild-type C57BL/6N and 129/Sv mice (both 2E1<sup>+/+</sup>). The results show that *CYP2E1* plays a negligible role in the metabolism of these ethers in the mouse liver.

[Hong et al. \(1999a\)](#) also studied the metabolism of gasoline ethers by human liver microsomes and heterologously expressed human CYP enzymes. Large interindividual variation in metabolism was seen in 15 samples of human liver microsomes. The microsomal metabolizing activities for MTBE, ETBE, and TAME were highly correlated ( $r=0.91-0.96$ ), suggesting that these ethers are metabolized by the same enzyme(s). Correlation analysis of the ether-metabolizing activities with individual CYP enzyme activities in the liver microsomes showed that the highest degree of correlation was with human CYP2A6 ( $r=0.90-0.95$ ), which is known to be polymorphic. Of a battery of human CYP enzymes expressed in human B-lymphoblastoid

cells, CYP2A6 had the highest turnover number for metabolizing gasoline ethers. Metabolism of the three ethers by human liver microsomes was inhibited by coumarin, a known substrate of human CYP2A6, in a concentration-dependent manner. Moreover, monoclonal antibody treatment against human CYP2A6 caused a significant inhibition (75–95%) of the metabolism of the three ethers. These results clearly indicate that CYP2A6 is the major enzyme responsible for the metabolism of the three ethers.

[Le Gal et al. \(2001\)](#) studied the metabolism of MTBE, ETBE, and TAME in microsomes prepared from 12 human livers. Two apparent  $K_m$  values, averaging 0.10 mM and 1.7 mM, were identified for TAME. [The Working Group noted that according to kinetic data, correlation studies, chemical inhibition, and metabolism by heterologously expressed human CYPs, the major enzyme involved in the metabolism of TAME is CYP2A6, with a minor contribution of CYP3A4 at low substrate concentrations.]

(c) *Physiologically based pharmacokinetic modelling*

[Collins et al. \(1999\)](#) developed a physiologically based pharmacokinetic (PBPK) model for TAME and TAA in male F344 rats. The TAME model had six compartments: lung, liver, rapidly perfused tissues, slowly perfused tissues, fat, and kidney. The TAA model had three compartments: lung, liver, and total body water. The two models were linked by the metabolism of TAME to TAA in the liver. Model simulations were compared with blood toxicokinetic data for TAME and TAA from male F344 rats exposed to TAME at 2500, 500, or 100 ppm, previously reported by [Sumner et al. \(2003a\)](#). The PBPK model could describe the toxicokinetic profile of TAME when two saturable pathways for TAME oxidation were included. The TAA model, which included pathways for oxidation and glucuronide conjugation of TAA, underpredicted the experimental data collected at later times

post-exposure. Three hypotheses were tested to explain the deviation: nonspecific binding of TAA, diffusion-limited transport of TAA, and enterohepatic circulation of TAA glucuronide. These hypotheses were tested using three different model structures. Of these, the model with nonspecific binding of TAA gave the best fit to the data.

### Synopsis

[Overall, the Working Group noted that TAME is efficiently absorbed in humans exposed via inhalation, and it is excreted as the unchanged form via exhalation. The first step in the metabolism of TAME is oxidation of the methyl group, which decomposes to the alcohol TAA. TAA is conjugated with glucuronic acid or further oxidized to different diols, which are then conjugated or, in the case of 2-methyl-1,2-butanediol, oxidized to the corresponding acid. In rodents, TAME presented a similar toxicokinetic profile to that in humans.]

## 4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens ([Smith et al., 2016](#)) encompassed by the agent TAME. Few studies were available for the key characteristics “is genotoxic” and “is immunosuppressive”. There were no data for the remaining key characteristics of carcinogens.

### 4.2.1 Experimental systems

See [Table 4.1](#).

[Daughtrey and Bird \(1995\)](#) assessed micronuclei formation at 24, 48 and 72 hours, in bone marrow cells of CD-1 mice exposed to a single dose of TAME at 0.15, 0.375, or 0.75 g/kg. No significant increase in the frequency of micronuclei was observed. In the same study,

**Table 4.1 End-points relevant to the key characteristics of carcinogens in non-human mammalian systems in vivo exposed to *tert*-amyl-methyl ether**

| End-point                                           | Species, strain (sex)                            | Tissue                | Results <sup>a</sup>                                     | Dose (LED or HID)                 | Route, duration, dosing regimen                                                                                                                                                                       | Comments                                                             | Reference                                 |
|-----------------------------------------------------|--------------------------------------------------|-----------------------|----------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|-------------------------------------------|
| Micronucleus formation<br>Blood cell count          | Mouse, CD-1 (M, F)<br>Rat, Sprague-Dawley (M, F) | Bone marrow;<br>blood | –<br>No change, haematological end-point                 | Mice, 0.75 g/kg<br>Rats, 1.0 g/kg | Mice: single i.p. injection of TAME (0.15, 0.375 or 0.75 g/kg); evaluation at 24, 48 and 72 h after dosing.<br>Rats: oral administration of TAME (0.125, 0.5, or 1.0 g/kg) for 7 days/wk for 28 days. | Relevant to KC2. <sup>b</sup>                                        | <a href="#">Daughtrey and Bird (1995)</a> |
| Micronucleus formation<br>Sister-chromatid exchange | Rat, Sprague-Dawley (M, F)                       | Blood                 | –, Immature erythrocytes<br>+, Sister-chromatid exchange | 20 g/m <sup>3</sup>               | Gasoline + 12% TAME (2, 10, or 20 g/m <sup>3</sup> ) via inhalation for 4 wk (5 days/wk).                                                                                                             | Did not evaluate TAME as single agent. Relevant to KC2.              | <a href="#">Schreiner et al. (2014)</a>   |
| IgM antibody-forming cell response                  | Rat, Sprague-Dawley (F)                          | Spleen                | No change                                                | 20 g/m <sup>3</sup>               | Gasoline + 12% TAME (2, 10, or 20 g/m <sup>3</sup> ) via inhalation for 4 wk (5 days/wk).                                                                                                             | Did not evaluate TAME as single agent. Relevant to KC7. <sup>c</sup> | <a href="#">White et al. (2014)</a>       |

F, female(s); h, hour(s); HID, highest ineffective dose; IgM, immunoglobulin M; i.p., intraperitoneal; KC, key characteristic of carcinogens; LED, lowest effective dose; M, male(s); TAME, *tert*-amyl methyl ether; wk, week(s).

<sup>a</sup> +, positive; –, negative; or no change.

<sup>b</sup> KC2, key characteristic of carcinogens 2, “is genotoxic”.

<sup>c</sup> KC7, key characteristic of carcinogens 7, “is immunosuppressive”.

no mutagenicity was observed in five different *Salmonella* strains (TA98, TA100, TA1535, TA1537, and TA1538) exposed to TAME either with or without metabolic activation (from S9, 9000×g supernatant fraction from the livers of Aroclor-pretreated Sprague-Dawley rats) (Daughtrey and Bird, 1995). Finally, the authors conducted a 28-day study in Sprague-Dawley rats. At the highest dose of 1.0 g/kg in male rats, TAME induced a decrease in food consumption and body weight but an increase in adrenal gland weight. Clinical alterations were minimal; no haematological or histopathological alterations were observed. [The Working Group noted that this study could be informative for the key characteristic of carcinogens “is genotoxic” (KC2).]

Micronucleus formation and sister-chromatid exchange in blood cells were assessed in Sprague-Dawley rats exposed to gasoline in combination with 12% TAME for 4 weeks (Schreiner et al., 2014). The gasoline combination at 20 g/m<sup>3</sup> (the highest dose tested) caused an increase in sister-chromatid exchange in peripheral lymphocytes in both sexes. None of the doses tested altered the frequency of micronucleated immature erythrocytes. The proportion of immature erythrocytes was also evaluated as a measure of cytotoxicity but did not significantly vary. [The Working Group noted that the effects of TAME alone were not assessed. The Working Group noted that this study could be informative for the key characteristic of carcinogens “is genotoxic” (KC2).]

In a similar study in female Sprague-Dawley rats, White et al. (2014) exposed the animals to a gasoline mixture plus TAME (vapour of baseline gasoline combined with 11.9% TAME) at doses of 2, 10, or 20 g/m<sup>3</sup> via inhalation for 4 weeks (5 days per week). The humoral response was not altered: the immunoglobulin M (IgM) antibody-forming cell response, total body weight, and organ weights were not affected. [The Working Group noted that by evaluating the antibody-forming cell response this study was informative to the

key characteristic of carcinogens “is immuno-suppressive” (KC7). However, only females were analysed, and TAME alone was not tested.]

## Synopsis

[The Working Group noted that the evidence across the key characteristics of carcinogens was scarce.]

### 4.2.2 Evaluation of high-throughput in vitro screening data

The mechanistic characteristics common to carcinogens (the 10 key characteristics of carcinogens) can be investigated through biochemical and cell-based assays run by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health Toxicity Forecaster/Toxicology in the 21st Century (ToxCast/Tox21) high-throughput screening programmes (Chiu et al., 2018) (see Section 4.2.10 of the monograph on MTBE in the present volume and Annex 4, Supplementary material for Section 4, Mechanistic evidence, online only, available from: <https://publications.iarc.who.int/652>). TAME (CAS No. 994-05-8, DTXSID8024521) has been evaluated in the US EPA and United States National Institutes of Health high-throughput screening assays.

Results were available for 111 assay end-points mapped to key characteristics for TAME. TAME was considered to be active in two out of 35 assay end-points, mapped to the key characteristic “modulates receptor-mediated effects” (KC8). Assays in which end-points were identified as active included TOX21\_ERa\_LUC\_VM7\_Agonist and TOX21\_ERb\_BLA\_Antagonist\_ratio. The TOX21\_ERa\_LUC\_VM7\_Agonist assay is designed to make measurements of induction of luciferase, an inducible reporter gene, as detected via bioluminescence signals by a luciferase-coupled ATP quantitation technology. Changes are indicative of alterations



in transcriptional gene expression caused by agonist activity regulated by human estrogen receptor 1 (*ESR1*). This assay uses VM7, a human breast cancer cell line, with chemical dosing in a 1536-well plate. The active call was noted with the flag “Less than 50% efficacy”.

The TOX21\_ERb\_BLA\_Agonist\_ratio is an assay readout measuring the expression of a reporter gene via receptor activity; it uses an inducible reporter (beta-lactamase induction) detected with the GAL4 beta-lactamase reporter gene. It is designed to target nuclear receptor activity at the protein (receptor) level, specifically mapping to the *ESR2* gene(s), using a positive control of 4-hydroxytamoxifen. This assay uses HEK293T, a human embryonic kidney cell line, with chemical dosing in a 1536-well plate. The active call was noted with the flags “Borderline active” and “Less than 50% efficacy”.

## 5. Summary of Data Reported

### 5.1 Exposure characterization

*tert*-Amyl methyl ether (TAME) is a volatile ether and a high-production-volume chemical that is produced from methanol and isoam- ylenes. TAME is used as an additive in gasoline to increase combustion efficiency; no uses unre- lated to fuels were identified. TAME is typically produced together with methyl *tert*-butyl ether (MTBE), but the production volume is small compared with MTBE. Data on the occurrence of TAME in products including gasoline or the environment were limited. TAME has been detected in groundwater near leaking under- ground fuel tanks and via routine groundwater monitoring. Worker exposure to TAME has been measured during tanker truck loading and in gasoline pump repairers and inspectors at service stations and repair shops. Exposure via inhalation may occur for people who refuelling their vehicles with gasoline that contains TAME,

or in areas near sources of TAME releases, and exposure via ingestion or dermal routes may occur from TAME-contaminated water. A few countries have established occupational expo- sure limits for TAME.

### 5.2 Cancer in humans

No data were available to the Working Group.

### 5.3 Cancer in experimental animals

Only one study was available, and it complied with Good Laboratory Practice (GLP). In this study, treatment with TAME did not cause an increase in the incidence of malignant neoplasms in rats. This study could not be interpreted as showing either the presence or the absence of a carcinogenic effect. The main limitation in this study was the inadequate criteria used for the classification of neoplasms of the haemolympho- poietic tissues.

### 5.4 Mechanistic evidence

Regarding absorption, distribution, metab- olism, and excretion of TAME, the agent is efficiently absorbed in humans exposed via inhalation, and it is excreted as the unchanged form via exhalation. The toxicokinetics are similar to those of MTBE. The first step in metab- olism is oxidation of the methyl group to form an unstable hemi-acetal that decomposes to the alcohol *tert*-amyl alcohol (TAA). TAA is conju- gated with glucuronic acid or further oxidized to various diols, which are then conjugated or, in the case of 2-methyl-1,2-butanediol, oxidized to the corresponding acid. In rodents, the absorp- tion, distribution, metabolism, and excretion of TAME is similar to that in humans.



The mechanistic evidence on whether TAME exhibits the key characteristics of carcinogens is scarce. Sister-chromatid exchanges were observed in a single study in vivo; however, TAME was administered in combination with gasoline.

TAME was essentially without effects in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

## 6. Evaluation and Rationale

### 6.1 Cancer in humans

There is *inadequate* evidence in humans regarding the carcinogenicity of *tert*-amyl methyl ether.

### 6.2 Cancer in experimental animals

There is *inadequate* evidence in experimental animals regarding the carcinogenicity of *tert*-amyl methyl ether.

### 6.3 Mechanistic evidence

The mechanistic evidence is *inadequate*.

### 6.4 Overall evaluation

*tert*-Amyl methyl ether is *not classifiable as to its carcinogenicity to humans* (Group 3).

### 6.5 Rationale

The Group 3 evaluation of *tert*-amyl methyl ether (TAME) is based on *inadequate* evidence regarding cancer in humans, *inadequate* evidence regarding cancer in experimental animals, and *inadequate* mechanistic evidence. The evidence regarding cancer in humans is *inadequate*

because no studies were available. The evidence regarding cancer in experimental animals is *inadequate* because in the sole available study, treatment with TAME did not cause an increase in the incidence of malignant neoplasms in rats. This study could not be interpreted as showing either the presence or the absence of a carcinogenic effect. The mechanistic evidence is *inadequate* because there is a paucity of data.

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# LIST OF ABBREVIATIONS

|        |                                                               |
|--------|---------------------------------------------------------------|
| ACGIH  | American Conference of Governmental Industrial Hygienists     |
| ADME   | absorption, distribution, metabolism, and excretion           |
| AFC    | antibody-forming cell                                         |
| AHS    | Agricultural Health Study                                     |
| AIDS   | acquired immunodeficiency syndrome                            |
| ALDH2  | aldehyde dehydrogenase 2                                      |
| ALL    | acute lymphoblastic leukaemia                                 |
| ALP    | alkaline phosphatase                                          |
| ALT    | alanine aminotransferase                                      |
| AML    | acute myeloid leukaemia                                       |
| ANLL   | acute non-lymphocytic leukaemia                               |
| ANOVA  | analysis of variation                                         |
| AOPP   | advanced oxidation protein product                            |
| ARB    | angiotensin receptor II blocker                               |
| AST    | aspartate aminotransferase                                    |
| ATSDR  | Agency for Toxic Substances and Disease Registry              |
| ATSM   | American Society for Testing and Materials                    |
| AUC    | area under the curve                                          |
| BAR    | biological reference value for a workplace substance [German] |
| BAT    | biological tolerance value for occupational exposure [German] |
| BBN    | <i>N</i> -butyl- <i>N</i> -(4-hydroxybutyl)nitrosamine        |
| BCC    | basal cell carcinoma                                          |
| BGVC   | vapour condensate of baseline gasoline                        |
| BMD    | benchmark dose                                                |
| BMI    | body mass index                                               |
| BMR    | benchmark response                                            |
| BrdU   | bromodeoxyuridine                                             |
| BTEX   | benzene, toluene, ethylbenzene, and xylene                    |
| BTX    | benzene, toluene, and xylene                                  |
| bw     | body weight                                                   |
| cAMP   | cyclic adenosine monophosphate                                |
| CANJEM | Canadian Job-Exposure Matrix                                  |
| CAR    | constitutive androstane receptor                              |
| CAS    | Chemical Abstracts Service                                    |

|                  |                                                                                                                                                                                                           |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CAT              | catalase                                                                                                                                                                                                  |
| CDK              | cyclin-dependent kinase                                                                                                                                                                                   |
| CHL              | Chinese hamster lung                                                                                                                                                                                      |
| CHO              | Chinese hamster ovary                                                                                                                                                                                     |
| CI               | confidence interval                                                                                                                                                                                       |
| CLL              | chronic lymphocytic leukaemia                                                                                                                                                                             |
| C <sub>max</sub> | maximum concentration                                                                                                                                                                                     |
| CML              | chronic myeloid leukaemia                                                                                                                                                                                 |
| CMV              | cytomegalovirus                                                                                                                                                                                           |
| CNODES           | Canadian Network for Observational Drug Effect Studies                                                                                                                                                    |
| CNS              | central nervous system                                                                                                                                                                                    |
| COPD             | chronic obstructive pulmonary disease                                                                                                                                                                     |
| COVID-19         | disease caused by SARS-CoV-2-infection                                                                                                                                                                    |
| COX              | cyclooxygenase                                                                                                                                                                                            |
| CpG              | cytosine-phosphate-guanine                                                                                                                                                                                |
| CPN              | chronic progressive nephropathy                                                                                                                                                                           |
| CPRD             | Clinical Practice Research Datalink                                                                                                                                                                       |
| CRP              | C-reactive protein                                                                                                                                                                                        |
| CTCL             | cutaneous T-cell lymphoma                                                                                                                                                                                 |
| CYP              | cytochrome P450                                                                                                                                                                                           |
| DAG              | directed acyclic graph                                                                                                                                                                                    |
| DCFH-DA          | 2',7'-dichlorodihydrofluorescein diacetate                                                                                                                                                                |
| DEN              | <i>N</i> -nitrosodiethylamine                                                                                                                                                                             |
| DIPE             | diisopropyl ether                                                                                                                                                                                         |
| DLBCL            | diffuse large B-cell lymphoma                                                                                                                                                                             |
| DMBDD            | diethylnitrosamine, <i>N</i> -methyl- <i>N</i> -nitrosourea, <i>N</i> -butyl- <i>N</i> -(4-hydroxybutyl)nitrosamine, 1,2-dimethylhydrazine dihydrochloride, and <i>N</i> -bis(2-hydroxypropyl)nitrosamine |
| DMSO             | dimethyl sulfoxide                                                                                                                                                                                        |
| DSB              | double-strand break                                                                                                                                                                                       |
| E2               | 17 $\beta$ -estradiol                                                                                                                                                                                     |
| EAF              | ethyl acetate fraction                                                                                                                                                                                    |
| EAGLE            | Environment And Genetics in Lung cancer Etiology                                                                                                                                                          |
| EC               | European Community                                                                                                                                                                                        |
| EDTA             | ethylenediaminetetraacetic acid                                                                                                                                                                           |
| EE2              | ethinyl estradiol                                                                                                                                                                                         |
| EEA              | European Economic Area                                                                                                                                                                                    |
| EFIM             | European Federation of Internal Medicine                                                                                                                                                                  |
| EGFR             | epidermal growth factor receptor                                                                                                                                                                          |
| EHEN             | <i>N</i> -ethyl- <i>N</i> -hydroxyethylnitrosamine                                                                                                                                                        |
| EHT              | European High Test                                                                                                                                                                                        |
| EI               | effort index                                                                                                                                                                                              |
| EKA              | exposure equivalent for carcinogenic substance [German]                                                                                                                                                   |
| ELISA            | enzyme-linked immunosorbent assay                                                                                                                                                                         |
| EMA              | European Medicines Agency                                                                                                                                                                                 |
| EPIC             | European Prospective Investigation into Cancer and Nutrition                                                                                                                                              |
| ERA              | European Renal Association                                                                                                                                                                                |
| EROD             | ethoxyresorufin- <i>O</i> -deethylase                                                                                                                                                                     |
| ETBE             | ethyl <i>tert</i> -butyl ether                                                                                                                                                                            |
| ETS              | environmental tobacco smoke                                                                                                                                                                               |
| EU               | European Union                                                                                                                                                                                            |



|                  |                                                                               |
|------------------|-------------------------------------------------------------------------------|
| EWAS             | epigenome-wide association study                                              |
| FEN              | final effective number                                                        |
| FEV1             | forced expiratory volume in the first second                                  |
| FIMEA            | Finnish Medicines Agency                                                      |
| FINJEM           | Finnish job-exposure matrix                                                   |
| FISH             | fluorescence in situ hybridization                                            |
| Fpg              | formamidopyrimidine/8-oxoguanine DNA <i>N</i> -glycosylase                    |
| FRAP             | ferric reducing ability of plasma                                             |
| FSH              | follicle-stimulating hormone                                                  |
| FVC              | forced vital capacity                                                         |
| GC               | gas chromatography                                                            |
| GENRES           | Genetics of Drug Responsiveness in Essential Hypertension                     |
| GERA             | Genetic Epidemiology of Responses to Antihypertensives                        |
| GFP              | green fluorescent protein                                                     |
| GFR              | glomerular filtration rate                                                    |
| GGT              | gamma-glutamyl transferase                                                    |
| GIS              | geographic information system                                                 |
| GLP              | Good Laboratory Practice                                                      |
| GLS1             | glutaminase 1 isoform                                                         |
| GPx              | glutathione peroxidase                                                        |
| GR               | glutathione reductase                                                         |
| GSH              | glutathione                                                                   |
| GST              | glutathione <i>S</i> -transferase                                             |
| GVC              | gasoline vapour condensate                                                    |
| GWAS             | genome-wide association study                                                 |
| γH2AX            | phosphorylated H2A histone family member X                                    |
| HBEL             | health-based exposure limit                                                   |
| HDAC             | histone deacetylase                                                           |
| HDF              | human dermal fibroblast                                                       |
| HDL-C            | high-density lipoprotein cholesterol                                          |
| HERV             | human endogenous retrovirus                                                   |
| 2-HIBA           | 2-hydroxyisobutyric acid                                                      |
| HIC              | highest ineffective concentration                                             |
| HID              | highest ineffective dose                                                      |
| HIF              | hypoxia inducible factor                                                      |
| HIV              | human immunodeficiency virus                                                  |
| HMG-CoA          | 3-hydroxy-3-methyl-glutaryl-coenzyme A                                        |
| 4-HPA            | 4-hydroxyphenylacetate                                                        |
| HPLC             | high-performance liquid chromatography                                        |
| HPV              | human papillomavirus                                                          |
| HR               | hazard ratio                                                                  |
| hsCRP            | high-sensitivity C-reactive protein                                           |
| HSCT             | haematopoietic stem cell transplant                                           |
| 11 β-HSD2        | 11 β-hydroxysteroid dehydrogenase type 2                                      |
| HWE              | healthy-worker effect                                                         |
| HWSB             | healthy-worker survival bias                                                  |
| IARC             | International Agency for Research on Cancer                                   |
| IC <sub>50</sub> | median inhibitory concentration                                               |
| ICARE            | Investigation of occupational and environmental CAuses of REspiratory cancers |
| ICD              | International Classification of Diseases                                      |

|                 |                                                                             |
|-----------------|-----------------------------------------------------------------------------|
| IFN $\gamma$    | interferon gamma                                                            |
| Ig              | immunoglobulin                                                              |
| IL              | interleukin                                                                 |
| INTEROCC        | International study of occupational exposures and brain cancer              |
| IPA             | ingenuity pathway analysis                                                  |
| ISCO            | International Standard Classification of Occupations                        |
| ISIC            | International Standard Industrial Classification of All Economic Activities |
| ISO             | International Organization for Standardization                              |
| IUPAC           | International Union of Pure and Applied Chemistry                           |
| JEM             | job-exposure matrix                                                         |
| KC              | key characteristic of carcinogens                                           |
| $K_d$           | dissociation constant                                                       |
| LC              | liquid chromatography                                                       |
| LEC             | lowest effective concentration                                              |
| LED             | lowest effective dose                                                       |
| LFG             | light fraction of unleaded gasoline                                         |
| LH              | luteinizing hormone                                                         |
| LINE            | long interspersed nuclear element                                           |
| LMIC            | low- and middle-income country                                              |
| LOD             | limit of detection                                                          |
| LOQ             | limit of quantification                                                     |
| LPG             | liquefied petroleum gas                                                     |
| LPO             | lipid peroxidation                                                          |
| LPS             | lipopolysaccharide                                                          |
| <i>t,t</i> ,-MA | <i>trans,trans</i> -muconic acid                                            |
| MAFLD           | metabolic-associated fatty liver disease                                    |
| MASLD           | metabolic dysfunction-associated steatotic liver disease                    |
| MDA             | malondialdehyde                                                             |
| MDCK            | Madin Darby canine kidney                                                   |
| MDS             | myelodysplastic syndrome                                                    |
| MIC             | minimum inhibitory concentration                                            |
| miRNA           | microRNA                                                                    |
| MMT             | methylcyclopentadienyl manganese tricarbonyl                                |
| MOAH            | mineral oil aromatic hydrocarbon                                            |
| MoBa            | Norwegian Mother and Child Cohort Study                                     |
| MPD             | 2-methyl-1,2-propanediol                                                    |
| MRL             | minimum risk level                                                          |
| mRNA            | messenger RNA                                                               |
| MS              | mass spectrometry                                                           |
| MS/MS           | tandem mass spectrometry                                                    |
| MTBE            | methyl <i>tert</i> -butyl ether                                             |
| MTD             | maximum tolerated dose                                                      |
| mtDNA           | mitochondrial DNA                                                           |
| MTT             | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide                |
| NADP            | nicotinamide adenine dinucleotide phosphate                                 |
| NADPH           | reduced form of nicotinamide adenine dinucleotide phosphate                 |
| NAF             | French classification of activities                                         |
| NAFLD           | non-alcoholic fatty liver disease                                           |
| ND              | not detected                                                                |
| NDSRI           | nitrosamine drug substance-related impurity                                 |

|                   |                                                                     |
|-------------------|---------------------------------------------------------------------|
| NER               | nucleotide excision repair                                          |
| NET               | neutrophil extracellular trap                                       |
| NHANES            | National Health and Nutrition Examination Survey                    |
| NHIRD             | National Health Insurance Research Database                         |
| NHL               | non-Hodgkin lymphoma                                                |
| NIOSH             | National Institute for Occupational Safety and Health               |
| NIPH              | Norwegian Institute of Public Health                                |
| NK                | natural killer                                                      |
| NMOC              | non-methane organic compounds                                       |
| NMR               | nuclear magnetic resonance                                          |
| NMSC              | non-melanoma skin cancer                                            |
| NMVOC             | non-methane volatile organic compound                               |
| NOAEC             | no-observed-adverse-effect concentration                            |
| NOCCA             | Nordic Occupational Cancer Study Cohort                             |
| NOCCA-JEM         | Nordic Occupational Cancer Study Cohort job-exposure matrix         |
| NSAID             | nonsteroidal anti-inflammatory drug                                 |
| NTP               | National Toxicology Program                                         |
| O-GlcNAcylation   | attachment of <i>O</i> -linked <i>N</i> -acetylglucosamine moieties |
| OAT               | organic anion transporter                                           |
| OCT               | organic cation transporter                                          |
| OECD              | Organisation for Economic Co-operation and Development              |
| OEL               | occupational exposure limit                                         |
| 8-OHdG            | 8-hydroxy-2'-deoxyguanosine                                         |
| OR                | odds ratio                                                          |
| ORVR              | onboard refuelling vapour recovery                                  |
| 8-oxodG           | 8-oxo-7,8-dihydro-2'-deoxyguanosine                                 |
| 8-oxoGua          | 8-oxoguanine                                                        |
| 8-oxoGuo          | 8-oxo-7,8-dihydroguanosine                                          |
| PAH               | polycyclic aromatic hydrocarbon                                     |
| PBPK              | physiologically based pharmacokinetic                               |
| PBMC              | peripheral blood mononuclear cell                                   |
| PCNA              | proliferating cell nuclear antigen                                  |
| PCR               | polymerase chain reaction                                           |
| PCT               | proximal convoluted tubule                                          |
| PDE               | permitted daily exposure                                            |
| PDFID             | preconcentration and direct flame ionization detection              |
| PDGF              | platelet-derived growth factor                                      |
| PEX               | cross-linked polyethylene                                           |
| PGE2              | prostaglandin E2                                                    |
| Ph+               | Philadelphia chromosome-positive                                    |
| PHG               | Public Health Goal                                                  |
| PHK               | primary human keratinocyte                                          |
| PI3K              | phosphatidylinositol 3-kinase                                       |
| PID               | photoionization detector                                            |
| PIR               | proportionate incidence ratio                                       |
| PKC $\zeta$       | protein kinase C zeta                                               |
| PM <sub>10</sub>  | particle matter of diameter < 10 $\mu\text{m}$                      |
| PM <sub>2.5</sub> | particle matter of diameter < 2.5 $\mu\text{m}$                     |
| PMA               | phorbol 12-myristate 13-acetate                                     |
| PMR               | proportionate mortality ratio                                       |

|              |                                                                      |
|--------------|----------------------------------------------------------------------|
| ppb          | parts per billion                                                    |
| ppm          | parts per million                                                    |
| ppt          | parts per trillion                                                   |
| PXR          | pregnane-X receptor                                                  |
| qPCR         | quantitative polymerase chain reaction                               |
| QSPR         | quantitative structure–property relationship                         |
| QuEChERS     | quick, easy, cheap, effective, rugged, and safe                      |
| RAEB         | refractory anaemia with excess of blasts                             |
| RCMD         | refractory cytopenia with multiple dysplasia                         |
| RDS          | replicative DNA synthesis                                            |
| REACH        | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| RON          | research octane number                                               |
| ROS          | reactive oxygen species                                              |
| RR           | relative risk                                                        |
| rRNA         | ribosomal RNA                                                        |
| rT3          | reverse triiodothyronine                                             |
| S9           | 9000 × g supernatant                                                 |
| SBMA         | S-benzylmercapturic acid                                             |
| SCC          | squamous cell carcinoma                                              |
| SCE          | sister-chromatid exchange                                            |
| SD           | standard deviation                                                   |
| SDS-PAGE     | sodium dodecyl sulfate–polyacrylamide gel electrophoresis            |
| SE           | standard error                                                       |
| SEER         | Surveillance, Epidemiology and End Results                           |
| SES          | socioeconomic status                                                 |
| SINE         | short interspersed nuclear element                                   |
| SIR          | standardized incidence ratio                                         |
| SMR          | standardized mortality ratio                                         |
| SOC          | Standard Occupational Classification                                 |
| SOD          | superoxide dismutase                                                 |
| SPE          | solid-phase extraction                                               |
| SPME         | solid-phase microextraction                                          |
| SPMA         | S-phenylmercapturic acid                                             |
| SRBC         | sheep red blood cell                                                 |
| SRR          | standardized registration ratio                                      |
| SSB          | single-strand break                                                  |
| STEL         | short-term exposure limit                                            |
| T3           | triiodothyronine                                                     |
| T4           | thyroxine                                                            |
| TAA          | <i>tert</i> -amyl alcohol                                            |
| TAME         | <i>tert</i> -amyl methyl ether                                       |
| TBA          | <i>tert</i> -butyl alcohol                                           |
| TBARS        | thiobarbituric acid-reactive substances                              |
| TK           | thymidine kinase                                                     |
| TLR          | toll-like receptor                                                   |
| TLV          | threshold limit value                                                |
| $T_{\max}$   | time to maximum plasma concentration                                 |
| TMP          | trimethylpentane                                                     |
| TNF $\alpha$ | tumour necrosis factor alpha                                         |
| Tox21        | Toxicology in the 21st Century                                       |

|         |                                                                      |
|---------|----------------------------------------------------------------------|
| ToxCast | Toxicity Forecaster                                                  |
| Treg    | regulatory T cell                                                    |
| TRI     | Toxics Release Inventory                                             |
| tRNA    | transfer RNA                                                         |
| TSH     | thyroid-stimulating hormone                                          |
| TUNEL   | terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling |
| TVOC    | total amount of volatile organic compound                            |
| TWA     | time-weighted average                                                |
| TyG     | triglyceride index                                                   |
| UBA     | Umwelt Bundesamt (German Environment Agency)                         |
| UDS     | unscheduled DNA synthesis                                            |
| UK      | United Kingdom                                                       |
| UKCCS   | United Kingdom Childhood Cancer Study                                |
| US      | United States                                                        |
| USA     | United States of America                                             |
| USP     | United States Pharmacopeia                                           |
| UV      | ultraviolet                                                          |
| UV-DAD  | ultraviolet-diode array detection                                    |
| UVA     | ultraviolet A                                                        |
| UVB     | ultraviolet B                                                        |
| UVC     | ultraviolet C                                                        |
| VASP    | vasodilator-stimulated phosphoprotein                                |
| VDR     | vitamin D receptor                                                   |
| VEGF    | vascular endothelial growth factor                                   |
| VHL     | von Hippel–Lindau                                                    |
| VLDL    | very low-density lipoprotein                                         |
| VOC     | volatile organic compound                                            |
| VSMC    | vascular smooth muscle cell                                          |
| WBC     | white blood cell                                                     |
| WHO     | World Health Organization                                            |
| WSFG    | water-soluble fractions of gasoline                                  |
| XO      | xanthine oxidase                                                     |





# ANNEX 1. SUPPLEMENTARY MATERIAL FOR SECTION 1, EXPOSURE CHARACTERIZATION

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## Automotive gasoline

*The following tables were produced in draft form by the Working Group and were subsequently fact-checked and edited:*

|            |                                                                                    |
|------------|------------------------------------------------------------------------------------|
| Table S1.6 | Air concentrations of gasoline components in and around gas stations               |
| Table S1.7 | Occupational exposure to gasoline, assessed using area air monitoring              |
| Table S1.8 | Occupational exposure to gasoline, assessed using a personal air monitoring device |
| Table S1.9 | Occupational exposure to gasoline, assessed using exposure biomarkers in urine     |

*The following table was produced in draft form by the Working Group and was subsequently fact-checked but not edited:*

|             |                                                                                                        |
|-------------|--------------------------------------------------------------------------------------------------------|
| Table S1.15 | Exposure assessment review and critique for studies on cancer in humans exposed to automotive gasoline |
|-------------|--------------------------------------------------------------------------------------------------------|



## ANNEX 2. SUPPLEMENTARY TABLES FOR SECTION 2, CANCER IN HUMANS

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### Automotive gasoline

*The following tables were produced in draft form by the Working Group and were subsequently fact-checked and edited:*

|            |                                                                                                  |
|------------|--------------------------------------------------------------------------------------------------|
| Table S2.5 | Epidemiological studies on exposure to automotive gasoline and digestive and respiratory cancers |
| Table S2.6 | Epidemiological studies on exposure to automotive gasoline and cancer of the breast              |
| Table S2.7 | Epidemiological studies on exposure to automotive gasoline and other solid tumours               |



## ANNEX 3. SUPPLEMENTARY FIGURES FOR SECTION 2, CANCER IN HUMANS

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This Annex contains supplementary figures related to the Working Group's random-effects meta-analyses of epidemiological studies on the association between gasoline exposure and childhood acute lymphoblastic leukaemia, adult haematolymphoid malignancies (non-Hodgkin lymphoma, multiple myeloma; chronic lymphocytic leukaemia, acute myeloid leukaemia, myelodysplastic syndrome, and Hodgkin lymphoma), and cancers of the kidney, urinary bladder, lung, larynx, nasal cavity, stomach, and female breast. The meta-analysis is described in Section 2.8 and results are discussed in Section 2.9 of the monograph on automotive gasoline, in the present volume.

Figs S2.2 to S2.27 are forest plots and Figs S2.28–S2.40 are funnel plots.

### Automotive gasoline

|           |                                                                                                                                                                                                                                                                                                         |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fig. S2.2 | Meta-analysis for childhood acute lymphoblastic leukaemia (using self-reported exposure after expert review in McKinney et al., 2008)                                                                                                                                                                   |
| Fig. S2.3 | Meta-analysis for childhood acute lymphoblastic leukaemia using leaded gasoline exposure in Infante-Rivard et al. (2005)                                                                                                                                                                                |
| Fig. S2.5 | Meta-analysis for childhood acute lymphoblastic leukaemia including only studies evaluating risk by distance from service stations: sensitivity analysis excluding Harrison et al. (1999), as they used other cancer as controls, and Brosselin et al. (2009), for the use of self-reported information |
| Fig. S2.7 | Meta-analysis for adult acute myeloid leukaemia or myelodysplastic syndrome (incidence only)                                                                                                                                                                                                            |

|            |                                                                                                                                          |
|------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Fig. S2.11 | Meta-analysis for adult chronic lymphocytic leukaemia (incidence only)                                                                   |
| Fig. S2.13 | Meta-analysis for adult multiple myeloma (incidence only)                                                                                |
| Fig. S2.14 | Meta-analysis for adult Hodgkin lymphoma                                                                                                 |
| Fig. S2.17 | Meta-analysis for kidney cancer (cohort)                                                                                                 |
| Fig. S2.18 | Meta-analysis for kidney cancer (case–control)                                                                                           |
| Fig. S2.21 | Meta-analysis for lung cancer                                                                                                            |
| Fig. S2.22 | Meta-analysis comparing estimates for bladder and lung cancer, including only studies that reported on both cancer sites                 |
| Fig. S2.23 | Meta-analysis for nasal cancer                                                                                                           |
| Fig. S2.24 | Meta-analysis for laryngeal cancer                                                                                                       |
| Fig. S2.27 | Meta-analysis for female breast cancer                                                                                                   |
| Fig. S2.28 | Funnel plot for childhood acute lymphoblastic leukaemia including only studies evaluating risk by distance from gasoline service station |
| Fig. S2.29 | Funnel plot for adult non-Hodgkin lymphoma                                                                                               |
| Fig. S2.30 | Funnel plot for adult multiple myeloma                                                                                                   |
| Fig. S2.31 | Funnel plot for adult acute myeloid leukaemia                                                                                            |
| Fig. S2.32 | Funnel plot for adult acute myeloid leukaemia, excluding Talbott et al. (2011)                                                           |
| Fig. S2.33 | Funnel plot for adult Hodgkin lymphoma                                                                                                   |
| Fig. S2.34 | Funnel plot for kidney cancer                                                                                                            |
| Fig. S2.35 | Funnel plot for urinary bladder cancer                                                                                                   |
| Fig. S2.36 | Funnel plot for lung cancer                                                                                                              |
| Fig. S2.37 | Funnel plot for laryngeal cancer                                                                                                         |
| Fig. S2.38 | Funnel plot for nasal cancer                                                                                                             |
| Fig. S2.39 | Funnel plot for stomach cancer                                                                                                           |
| Fig S2.40  | Funnel plot for female breast cancer                                                                                                     |



## ANNEX 4. SUPPLEMENTARY MATERIAL FOR SECTION 4, MECHANISTIC EVIDENCE

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These supplementary online-only tables (available from: <https://publications.iarc.who.int/652>) contain summaries of the findings (including the assay name, the corresponding key characteristic, the resulting “hit calls” both positive and negative, and any reported caution flags) for those chemicals evaluated in the present volume that have been tested in high-throughput screening assays performed by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health. The results were generated by the Working Group using the software “kc-hits” (key characteristics of carcinogens – high-throughput screening discovery tool) available from <https://gitlab.com/i1650/kc-hits.git> (Reisfeld et al., 2022), using the US EPA Toxicity Forecaster (ToxCast) assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for *IARC Monographs* Volume 138. Data were available for methyl *tert*-butyl ether, ethyl *tert*-butyl ether, diisopropyl ether, and *tert*-amyl methyl ether.

Please report any errors to [imo@iarc.who.int](mailto:imo@iarc.who.int).

1. Methyl *tert*-butyl ether: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
2. Ethyl *tert*-butyl ether: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
3. Diisopropyl ether: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens
4. *tert*-Amyl methyl ether: ToxCast/Tox21 assay results mapped to the key characteristics of carcinogens

### Reference

Reisfeld B, de Conti A, El Ghissassi F, Benbrahim-Tallaa L, Gwinn W, Grosse Y, et al. (2022). kc-hits: a tool to aid in the evaluation and classification of chemical carcinogens. *Bioinformatics*. 38(10):2961–2. doi:[10.1093/bioinformatics/btac189](https://doi.org/10.1093/bioinformatics/btac189) PMID:[35561175](https://pubmed.ncbi.nlm.nih.gov/35561175/)



# SUMMARY OF FINAL EVALUATIONS

## Summary of final evaluations for Volume 138

| Agent                                  | Evidence stream   |                                |                           | Overall evaluation |
|----------------------------------------|-------------------|--------------------------------|---------------------------|--------------------|
|                                        | Cancer in humans  | Cancer in experimental animals | Mechanistic evidence      |                    |
| Automotive gasoline                    | <i>Sufficient</i> | <i>Sufficient</i>              | <i>Strong<sup>a</sup></i> | Group 1            |
| Methyl <i>tert</i> -butyl ether (MTBE) | <i>Inadequate</i> | <i>Sufficient</i>              | <i>Limited</i>            | Group 2B           |
| Ethyl <i>tert</i> -butyl ether (ETBE)  | <i>Inadequate</i> | <i>Sufficient</i>              | <i>Strong<sup>b</sup></i> | Group 2B           |
| <i>tert</i> -Butyl alcohol (TBA)       | <i>Inadequate</i> | <i>Limited</i>                 | <i>Limited</i>            | Group 3            |
| Diisopropyl ether (DIPE)               | <i>Inadequate</i> | <i>Limited</i>                 | <i>Inadequate</i>         | Group 3            |
| <i>tert</i> -Amyl methyl ether (TAME)  | <i>Inadequate</i> | <i>Inadequate</i>              | <i>Inadequate</i>         | Group 3            |

<sup>a</sup> There was *strong* mechanistic evidence that the agent exhibits key characteristics of carcinogens in exposed humans and experimental systems.

<sup>b</sup> There was *strong* mechanistic evidence that the agent exhibits key characteristics of carcinogens in experimental systems.



This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of automotive gasoline and five oxygenated gasoline additives – methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-butyl alcohol (TBA), diisopropyl ether (DIPE), and *tert*-amyl methyl ether (TAME).

Automotive gasoline (referred to hereafter as “gasoline”) is a commercial product primarily used in internal combustion engines. A complex mixture with a composition that has changed over time, it typically comprises volatile, petroleum-derived hydrocarbons, including alkanes, alkenes, and aromatics, which are blended with various additives. The oxygenated additives MTBE, ETBE, TBA, TAME, and DIPE are volatile compounds that have been used in gasoline to increase combustion efficiency, especially since the elimination of lead. All are listed as high-production-volume chemicals. The most widely used, MTBE and ETBE, are no longer added to gasoline in the USA, but are currently used in Europe, Asia, and elsewhere.

Occupational exposure to gasoline is expected mainly during the production and transport of gasoline and during vehicle refuelling, and includes occupations such as service station attendants, refinery workers, tanker truck drivers, automobile mechanics, and gasoline pump repairers and inspectors. For the oxygenated additives, workers may be exposed during production and via gasoline vapour in any of the above occupations. The general population is mainly exposed via gasoline vapour occurring at service stations, in air pollution, or in water and soil contaminated by gasoline spills.

An *IARC Monographs* Working Group reviewed evidence from epidemiological studies in humans, cancer bioassays in experimental animals, and mechanistic studies to assess the carcinogenic hazard to humans of exposure to these agents and concluded that:

- Automotive gasoline is *carcinogenic to humans* (Group 1);
- MTBE and ETBE are *possibly carcinogenic to humans* (Group 2B);
- TBA, DIPE, and TAME are each *not classifiable as to its carcinogenicity to humans* (Group 3).

