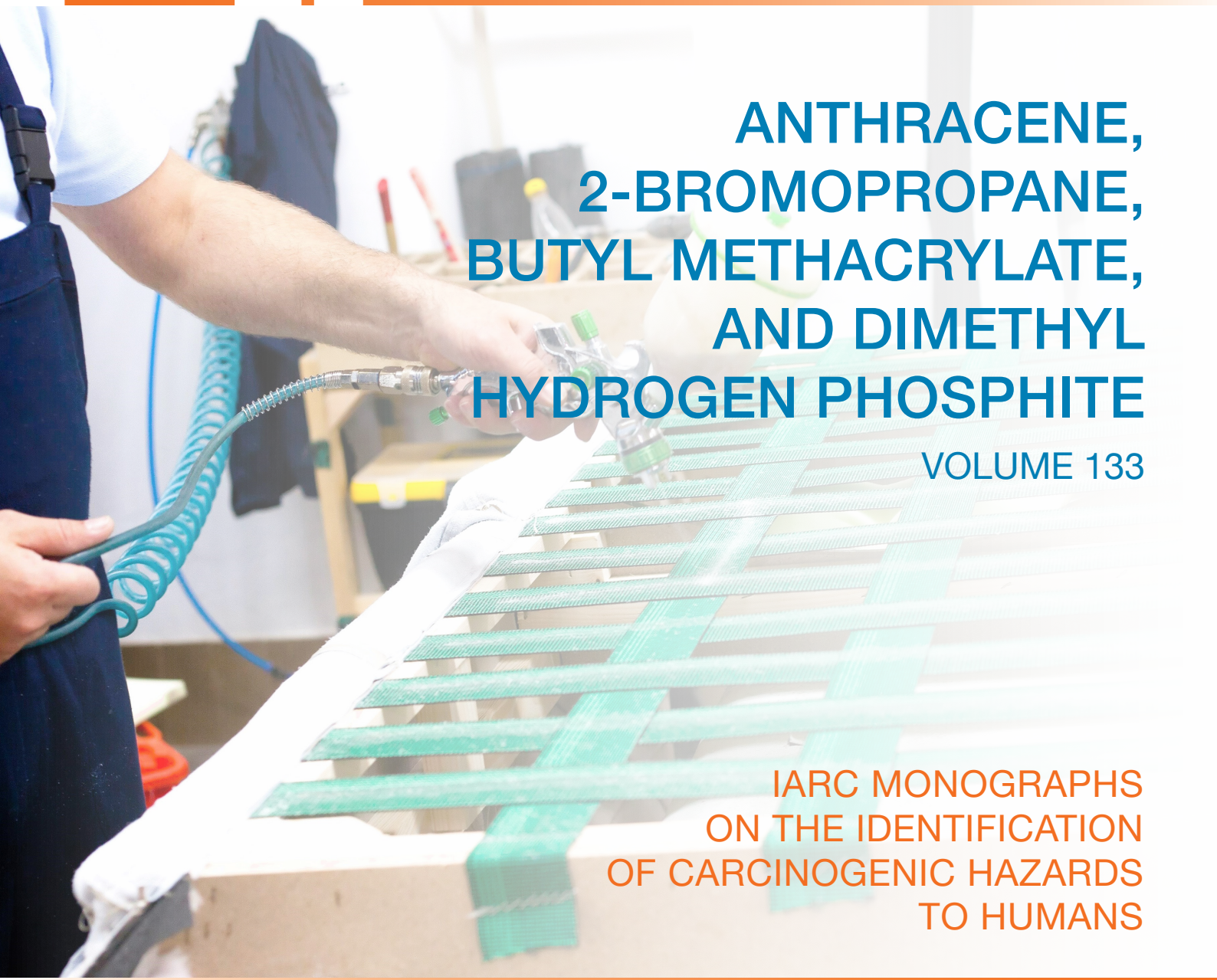
IARC MONOGRAPHS



ANTHRACENE, 2-BROMOPROPANE, BUTYL METHACRYLATE, AND DIMETHYL HYDROGEN PHOSPHITE

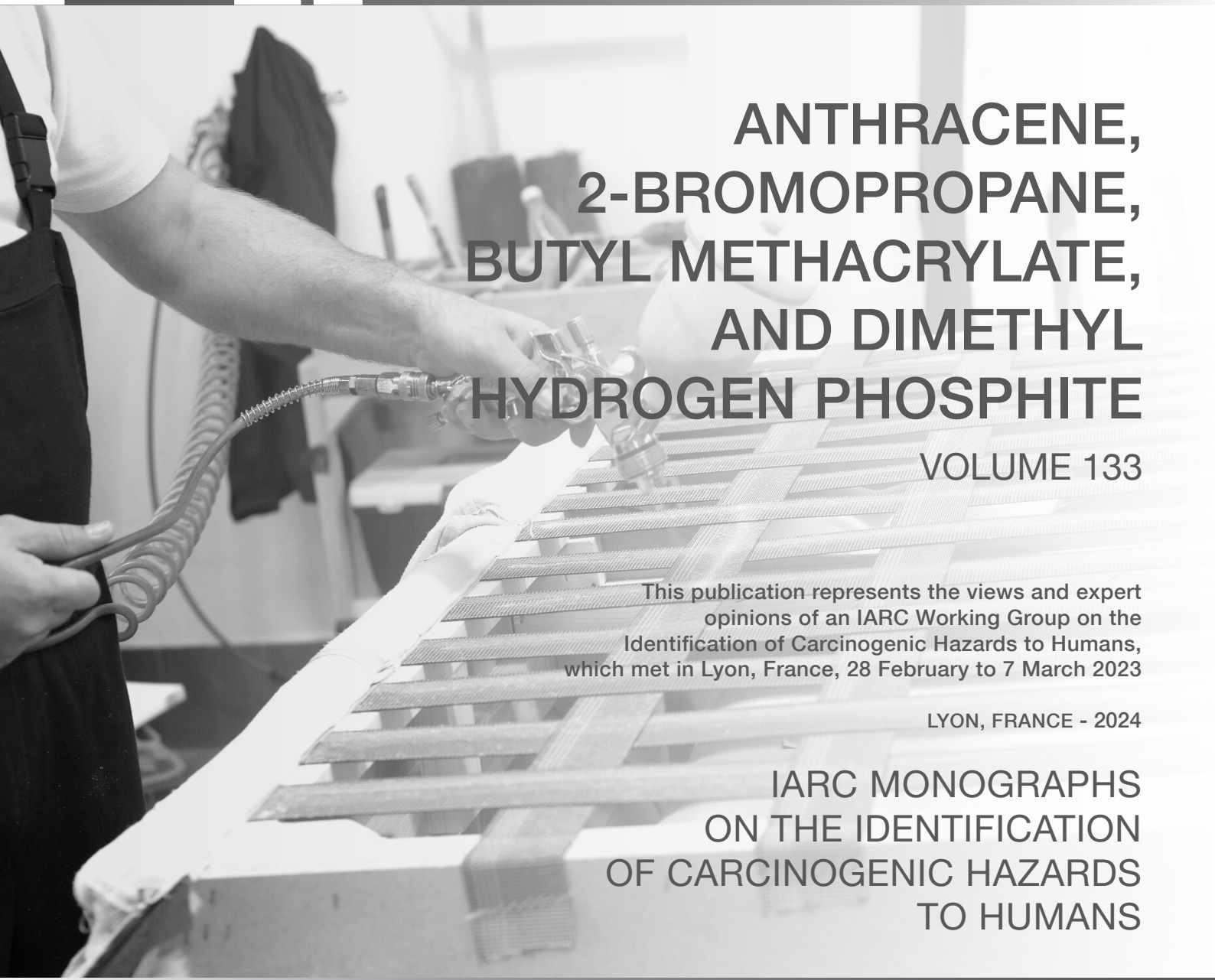
VOLUME 133

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



ANTHRACENE, 2-BROMOPROPANE, BUTYL METHACRYLATE, AND DIMETHYL HYDROGEN PHOSPHITE

VOLUME 133

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, 28 February to 7 March 2023

LYON, FRANCE - 2024

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

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/.

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission Directorate-General for Employment, Social Affairs, and Inclusion, initially by the Unit of Health, Safety and Hygiene at Work, and since 2014 by the European Union Programme for Employment and Social Innovation "EaSI" (for further information please consult: https://ec.europa.eu/social/easi). Support has also been provided since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the United States National Cancer Institute, the United States National Institute of Environmental Health Sciences, the United States Department of Health and Human Services, or the European Commission.

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About the cover: The agents evaluated in this volume have varied industrial uses, including in coatings and adhesives, such as those used in furniture manufacture.

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NOTE TO THE READER

The evaluations of carcinogenic hazard in the *IARC Monographs on the Identification of Carcinogenic Hazards to Humans* series are made by international working groups of independent scientists. The *IARC Monographs* classifications do not indicate the level of risk associated with a given level or circumstance of exposure. The *IARC Monographs* do not make recommendations for regulation or legislation.

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, France, or via email at imo@iarc.who.int, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the *IARC Monographs* programme. Corrigenda are published online on the relevant webpage for the volume concerned (IARC Publications: https://publications.iarc.who.int/).

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[3] Il Je Yu reported being a salaried employee of HCT Co., Ltd, a spin-off of Hyundai Electronics, whose work may involve competing interests for the topics covered by this *IARC Monographs* volume.

[4] Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers do not serve as Meeting Chair or Subgroup Chair, draft any part of a monograph, or participate in the evaluations. They also agree not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.

[5] Dr Tuschl attended as an Observer for the Methacrylate REACH Task Force (MRTF). He is employed by Röhm GmbH, which manufactures butyl methacrylate. Dr Tuschl noted that an IARC classification may have a negative impact on sales for the manufacturers organized within the MRTF, including his employer.

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PREAMBLE

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 Cogliano, 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	✓[a]	✓		
Representatives of health agencies		✓[b]		
Observers		✓[b]		
IARC Secretariat	✓[c]	✓	✓[d]	

[a] Only for the section on exposure characterization.
[b] Only at times designated by the Meeting Chair and Subgroup Chairs.
[c] When needed or requested by the Meeting Chair and Subgroup Chairs.
[d] 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 *Monographs* meeting	IARC selects agents for review in a new volume of the *Monographs* IARC posts on its website: Preliminary List of Agents to be reviewed Call for Data and Call for Experts Request for Observer Status WHO Declaration of Interests form
~8 months before a *Monographs* meeting	Call for Experts closes
~4 months before a *Monographs* meeting	Request for Observer Status closes
~2 months before a *Monographs* 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 *Monographs* meeting	Call for Data closes
~2–4 weeks after a *Monographs* meeting	IARC publishes a summary of evaluations and key supporting evidence
~9 months after a *Monographs* meeting	IARC Secretariat publishes the verified and edited master copy of plenary drafts as a *Monographs* 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 preneoplastic 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[a]	**Evidence of cancer in experimental animals**	**Mechanistic evidence**	
Sufficient	Not necessary	Not necessary	**Carcinogenic to humans (Group 1)**
Limited or Inadequate	***Sufficient***	***Strong (b)(1) (exposed humans)***	
Limited	***Sufficient***	Strong (b)(2–3), Limited, or Inadequate	**Probably carcinogenic to humans (Group 2A)**
Inadequate	***Sufficient***	***Strong (b)(2) (human cells or tissues)***	
Limited	Less than Sufficient	***Strong (b)(1–3)***	
Limited or Inadequate	Not necessary	***Strong (a) (mechanistic class)***	
Limited	Less than Sufficient	Limited or Inadequate	**Possibly carcinogenic to humans (Group 2B)**
Inadequate	***Sufficient***	Strong (b)(3), Limited, or Inadequate	
Inadequate	Less than Sufficient	***Strong (b)(1–3)***	
Limited	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	
Inadequate	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	**Not classifiable as to its carcinogenicity to humans (Group 3)**
All other situations not listed above			

[a] Human cancer(s) with highest evaluation.

[b] 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

General remarks

This one-hundred-and-thirty-third volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of anthracene, 2-bromopropane, butyl methacrylate, and dimethyl hydrogen phosphite.

Anthracene and dimethyl hydrogen phosphite were each previously evaluated by the *IARC Monographs* programme as *not classifiable as to its carcinogenicity to humans (Group 3)* (IARC, 1999, 2010). Two of these agents – 2-bromopropane and butyl methacrylate – were evaluated by the *IARC Monographs* programme for the first time.

The Advisory Group to Recommend Priorities for the *IARC Monographs* during 2020–2024, which met in 2019, recommended that anthracene and dimethyl hydrogen phosphite be evaluated with medium priority and butyl methacrylate with low priority (IARC, 2019a; Marques et al., 2019). 2-Bromopropane was not recommended for evaluation, but it was the subject of a recent cancer bioassay that gave positive results and was thus accorded priority for evaluation in forthcoming meetings (IARC, 2019a; Marques et al., 2019).

A summary of the findings of this volume appears in *The Lancet Oncology* (Cattley et al., 2023).

Evaluation of anthracene

Exposure data for anthracene

The lack of data on anthracene concentrations from food surveys, particularly in geographical areas where agricultural lands are polluted and/or biomass is widely used for cooking (e.g. Africa), hampers the assessment of anthracene dietary intake by the general population. The Working Group considered this information highly relevant for assessing general population exposure because ingestion has been identified as one of the most significant routes of exposure to anthracene for people who do not smoke and are not exposed occupationally. There is a general lack of data on anthracene concentrations in consumer products, such as those incorporating coal tar or those derived from pitch or coal tar (e.g. over-the-counter shampoos and hair care products for the treatment of seborrheic dermatitis and psoriasis) and that are likely to contain anthracene. Dermal absorption is also poorly characterized for these products.

Anthracene's potential for phototoxicity

The Working Group noted the lack of specific epidemiological literature on (i) investigating the effects of anthracene among people who work

outside and are exposed to anthracene under sunlight; and (ii) identifying specific biomarkers. Phototoxicity is a concern for anthracene, given that common exposures are from outdoor air pollution and in sunlight; therefore, the photo-modifications that anthracene undergoes should not be overlooked when considering the carcinogenic potential of anthracene in combination with sunlight (Mujtaba et al., 2011; Choi & Oris, 2000a, b; Forbes et al., 1976). There are knowledge gaps and research opportunities for anthracene, including: (i) mechanistic studies with primary human cells; (ii) studies to evaluate the tumour promotion potential of anthracene in a two-stage initiation–promotion animal model with anthracene as the promoter; and (iii) additional exposure studies to better evaluate the effects of anthracene on human health specifically for, but not limited to, cancer.

Evaluation of 2-bromopropane

The classification of 2-bromopropane in Group 2A

In the case of 2-bromopropane, the Working Group recognized that the strict application of the framework given in the Preamble to the *IARC Monographs* (IARC, 2019b) would have led to an assignment to Group 2B (*possibly carcinogenic to humans*) and that the assignment of 2-bromopropane to Group 2A (*probably carcinogenic to humans*) was exceptional. This Group 2A evaluation was based upon two circumstances within two streams of evidence: cancer in experimental animals and mechanistic evidence. First, there was an unusually high degree of carcinogenic activity in both sexes of animals in a study that complied with Good Laboratory Practice, based upon the occurrence of malignant tumours of various types with a high incidence and at numerous sites. Second, the evidence for key characteristics of carcinogens in experimental systems, which was judged to be *strong* for "is immunosuppressive" and "modulates receptor-mediated effects", was supported by suggestive evidence for these two key characteristics in studies of exposed humans.

Addressing bias in exposure assessments for 2-bromopropane

In its evaluation of 2-bromopropane, the Working Group re-analysed a study on biomarkers of effect among workers exposed to 2-bromopropane that clearly showed that the applied individual-based assessment of exposure resulted in a strong attenuation of exposure–response associations. Re-analysing the data using a group-based approach resulted in stronger and unbiased estimates of the exposure–response association (see Fig. 4.1 in the monograph on 2-bromopropane). In addition to evaluating the quality of the exposure assessment in observational studies in humans, important bias should be addressed and, where possible, corrected (Schubauer-Berigan et al., 2023). Thus, the Working Group considered that the assessment and post hoc correction of bias caused by measurement error in observational studies in humans was essential for a proper assessment of the carcinogenic hazard of 2-bromopropane.

Mechanistic considerations for 2-bromopropane and similar agents

The Working Group noted that 2-bromopropane is structurally similar to at least two other agents previously evaluated for carcinogenic hazard by the *IARC Monographs* programme – 1-bromopropane and bromodichloromethane – both of which were classified in Group 2B (*possibly carcinogenic to humans*). In addition, all three chemicals have mechanistic features in

common, including genotoxicity. The Working Group further noted similarities in reproductive toxicity among these chemicals.

End-points related to immunosuppression of 2-bromopropane

The Working Group found consistent and coherent mechanistic evidence that 2-bromopropane is immunosuppressive in experimental systems and suggestive evidence for this key characteristic in exposed humans (see Section 5.4 in the monograph on 2-bromopropane).

Host immunity represents an important barrier to tumour formation and progression, and immunosuppression is recognized as one of the 10 key characteristics commonly exhibited by human carcinogens (Smith et al., 2016). Multiple pathways are involved in evading innate and adaptive immune responses, and a broad spectrum of chemicals display the potential to adversely influence immunosurveillance (Kravchenko et al., 2015). Many of the mechanisms through which environmental chemicals or therapeutic drugs modulate immune function are well recognized, and 10 key characteristics exhibited by immunotoxic agents have recently been described (Germolec et al., 2022).

In the context of carcinogenicity, chemical-induced immunosuppression is a mechanism by which chemicals alter immune cell function such that immune cells fail to detect and destroy tumour cells, restrain tumour growth, or create a permissive environment for cancer via some other mechanism.

The immune system comprises a complex network of different cell types located in various organs and their mediators, which operate to maintain homeostasis. An immune response occurs through the coordination of many different cell types and can involve several tissues. The thymus and bone marrow are critical for immune cell development, and the lymph nodes and spleen are organs in which many immune responses occur. Chemical exposure can influence various components of the immune system via different mechanisms, eventually leading to adverse health outcomes.

Factors such as age at onset, sex, dose, duration, and route of exposure may result in differing effects on the immune system and skew the adverse response in the direction of immunosuppression or immunostimulation. Immunotoxicity can manifest in a variety of ways, with one of the most prominent effects being immunosuppression (Vos & Moore, 1977; Dean et al., 1982).

The consequences of immunosuppression after exposure to environmental chemicals or therapeutic drugs are increased sensitivity to infections and cancer (Germolec et al., 2017). A drug or chemical that causes immunosuppression might alter the number of cells (innate or adaptive); the ability of the cells to produce cytokines, chemokines, antibodies, or growth factors; the composition of the subpopulations of cells present at the site of the response; or the cell function (e.g. kill infected cells or cause proliferation). Signs of immunotoxic potential caused by agents in standard toxicology studies in experimental animals can be defined by haematological changes (i.e. leukocytopenia/leukocytosis, granulocytopenia/granulocytosis, or lymphopenia/lymphocytosis), alterations in immune system organ weights or histology, changes in serum antibodies, or changes in the incidence of infections or tumours. Specifically, the following parameters should be evaluated for signs of immunotoxicity: (i) changes in total and differential leukocyte counts; (ii) alterations in immune organ weights and histology; (iii) decreased levels of basal plasma immunoglobulins; (iv) increased incidence of infection; (v) increased occurrence of tumours in the absence of genotoxicity, hormonal effects, or liver enzyme induction; and (vi) retention of

Table 1. Testing battery to assess chemical-induced immunotoxicity in rodents (according to National Toxicology Program guidelines)

Screen (tier I)	Immunopathology (haematology, organ weights, spleen cellularity, histopathology) Cell quantification (surface marker analysis in spleen) Humoral immunity (IgM TDAR) Cell-mediated immunity (CTL, DTH) Nonspecific immunity (NK cell assay)
Definitive (tier II)	Humoral immunity (IgG TDAR) Nonspecific immunity (macrophage function) Host-resistance assays

TDAR, T-cell dependent antibody response; CTL, cytotoxic T lymphocytes; DTH, delayed-type hypersensitivity; NK, natural killer; IgM, immunoglobulin M.
Adapted from Hinton (2000), Luster et al. (1988).

the chemical in organs or cells of the immune system.

Myelotoxicity or bone marrow toxicity is characterized by a decrease in the production of cells responsible for providing immunity (leukocytes), carrying oxygen (erythrocytes), and/or those responsible for normal blood clotting (thrombocytes) (in each monograph, this information is reported in Section 3, Cancer in experimental animals, and Section 4, Mechanistic evidence). In the context of immunotoxicity, myelotoxicity would refer to toxicity to precursors of immune cells. Compounds that are capable of damaging or destroying the bone marrow will have a profound immunotoxic effect, since the effectors of the immune system itself will no longer be available. Therefore, if a compound is myelotoxic, according to the specific assay performed, the chemical will de facto be an immunotoxicant (Gennari et al., 2005; OECD, 2022).

Thus, useful information on potential immunosuppressive hazard can be derived from histopathology of immune organs, enumeration of immune cells, or mostly from functional immune tests, which may be used in various tiers (Hinton, 2000; Luster et al., 1988). An example of a testing battery to assess chemical-induced immunotoxicity, from the National Toxicology Program guidelines for immunotoxicity evaluation in rodents, is shown in Table 1.

Standard assessments of immunotoxicity use both in vitro and ex vivo assays that evaluate different functional parameters of the immune response; of these assays, those for lymphocyte proliferation, mixed lymphocyte reaction, cytotoxic T lymphocytes, and natural killer cell activity are relevant to immunosurveillance of cancer (Germolec et al., 2017).

Modulation of receptor-mediated effects by 2-bromopropane

Together with myelotoxicity, there is suggestive evidence that 2-bromopropane modulates receptor-mediated effects, a key characteristic of carcinogens; this is based on alterations in serum levels of several hormones, namely, follicle-stimulating hormone (FSH), luteinizing hormone-releasing hormone (LHRH), luteinizing hormone (LH), estradiol, and testosterone in exposed workers. Alterations in hormone levels can have significant effects on their respective target receptors. However, the Working Group considered that there is only very limited evidence of cancer causation associated with levels of LH and LHRH, since their role is yet to be fully elucidated. In addition, while there are known associations between estradiol and cancers in the female reproductive tract and between testosterone and cancers in the male reproductive tract,

these are generally shown as positive associations with increased receptor activity. The Working Group observed increased levels of FSH and LH and decreased levels of estradiol in women, and decreased levels of testosterone in men. For this reason, and because of the lack of further information on the activities of various receptors, the evidence for modulation of receptor-mediated effects, and the link to carcinogenesis, was found to be only suggestive for 2-bromopropane.

Carcinogenicity in experimental animals

Trend tests

In its evaluation of studies of cancer in experimental animals for three of the agents considered (anthracene, 2-bromopropane, and butyl methacrylate), the Working Group took into account, in addition to the Cochran–Armitage trend test, the data analysis methodology applied by the Japan Bioassay Research Center (JBRC, 1998, 2018a, b, 2019). This included three Peto test methods: the standard method (referred to as "death analysis"), the prevalence method (referred to as "incidental tumour test"), and combined analysis (referred to as "death analysis plus incidental tumour test"). The Working Group considered that a significant *P* value in any trend test was relevant for the detection of treatment-related increases in tumour incidence.

Combination of tumours

When considering the data for anthracene and 2-bromopropane, the Working Group consulted a publication by Brix et al. (2010) on appropriate combinations of lung neoplasms and combinations of mammary gland neoplasms in rodents for the purposes of evaluating the statistical and biological significance of these neoplasms. Specifically, the incidence data for squamous cell neoplasms of the lung should not be combined with those for bronchioloalveolar neoplasms. Similarly, the incidence data for fibroadenoma of the mammary gland should not be combined with those for adenoma, except when there is evidence that an adenoma or carcinoma of the mammary gland has arisen from a fibroadenoma. In the studies by the Japan Bioassay Research Center, no information was provided regarding why these tumour types were combined or the criteria used (JBRC, 1998; 2019). Therefore, these combinations of tumour incidence data were not considered by the Working Group in its evaluation of the evidence on carcinogenic activity.

Scope of the systematic review

Standardized searches of the PubMed database (NCBI, 2023) were conducted for each agent and for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). For cancer in humans, searches were also conducted in the Web of Science (Clarivate, 2023) and Embase (Elsevier, 2023) databases. The literature trees for the agents, including the full set of search terms for the agent name and each outcome type, are available online.[a]

[a] The literature trees for the present volume are available at: https://hawcproject.iarc.who.int/assessment/660/ (anthracene), https://hawcproject.iarc.who.int/assessment/695/ (2-bromopropane), https://hawcproject.iarc.who.int/assessment/696/ (butyl methacrylate), and https://hawcproject.iarc.who.int/assessment/697/ (dimethyl hydrogen phosphite).

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ANTHRACENE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

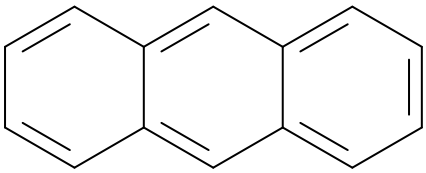
Chem. Abstr. Serv. Reg. No.: 120-12-7 (NCBI, 2022)

EC/List No.: 204-371-1 (NCBI, 2022)

IUPAC systematic name: anthracene (NCBI, 2022)

Synonyms: paranaphthalene; anthracin; Green Oil; Tetra Olive N2G (NCBI, 2022)

1.1.2 Structural and molecular information



Molecular formula: $C_{14}H_{10}$ (NCBI, 2022)

Relative molecular mass: 178.23 (NCBI, 2022).

1.1.3 Chemical and physical properties of the pure substance

Description: tablets or monoclinic prisms (from alcohol recrystallization) with weak aromatic odour. When pure, it is colourless with violet fluorescence; if impure (due to the presence of tetracene or naphthacene), it is yellow with green fluorescence (NCBI, 2022).

Boiling-point: 340–342 °C (NCBI, 2022; Royal Society of Chemistry, 2022)

Melting-point: 214 °C [the melting point of anthracene ranges from 208 °C to 218 °C, mainly depending on the grade of purity] (NCBI, 2022; Royal Society of Chemistry, 2022; ECHA, 2023)

Flash-point: 121 °C at 101.3 kPa (NCBI, 2022; Royal Society of Chemistry, 2022; ECHA, 2023)

Density: 1.25 g/mL at 20 °C (NCBI, 2022; Royal Society of Chemistry, 2022)

Vapour pressure: 9.4×10^{-4} Pa at 25 °C (ECHA, 2008a)

Solubility: 4.34×10^{-5} g/L in water at 24 °C; 14.93 g/L in ethanol; 14.29 g/L in methanol; 16.13 g/L in benzene; 11.76 g/L in chloroform; 5 g/L in ether; 32.36 g/L in carbon disulfide; 11.63 g/L in carbon tetrachloride; 8 g/L in toluene (IARC, 1983; NCBI, 2022; Royal Society of Chemistry, 2022).

Octanol/water partition coefficient (P): log K_{ow} = 4.45 (NCBI, 2022; Royal Society of Chemistry, 2022)

Octanol/air partition coefficient (P): log K_{oa} = 7.55 (Royal Society of Chemistry, 2022)

Stability: Darkens in sunlight. Strongly triboluminescent and triboelectric; forms molecular addition products with nitrogen compounds (NCBI, 2022).

[The Working Group used a conversion factor of 1 ppm ≈ 7.29 mg/m^3 at 25 °C in air.]

1.1.4 Technical grade and impurities

A commercial grade (purity, 90–95% by weight), higher-purity reagent grade (purity, 97%), and sublimed anthracene (purity, ≥ 99%) are available (IARC, 1983; ECHA, 2008a; NCBI, 2022).

Reported impurities include phenanthrene, carbazole, naphthothiophene, dibenzo[*b*,*c*]thiophene, acridine, acetophenone, and chrysene (ECHA, 2008a). Anthracene was reported to be available until 1982 from one producer in the USA, as refined anthracene with the following specifications: purity, 90–95% by weight; carbazole, 3% maximum; sublimation residue, 0.5% maximum; pyridine, 0.2% maximum; ash, 0.1% maximum; and iron, 0.03% maximum (IARC, 1983). Typical properties of this refined anthracene were: melting-point, 216 °C; boiling-point, 340 °C; specific gravity, 1.25; and vapour pressure, < 3 × 10^{-4} mm Hg (20 °C) (IARC, 1983).

1.2 Production and use

1.2.1 Production process

Anthracene can be synthesized from benzyl chloride in a two-step reaction (Friedel–Crafts reaction); from *ortho*-methylbenzophenone (Elbs reaction); from 2,3-dihydronaphthalene-1,4-dione (Diels–Alder reaction); from benzene and phthalic anhydride (Haworth synthesis); from the reaction between two molecules of benzene with 1,1,2,2-tetrabromoethane in the presence of aluminium chloride; and from phthalic anhydride and substituted benzene (Sahoo et al., 2020; Baviera & Donate, 2021). However, anthracene is not generally synthesized industrially and is usually recovered from coal tar, specifically from one of its distillate fractions (known as anthracene oil or green oil), through the application of a set of sequential separation and purification techniques, namely vacuum distillation (concentration to about 50% anthracene), salting out, recrystallization in polar solvents (yielding > 95% anthracene) and sublimation or azeotropic distillation (ECHA, 2008a, 2022b; Chemicalbook, 2021; NCBI, 2022). [The Working Group noted that anthracene oil is a complex mixture containing anthracene and other two- to four-ring aromatic compounds, and is not the agent under evaluation in the present monograph.] Anthracene, as a polycyclic aromatic hydrocarbon (PAH; a compound that is exclusively composed of fused aromatic rings that share a pair of carbon atoms), is also involuntarily produced during combustion and in some industrial processes (see Section 1.4.1) and is, in these processes, included in the respective tailings.

1.2.2 Production volume

Anthracene has been classified by the Organisation for Economic Co-operation and Development (OECD) as a High Production Volume chemical (OECD, 2023). Estimates of the world production of anthracene from coal tar range from 10 000 to 20 000 tonnes per year (Collin et al., 2011; Chemicalbook, 2021), which are almost exclusively used in the manufacture of anthraquinone (Collin et al., 2011). On the basis of the available data, two active suppliers were identified in the European Union (EU) (ECHA,

2022a). Few data were available, but production volumes of < 2000 tonnes per year have been reported for Europe between 1995 and 2001 (European Chemicals Bureau, 2007). Under the Toxic Substances Control Act, the United States Environmental Protection Agency (US EPA) reported a nationally aggregated production volume of < 1 000 000 pounds [453.6 tonnes] in 2016, 2017, 2018, and 2019, with no obvious differences when compared with data from 1986, 1998, and 2002 (10 000–500 000 pounds [4.54–226.8 tonnes]) (US EPA, 2020; NCBI, 2022).

1.2.3 Uses

Anthracene is reported to be mainly used as an intermediate in the manufacture of dyes (anthraquinone-based products and alizarin dyes), wood preservatives, and pesticides (see also Section 1.4.1(c) for uses of anthracene in consumer products) (NCBI, 2022). The US EPA classifies industrial use of anthracene as a chemical ingredient in the composition of propellants, e.g. in the manufacture of pyrotechnics; no information on anthracene was presented in the database on consumer and commercial uses (US EPA, 2020). In the European Economic Area, anthracene is registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations for use as an intermediate in the manufacture of bulk, large-scale chemicals (including petroleum products), and fine chemicals, laboratory chemicals, and pharmaceuticals (ECHA, 2022b). In Nordic countries, anthracene was reported to be used during 2000–2003 in Denmark in rust inhibitors and anti-corrosion products, and during 2016–2017 in Finland in the manufacture of chemicals and chemical products (SPIN, 2022). Worldwide, anthracene is also used in research laboratories in the fields of polymers and semiconductor materials, including as a core component to obtain saturated deep blue organic light-emitting diodes to be used in flat-panel displays and lighting source technology (Park et al., 2012; Baviera & Donate, 2021).

Anthracene is likely to exist, simultaneously with additional PAHs, in commercial products such as coal tar, creosote, and asphalt (bitumen), and in their respective derived products (Government of Canada, 2022a).

1.3 Detection and quantification

Since anthracene is part of a complex mixture of PAHs that commonly occur as products of combustion/pyrolysis processes, it is usually detected and quantified together with other PAHs. A selection of methods used to detect and quantify anthracene in various matrices is reported in Table 1.1.

1.3.1 Air

Anthracene is a three-ring PAH and is therefore expected to be found mostly in the vapour phase in air, together with two-ring PAHs. A smaller proportion is found in the particle phase (Oliveira et al., 2016). The sampling of airborne PAHs requires the use of pumping systems in which adsorbent materials (for gaseous PAHs) are connected to filters for particle-phase PAHs. After collection, PAHs are desorbed from the adsorbents using various organic solvents (including dichloromethane, methanol, acetone, cyclohexane, and benzene). Chromatographic separation, either by gas chromatography (GC) or high-performance liquid chromatography (HPLC), is necessary to separate the different PAHs, usually followed by detection using universal methods such as mass spectrometry (MS) and fluorescence detection (FLD).

The sampling of anthracene in air is usually performed by means of an adsorbent material (including XAD-2 resins and polyurethane foam) placed downstream from the filter used to collect high-molecular-weight PAHs (National

Table 1.1 Analytical methods for the measurement of anthracene

Sample matrix	Sample preparation	Analytical method (LOD)	Comments	Reference
Air	Filter (glass fibre) + adsorption on sorbent material (XAD-7), extraction with methylene chloride in ultrasonic bath	GC-MS SIM (0.08 µg/sample)	NIOSH method 5528	NIOSH (1984)
Air	Filter (PTFE) + adsorption on sorbent material (XAD-2), extraction with acetonitrile	HPLC-FLD or UV (0.0010–0.090 µg/sample)	NIOSH method 5506	NIOSH (1998)
Air	Adsorption on sorbent material (XAD-2), extraction with alternate organic solvents depending on the sample matrix in ultrasonic bath	GC-FID (0.3–0.5 µg/sample)	NIOSH method 5515	NIOSH (1994)
Air	Adsorption on sorbent material (glass fibre filters), extraction with benzene	LC-FLD or UV (0.028 µg/m^3)	OSHA method 58	OSHA (1986)
Solid waste matrices, soils, and groundwater	Extraction with methylene chloride	GC-MS (ground water,10 µg/L; soil/sediment, 660 µg/kg)	US EPA method 8270D	US EPA (2014a)
Municipal and industrial discharge	Extraction with methylene chloride	HPLC-FLD (0.66 µg/L)	US EPA method 610	US EPA (1984)
Soil and sediments	Pressurized solvent extraction using a water/isopropyl alcohol mixture followed by SPE	GC-MS (11.9 µg/kg)	Method prepared by the United States Geological Survey Office of Water Quality	Zaugg et al. (2006)
Consumer products (polymer samples)	Extraction with toluene	GC-MS (0.2 mg/kg)	Method in the awarding of the GS mark	AfPS (2020)
Cosmetics	Extraction with acetone:hexane	GC-MS/MS (0.1 mg/kg)		Wang et al. (2019)
Edible fats and oils	Purification by donor–acceptor complex chromatography	HPLC-FLD (0.1 µg/kg)	Standard method ISO 22959	ISO (2009)
Seafood	Extraction with ethyl acetate followed by silica SPE clean-up	GC-MS (LOD not reported)	AOAC International	Mastovska et al. (2015)
Urine	HS-SPME	GC-MS (2.2 ng/L)		Campo et al. (2009)
Urine	SPME	GC-MS/MS (0.2 ng/L)		Campo et al. (2016)
Blood (serum)	Extraction with organic solvents, followed by silica SPE clean-up	GC-MS/MS (191 ng/L)		Yin et al. (2017)
Saliva	LLE-PTV	GC-MS (91 ng/L)		Santos et al. (2019)

Table 1.1 (continued)

Sample matrix	Sample preparation	Analytical method (LOD)	Comments	Reference
Saliva	HS-SPME	GC-MS/MS (13.4 ng/L)		Martín Santos et al. (2020)
Breast milk	Extraction with acetonitrile and filtration	HPLC-FLD (0.23 μg/L)		Oliveira et al. (2020)

AOAC, Association for Official Analytical Collaboration; GC-FID, gas chromatography-flame-ionization detection; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; GS, Geprüfte Sicherheit ("tested safety"), licensed by the German government for consumer products; HPLC-FLD, high-performance liquid chromatography method-fluorescence detection; HS-SPME, headspace solid-phase microextraction; LLE-PTV, liquid–liquid extraction and programmed temperature vapourizer; LOD, limit of detection; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PTFE, polytetrafluoroethylene; SIM, selected ion monitoring; SPE, solid-phase extraction; SPME, solid-phase microextraction;; US EPA, United States Environmental Protection Agency; UV, ultraviolet.

Institute for Occupational Safety and Health, NIOSH methods 5528, 5506, 5515; Occupational Safety and Health Administration (OSHA) method 58) (NIOSH, 1984, 1994, 1998; OSHA 1986; see Table 1.1). For NIOSH method 5528, which uses gas chromatography-mass spectrometry (GC-MS) in the selected ion monitoring (SIM) mode, the limit of detection (LOD) for anthracene (LOD, 0.08 µg/sample) is comparable to that for NIOSH method 5506, which uses HPLC-FLD (LOD, 0.010–0.090 µg/sample). For other PAHs, the LODs for NIOSH method 5528 are on average twice as low as for NIOSH method 5506. Moreover, NIOSH method 5528 is similar to NIOSH method 5515, the primary differences including a more efficient sampling device and the use of GC-MS SIM rather than GC-FID, thus leading to a lower LOD (0.08 µg/sample versus 0.3–05 µg/sample) and a higher specificity. OSHA method 58 is used in the assessment of exposure to coal tar pitch volatiles and coke-oven emissions. Analytes, including anthracene, are collected with glass fibre filters and desorbed with benzene. The LOD for OSHA method 58 is 0.028 µg/m^3, but the reliable limit of quantification (LOQ) is 0.066 µg/m^3.

1.3.2 Water and soil

In aqueous media (including municipal and industrial discharge), anthracene is extracted using solvents such as methylene chloride (US EPA methods 610 and 8270D, see Table 1.1) (US EPA, 1984, 2014a). In method EPA 610 (LOD, 0.66 µg/L), the extract is concentrated and then separated by HPLC or GC, but the GC procedure does not adequately resolve the pair anthracene/phenanthrene, so HPLC should be preferred (US EPA, 1984). Moreover, both FLD and ultraviolet (UV) detection coupled to HPLC could be used, but FLD is recommended for the determination of anthracene (US EPA, 1984). A less sensitive method is US EPA method 8270D, which uses GC-MS to quantify a series of semivolatile organic compounds (including PAHs) in extracts prepared from different types of matrix, including groundwater samples (LOD, 10 µg/L), sediment and soil matrices (LOD, 660 µg/kg), and wastes (LOD, 1–200 mg/kg, depending on matrix and method preparation) (US EPA, 2014a). For sediment and soil matrices, an extraction procedure consisting of pressurized solvent extraction using a water/isopropyl alcohol mixture followed by solid-phase extraction is described by the United States Geological Survey Office of Water Quality. Anthracene, together with 38 other PAHs and semivolatile organic compounds, is detected by GC-MS, with an LOD of 11.9 µg/kg (Zaugg et al., 2006), making this method more sensitive than US EPA method 8270D.

1.3.3 Consumer products

A method to test and assess anthracene (together with 14 other PAHs) in the awarding of the GS mark (the "Geprüfte Sicherheit" or "tested safety" mark licensed by the German government for consumer products) has been proposed by the German Federal Institute for Occupational Safety and Health (AfPS, 2020). Products (polymer samples) are extracted with toluene (a further purification step using silica gel columns may be necessary) and quantified by GC-MS. The LOD (0.2 mg/kg) achieved with this procedure meets the maximum PAH limits for materials with relevant contact/grip and operating surfaces (i.e. materials intended to be placed in the mouth, or materials coming into long-term contact with skin (more than 30 seconds) during the intended use according to EU legislation Directive 2009/48/EC for toys (European Parliament and Council, 2009a). A validated method to quantify PAHs in cosmetics has been proposed: anthracene is extracted, together with 17 other PAHs, by means of an acetone:hexane mixture and quantified by gas chromatography coupled to tandem mass spectrometry (GC-MS/

MS) with an LOQ as low as 0.1 mg/kg (Wang et al., 2019).

1.3.4 Food

ISO 15753 focuses on the determination of 16 PAHs (including anthracene) in animal and vegetable fats and oils, and has an LOD of 0.2 µg/kg (ISO, 2016). This method cannot be used for the determination of PAHs in palm oil and olive pomace oil. The standard method, ISO 22959, which enables the quantification of 17 PAHs in edible fats and oil, is more sensitive and has an LOD of 0.1 µg/kg (ISO, 2009). Both ISO methods are based on HPLC-FLD. In a method using GC-MS, focused on the determination of PAHs in seafood and published by the Association for Official Analytical Collaboration (AOAC) International, anthracene is quantified together with 18 other PAHs (Mastovska et al., 2015). Several analytical methods for the determination of PAHs in various food products and with different extraction and clean-up procedures and analytical techniques have been published in the scientific literature. A review of analytical methods for PAHs in food can be found in Zelinkova & Wenzl (2015), and a review focusing on the determination of PAHs in olive oils can be found in Bertoz et al. (2021).

1.3.5 Biological samples

Some analytical methods have been developed to quantify anthracene in urine, blood, saliva, and hair. The validation of these methods is not always described appropriately. In urine, a method based on headspace solid-phase microextraction (HS-SPME) coupled to GC-MS, with an LOQ of 2.28 ng/L, has been developed and validated by Campo et al. (2009). The method has been further improved by using direct immersion solid-phase microextraction (SPME) instead of HS-SPME and GC coupled to triple quadrupole mass spectrometer (GC-MS/MS), obtaining a lower LOQ for anthracene (0.2 ng/L) (Campo et al., 2016).

In blood, a method based on extraction with organic solvents followed by clean-up using silica solid-phase extraction and analysis by GC-MS/MS (LOD, 191 ng/L) was applied to umbilical cord serum samples (Yin et al., 2017) and to blood samples from firefighters (Ekpe et al., 2021).

For saliva, Santos et al. developed and validated a method based on liquid–liquid extraction and programmed temperature vapourizer-GC-MS (LLE-PTV-GC-MS), with an LOQ for anthracene of 91 ng/L (Santos et al., 2019), and a more sensitive method (LOQ, 13.4 ng/L) based on headspace solid-phase microextraction (HS-SPME) and GC-MS/MS (Martín Santos et al., 2020).

For breast milk, Oliveira et al. developed a method using HPLC-FLD to detect anthracene together with other PAHs after solvent extraction and filtration (LOQ, 0.23 µg/L) (Oliveira et al., 2020).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Anthracene releases or disposal into the environment take place due to industrial use or unintended formation during production processes. Anthracene, as a PAH, is also a ubiquitous environmental pollutant since it is formed during the incomplete combustion or pyrolysis of organic matter (IARC, 2010). Thus, sources can be of both natural (e.g. forest fires, volcanic eruptions and seepage of petroleum or coal deposits) and anthropogenic (industrial, domestic, traffic, etc.) origins, with a predominance of the latter (Santonen et al., 2019). Several hundred PAHs exist, and they usually occur as complex mixtures that are produced during the combustion or pyrolysis processes. The US EPA (2005) has established a list of 16 PAHs, including anthracene, that are classified

Table 1.2 Total annual releases of anthracene to air, water, land disposal, or off-site disposal, in the USA, 2010–2020

	Releases (in pounds[b])										
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Air	8 647	8 509	6 985	5 850	5 604	5 376	4 556	5 971	9 390	8 792	7 479
Land[a] (placed in a disposal facility on site)	[487]	[57 556]	[99 883]	[1 364]	[1 848]	[42 853]	[46 906]	[25 342]	[18 937]	[1 301]	[731]
Off-site disposal (transferred off-site) or other releases	58 493	24 969	15 179	7 578	7 978	9 541	6 529	22 053	12 351	55 805	20 603
Water	400	981	136	155	135	143	169	214	240	1 317	272
Total	68 027	92 015	122 183	14 947	15 565	57 913	58 161	53 579	40 918	67 215	29 085

[a] Release to land was calculated by the Working Group by subtracting all other releases from the total releases.
[b] To convert pounds into kilograms, multiply by 0.4536.
From US EPA (2023).

as priority pollutants on the basis of their representativeness and frequency of (co)occurrence in environmental samples (IARC, 2010; Keith, 2015). The total releases of anthracene per year to air, water, land (placed in disposal, defined as any underground injection, placed in landfills or surface impoundments, land treatment, or other intentional land disposal), or transferred off-site for disposal (or other releases not quantified elsewhere) in the USA during 2010–2020 are presented in Table 1.2 (US EPA, 2023). [The mean total is 56 328 pounds (25.57 tonnes) per year, with variations of –48% to +19% in the later years (2015–2020).] According to these data, the lowest emissions have been consistently to water (except for 2010 and 2019) and the highest to land (2011–2012, 2015–2018) or off-site disposal (2010, 2013–2014, 2019–2020). Emissions to air have been the second highest when off-site disposal was predominant (US EPA, 2023). The Toxics Release Inventory data from the 2019 national analysis in the USA (US EPA, 2023) show that releases by industry sector were, in descending order, petroleum (87.1%), chemical manufacturing (8.5%), primary metals (2.4%), electrical equipment (1.8%), petroleum bulk terminals (0.16%), electric utilities (0.03%), and non-metallic mineral products (0.009%); data from 2020 generally follow the same order (US EPA, 2023).

(a) Air

(i) Ambient air

Anthracene in the air originates mainly from incomplete combustion and industrial processes such as coking and primary aluminium production (Ravindra et al., 2008; Shen et al., 2014; Government of Canada, 2022b; US EPA, 2022). Since anthracene is a low-molecular-weight PAH, the majority of airborne anthracene is present in the gas phase, with a small fraction adsorbed on particles. Anthracene in the gas phase is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals, and nitrate radicals and ozone (half-lives ranged from 2.1 to 10 hours) (Atkinson et al., 1989; ECHA, 2008a).

Anthracene is ubiquitous in the atmosphere and has even been detected at several global background sites, including the High Arctic (Norwegian Institute for Air Research 2022). There are numerous studies on measurements of anthracene in ambient air (selected references are listed in Table 1.3). Relatively high

Table 1.3 Occurrence of anthracene in ambient air

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Chicago, USA, 1995	12	14.1 ng/m³ (NR)	NR	HPLC-MS (NR)	Active sampling, 39 L/minute, QFF medium, gas and particle	Odabasi et al. (1999)
Gandy Bridge, USA, 2002	7–9	0.5 ng/m³ (NR)	NR	GC-MS (0.002 ng/m³)	Passive sampling, QFF, gas	Poor et al. (2004)
Baltimore, USA, 1997	1	0.332 ng/m³ (NR)	NR	GC-MS (NR)	Passive sampling, QFF-PUF, gas and aerosol	Dachs et al. (2002)
Adjacent to Chesapeake Bay, USA, 1997	1	0.185 ng/m³ (NR)			Summer, passive sampling, QFF-PUF, gas and aerosol	
Adjacent to Chesapeake Bay, USA, 1997	1	0.145 ng/m³ (NR)			Winter, passive sampling, QFF-PUF, gas and aerosol	
Bursa, Turkey, 2004–2005	25	6.09 ng/m³ (NR)	NR	GC-MS (0.5–201 ng)	Winter, filters and PUFs	Vardar et al. (2008)
		0.30 ng/m³ (NR)			Summer, filters and PUFs	
Boston, USA, 1991	NR	2.1 ng/m³ (NR)	NR	GC-MS	Average of the four seasons	US EPA (1992)
Houston, USA, 1991	NR	1.5 ng/m³ (NR)			Average of the four seasons	
Brisbane, Australia, 1998	6	4.3 (1.2–8.8) ng/m³	NR	GC-ITD (0.01 ng/m³)	Urban, winter, gas and particle	Müller et al. (1998)
	1	1.0 ng/m³ (NR)			Urban, summer, gas and particle	
Flanders, Belgium, 2001	6	6.22 ng/m³ (NR)	NR	HPLC-FLD (NR)	Active sampling, urban site, QF + PUF, winter	Du Four et al. (2005)
	6	2.5 ng/m³ (NR)			Active sampling, industrial site, QF + PUF, winter	
	6	6.1 ng/m³ (NR)			Active sampling, rural site, QF + PUF, winter	
Indigenous Nations' Park, Brazil, 2003	8	0.39 (0.05–1.77) ng/m³	NR	NR	Urban, active sampling, PUF, gas	Ströher et al. (2007)
Indubrasil, Brazil, 2003	9	0.51 (0.02–1.66) ng/m³			Industry, active sampling, PUF, gas	
Ary Coelho Square, Brazil, 2003	8	0.22 (0.03–0.87) ng/m³			Active sampling, square downtown, PUF, gas	
São Paulo City, Brazil, 2000	41	[0.021] (0.007–0.031) ng/m³		GC-MS (0.6 µg/mL)	Passive sampling, gas and particle	Vasconcellos et al. (2003)
Porto Alegre, Brazil, November 2001 to November 2002	73	[0.131] (0.010–5.120) ng/m³	NR	GC-MS (LOQ, 0.01 µg/mL)	Active sampling, gas and particle	Dallarosa et al. (2005b)
Metropolitan Area of Porto Alegre (MAPA), Brazil, 2002 and 2005	69	[0.056] (0.005–0.474) ng/m³	NR	GC-MS (0.001 ng/mL)	Traffic, active sampling, HV and dichotomous, PM_{10}	Dallarosa et al. (2008)

Table 1.3 (continued)

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Candiota region, Brazil, 2001	19	0.25 (0.010–2.420) ng/m³	0.060 ng/m³ (NR)	GC-MS (0.011 ng/m³)	Industry, active sampling, particle	Dallarosa et al. (2005a)
Santiago, Chile, 1997	60	NR (0.00–0.04) ng/m³	NR	GC-MS (NR)	Background, active sampling, particle	Kavouras et al. (1999)
Guangzhou, China, April 2001 to March 2002	NR	30 ng/m³	NR	GC-MS	Active sampling GFF-PUF, total in vapour and particle	Li et al. (2006)
Beijing, China, December 2005 to January 2006	30	1.15 ng/m³	NR	GC-MS (NR)	Active sampling (NR), GFF, particle $PM_{2.5}$	Wang et al. (2008)
Nanjing, urban, China, 2001–2002	40	0.52 (ND–1.83) ng/m³ 0.41 (ND–1.49) ng/m³	NR	GC-MS	Urban, active sampling, PM_{10} Urban, active sampling, $PM_{2.5}$	Wang et al. (2006)
Guangzhou, China, 2004	10	0.06 ng/m³ (NR)	NR	GC-MS (NR)	Active sampling, MOUDI, aerosol	Duan et al. (2007)
Seine estuary, France, 2001	26	[9.98] ng/m² per week (NR)	NR	HPLC-FLD-UV	Active sampling, GFF bulk atmospheric deposition, gas and particle	Motelay-Massei et al. (2007)
Marseilles, France, 2004	12	0.767 (0.003–4.343) ng/m³	NR	HPLC-FLD-UV	Urban, active sampling, gas and particle	Albinet et al. (2007)
	14	0.998 (ND–6.595) ng/m³		(NR)	Rural, active sampling, gas	
Essen, Germany, 1981	NR	About 10 ng/m³ (NR)	NR	Glass-capillary-GC	Winter, gas	Grimmer et al. (1981)
	NR	6.7 ng/m³ (NR)		Glass-capillary-GC	Summer, gas	
Athens, Greece, 2006	7	35.6 ng/m³ (NR)		HPLC-FLD (NR)	Winter, GFF, particle	Valavanidis et al. (2006)
	7	26.5 ng/m³ (NR)			Summer, GFF, particle	
Greater Athens area, 2001–2002	58	0.079 ng/m³ (NR)	NR	HPLC-FLD (NR)	Urban, active sampling, particle	Mantis et al. (2005)
	64	0.246 ng/m³ (NR)			Downtown, active sampling, particle	
	35	0.206 ng/m³ (NR)			Industry, active sampling, particle	
	29	0.01 ng/m³(NR)			Background, active sampling, particle	
Heraklion, Greece, 2000–2002	16	3.3 ng/m³ (NR)	NR	GC-MS (0.001 ng/m³)	Passive sampling, GFF-PUF, gas and particle	Tsapakis & Stephanou (2005)
Athens, Greece, 2003	55	3.18 ng/m³ (NR)	NR	GC-MS (NR)	Passive sampling, Koropi, gas and particle	Vasilakos et al. (2007)
	55	3.19 ng/m³ (NR)			Passive sampling, Spata, gas and particle	
Delhi, India, January 2002 to December 2003	24	63.6 ng/m³ (NR)	NR	GC-FID (NR)	Winter, particle	Sharma et al. (2007)
	24	17.1 ng/m³ (NR)			Summer, particle	

Table 1.3 (continued)

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Prato, Italy, 2002	11	4.48 ng/m³ (NR)	NR	HPLC-UV/FLD (NR)	Industry, active sampling, PUF-QFF, gas and particle	Cincinelli et al. (2007)
Nagasaki city, Japan, 1997–1998	42	0.09 ng/m³ (NR)	NR	HPLC-FLD (NR)	Traffic, active sampling, particle	Wada et al. (2001)
Higashi, Hiroshima, Japan, 2006–2007	21	0.035 ng/m³ (NR)	NR	GC-MS	Active sampling, particle	Tham et al. (2008)
Shimizu, Japan, 2000	NR	0.93 ± 0.88 (0.12–3.9 as min. and max.) ng/m³ [a] 0.51 ± 1.9 ng/m³	NR	HPLC-FLD (NR)	Summer, gas, and particle	Ohura et al. (2004)
Shimizu, Japan, 2001	NR	0.39 ± 0.26 (0.12–1.0 as min. and max.) ng/m³ [a] 0.31 ± 1.4 ng/m³			Winter, gas, and particle	
Sarajevo, (former) Jugoslavia, 2004	30	1.38 (0.50–2.39) ng/m³	NR	GC-MS (2.5 pg/m³)	Industry, active sampling, the average urban and rural areas, light industry, GFF-PUF, gas and particle	Škarek et al. (2007)
Tuzla, (former) Jugoslavia, 2004	30	4 ng/m³ (2.38–5.87) ng/m³			Industry, active sampling, the average urban and rural areas, heavy industrial, GFF-PUF, gas and particle	
Inchon, Seoul, Yangsuri, and Yangpyoung, Republic of Korea, 2002	NR	2.07 ng/m³ (NR)	NR	GC-MS (NR)	Active sampling, particle	Chang et al. (2006)
Changwon–Masan, Republic of Korea, 2004	18	NR (0.101–0.859) ng/m³	NR	HPLC-UV (NR)	Active sampling, particle, range of mean values	Lee & Lee (2008)
Daeyeon-dong, Republic of Korea, 2002–2004	NR	1.79 ng/m³ (NR)	NR	GC-MS (NR)	Urban, active sampling, GFF, particle	Moon et al. (2006)
Gijang-gun, Republic of Korea, 2002–2004	NR	1.23 ng/m³ (NR)			Suburban, active sampling, GFF, particle	
Seoul, Republic of Korea, 1998–1999	5	2.7 ng/m³ (NR)	NR	GC-MS	Passive sampling, gas and particle	Park et al. (2002)
Mount Halla site, Jeju Island, Republic of Korea, 1999–2002	36	0.004 (0.001–0.0185) ng/m³	NR	GC-MS (NR)	Passive sampling, QFF, particle	Lee et al. (2008)
Kuala Lumpur, Malaysia, 2001	19	0 ng/m³ (NR)	NR	GC-MS	Passive sampling, particle	Omar et al. (2006)

Table 1.3 (continued)

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Lahore, Pakistan, 1992–1993	62	4.99 ng/m³ (NR)	NR	HPLC-UV/FLD (NR)	Active sampling, city, QM/A QFF, particle	Smith et al. (1996)
	62	4.6 ng/m³ (NR)			Active sampling, industrial site, particle	
	62	2.93 ng/m³ (NR)			Active sampling, rural site, particle	
	62	4.17 ng/m³ (NR)			Traffic, active sampling, autumn, gas	
Balagtas, Bulacan, Philippines, 2005	2	1.631 ng/m³ (NR)	NR	GC-MS (0.24 ng/m³)	Rural, passive sampling, spring, gas	Santiago & Cayetano (2007)
	2	2.093 ng/m³ (NR)			Rural, passive sampling, summer, gas	
	2	3.719 ng/m³ (NR)			Rural, passive sampling, autumn, gas	
	2	8.574 ng/m³ (NR)			Rural, passive sampling, winter, gas	
Manila, Philippines, 2005	2	2.032 ng/m³ (NR)			Urban site, passive sampling, spring, gas	
	2	2.182 ng/m³ (NR)			Urban site, passive sampling, summer, gas	
	2	3.211 ng/m³ (NR)			Urban site, passive sampling, autumn, gas	
	2	7.318 ng/m³ (NR)			Urban site, passive sampling, winter, gas	
Rizal, Philippines, 2005	2	2.971 ng/m³ (NR)			Rural, passive sampling, spring, gas	
	2	2.635 ng/m³ (NR)			Rural, passive sampling, summer, gas	
	2	2.805 ng/m³ (NR)			Rural, passive sampling, autumn, gas	
	2	12.008 ng/m³ (NR)			Rural, passive sampling, winter, gas	
Laguna, Philippines, 2005	2	3.709 ng/m³ (NR)			Rural, passive sampling, spring, gas	
	2	2.907 ng/m³ (NR)			Rural, passive sampling, summer, gas	
	2	6.116 ng/m³ (NR)			Rural, passive sampling, autumn, gas	
	2	8.672 ng/m³ (NR)			Rural, passive sampling, winter, gas	
Errenteria, Spain, 1996–1997	167	0.05 ng/m³ (NR)	NR	GC-MS (0.02 ng/m³)	Traffic, active sampling (500 L/minute), particle	Mazquiarán & Cantón Ortiz de Pinedo (2007)
Valencia, eastern Spain, 2004–2005	126	0.03 ng/m³ (NR)	NR	NR	Active sampling, hospital, $PM_{2.5}$	Viana et al. (2008)
	120	0.03 ng/m³ (NR)			Local Sport Centre, $PM_{2.5}$	
	58	0.01 ng/m³ (NR)			Emergency Control Centre, $PM_{2.5}$	
	72	ND			School, $PM_{2.5}$	
	42	0.04 ng/m³ (NR)			Youth centre, $PM_{2.5}$	
	59	0.03 ng/m³ (NR)			Swimming pool, $PM_{2.5}$	

Table 1.3 (continued)

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Gothenburg, Sweden, 2000	NR	12 ng/m³ (NR)	NR	GC-FID (NR)	Active sampling (433 L/minute), gas and particle	Wingfors et al. (2001)
Hagfors, Sweden, 2003	8	1.2 (0.42–2.4) ng/m³	1.1 ng/m³ (NR)	GC-MS (NR)	Outdoors, gas and particle	Gustafson et al. (2008)
Thailand, Thailand, 2000	NR	0.21 ng/m³ (NR)	NR	GC-FID	Traffic, active sampling, particle	Chang et al. (2006)
Merinos, Turkey, 2004–2005	20	7 ng/m³ (NR)	NR	GC-MS (NR)	Industry, active sampling (161 L/minute), industrial residential area, gas and particle	Tasdemir & Esen (2007)
Bursa, Turkey, 2004–2005	20	120.6 ng/m³ (NR)	NR	GC-MS	Active sampling (9.8 m³/hour), gas and particle	Esen et al. (2008)
Aliaga industrial region, Turkey, 2004–2005	60	0.5 ng/m³ (NR)	NR	GC-MS (NR)	Industry, passive sampling, seasonal variation, summer, gas and particle	Bozlaker et al. (2008)
	60	1.5 ng/m³ (NR)			Industry, passive sampling, winter, gas and particle	
Bursa, Turkey, 2004–2005	25	6.09 ng/m³ (NR)	NR	GC-MS (NR)	Passive sampling, winter, GFF-PUF, gas and particle	Vardar et al. (2008)
	25	0.3 ng/m³ (NR)			Passive sampling, summer, GFF-PUF, gas and particle	
Birmingham, UK, 1996	NR	4.5 ng/m³ (NR)	NR	HPLC-UV/FLD (NR)	Winter, PUF, gas and particle	Harrison et al. (1996)
	NR	0.6 ng/m³ (NR)			Summer, PUF, gas and particle	
London, UK, 1992	26	5 ng/m³ (1.20-9.54 ng/m³)	NR	GC-MS (0.002 ng/m³)	PUF, gas and particle	Halsall et al. (1994)
Alert, High Arctic, Canada, 2004–2015	NR	3.78 pg/m³ (0.072–882 pg/m³ as min. and max.)	NR	GC-MS (NR)	GFF-PUF, gas and particle	Yu et al. (2019)

FID, flame ionization detection; FLD, fluorescence detection; GC-ITD, gas chromatography-ion trap detection; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; GFF, glass microfibre filter; HPLC-FLD, high-performance liquid chromatography method-fluorescence detection; HPLC-UV, high-performance liquid chromatography method-ultraviolet detection; HV, high volume; IQR, interquartile range; LOD, limit of detection; max., maximum, min., minimum; LOQ, limit of quantification; MS, mass spectrometry; ND, not detected; NR, not reported; $PM_{2.5}$, particulate matter with diameter ≤ 2.5 µm; PM_{10}, particulate matter with diameter ≤ 10 µm; PUF, polyurethane foam; QF, quartz filter; QFF, quartz fibre filter; QFF-PUF, quartz fibre filter-polyurethane foam; UV, ultraviolet.

[a] Geometric mean.

concentrations of anthracene have been found in east Asia and south Asia. For example, measurements showed annual average concentrations of [40.3] ng/m^3 and [30.0] ng/m^3 in Delhi, India, and in Guangzhou, China, respectively, which can be one or more orders of magnitude higher than those in the USA [0.26] ng/m^3 (measurements only in July) and the United Kingdom [2.5] ng/m^3 (Harrison et al., 1996; Dachs et al., 2002; Li et al., 2006; Sharma et al., 2007) [Averages were calculated by the Working Group.] High seasonal levels of anthracene were also reported in the Philippines (up to 12 ng/m^3) and some European cities, including Essen, Germany (about 10 ng/m^3) and Athens, Greece (35.6 ng/m^3 in total suspended particulate) (Grimmer et al., 1981; Dachs et al., 2002; Mantis et al., 2005; Tsapakis & Stephanou, 2005; Valavanidis et al., 2006; Santiago & Cayetano, 2007; Vasilakos et al., 2007). The levels vary spatially, depending on the source proximity and long-range transport (Shen et al., 2014; Shrivastava et al., 2017). Based on a collection of measurements listed in Table 1.3, concentrations measured at urban (range, 0.021–120 ng/m^3) and industrial (range, 0.2–30 ng/m^3) sites or near road traffic (range, 0.05–4.17 ng/m^3) tended to be higher than those measured at regional background sites (range, 0.01–6.0 ng/m^3). Concentrations at rural sites (range, 0.11–12.0 ng/m^3), however, are comparable with or even higher than concentrations at urban sites (Table 1.3), mainly because of indoor and open burning of biomass, which frequently occurs in rural areas (Shen et al., 2013). Concentrations detected in the High Arctic are very low, in the order of 0.1–1000 pg/m^3 (Hung et al., 2005; Yu et al., 2019). Airborne anthracene shows seasonal variation, with some reported concentrations in winter being more than four times as high as those in summer (Sharma et al., 2007; Akyüz & Çabuk, 2010; Ma et al., 2010).

(ii) Indoor air

Cooking, heating, and smoking are the main indoor sources of anthracene (Liu et al., 2001; Ohura et al., 2004). Infiltration of air from the outside environment also contributes to indoor anthracene levels (Ali, 2019). Examples of measured levels of anthracene in indoor air can be found in Table 1.4. Indoor air quality was assessed in a residential area in Sweden, and levels were compared in households that did or did not use wood burning as a heating system; anthracene concentrations in the air were found to be higher in the wood-burning homes (median, 1 ng/m^3 versus 0.40 ng/m^3) (Gustafson et al., 2008). In China, in rural households using solid fuels for cooking, the highest concentrations of anthracene tend to be found in kitchens [mean ± standard deviation, 198 ± 96 ng/m^3] (Ding et al., 2012). In a study on different indoor microenvironments in Saudi Arabia, higher concentrations were also reported in kitchens (mean, 0.7 μg/m^3) than in other rooms (range of means, 0.3–0.6 μg/m^3) (Ali, 2019). In a study that evaluated exposure from indoor smoking in public bars in Enerhen Warri, Nigeria, the mean concentration in multiple samples collected in six different bars was 0.30 ng/m^3 (Adesina et al., 2021).

Seasonal variations in measured indoor anthracene concentrations are not consistent among studies, with some measurements indicating higher concentrations in autumn or winter (Ding et al., 2012; Chen et al., 2022; Florencia et al., 2022), whereas others suggested that concentrations were higher in summer (Liu et al., 2001; Ohura et al., 2004).

(b) Water

During 2010–2020 (Table 1.2), the annual amounts released from industry to water in the USA varied from 9047 pounds [4.10 tonnes] in 2010 to 135 pounds [0.06 tonnes] in 2014, demonstrating a marked reduction since 2013 (US EPA,

Table 1.4 Occurrence of anthracene in indoor air

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Hong Kong Special Administrative Region, China, 2014–2016	63	0.25 ± 0.17 (SD) ng/m³	NR	TD-GC-MS (0.48 ng/sample)	Average, air samples were taken in 26 non-smoking homes, 25% did not cook at home; $PM_{2.5}$-bound anthracene; 95th percentile, 0.60 ng/m³	Chen et al. (2022)
		0.20 ± 0.13 (SD) ng/m³			Summer	
		0.31 ± 0.19 (SD) ng/m³			Winter	
A rural household in Zhuanghu, Hebei, China, 4 days in winter (16, 17, 19, and 20 January) and 3 days in summer (13–15 June) in 2010	6	120 ± 48 (SD) ng/m³	NR	GC-MS	Winter, kitchen	Ding et al. (2012)
		42 ± 27 (SD) ng/m³			Winter, outdoors	
		19 ± 7.2 (SD) ng/m³			Winter, bedroom	
		58 ± 45 (SD) ng/m³			Summer, kitchen	
		3.7 ± 3.6 (SD) ng/m³			Summer, outdoors	
		18 ± 2.0 (SD) ng/m³			Summer, bedroom	
	4	11 ± 3.6 (SD) ng/m³			Winter, control	
		18 ± 7.1 (SD) ng/m³			Winter, cooking	
		16 ± 12(SD) ng/m³			Summer, control	
		17 ± 23 (SD) ng/m³			Summer, cooking	
Hangzhou, China, 1999	56	0.234 (0.019–0.683) μg/m³	NR	HPLC (3.58 pg)	Summer, smoking and non-smoking homes, air samples were taken in bedroom, balcony, kitchen, and living room.	Liu et al. (2001)
		0.220 (0.063–0.437) μg/m³			Autumn	
Qujiang District, south-eastern Xi'an, China, 2011–2012	18	95.1 (30.2–177.0) ng/m³	NR	HPLC (NR)	Measurements were taken in six different restaurants, under low ventilation conditions in wintertime	Dai et al. (2018)
Stockholm, Sweden, 2016–2017	5	2.01 (ND–10.9) pg/m³	NR	LC-GC-MS (0.027 pg/m³)	Preschool, indoor, PM_{10}	Lim et al. (2021)
Hagfors, Sweden, 2003	13	1.3 (ND–2.8) ng/m³	1.0 ng/m³	GC-MS (0.15 ng/m³)	Winter, wood-burning homes	Gustafson et al. (2008)
	10	0.41 (ND–0.84) ng/m³	0.40 ng/m³		Reference homes	
Shimizu, Japan, 2000–2001	25	0.94 (0.23–4.0) ng/m³	NR	HPLC-FLD (NR)	Homes, summer, industrial area	Ohura et al. (2004)
	22	0.31 (0.12–1.4) ng/m³			Homes, winter, industrial area	

Table 1.4 (continued)

Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Preschools, Porto and Chaves, Portugal, May to June 2015	152 (8-hour)	NR	0.191 (0.00 471–0.665) ng/m³ 0.507 (0.336–0.638) ng/m³	LC-PAD (1 pg/m³)	Oporto city school Chaves city school	Oliveira et al. (2017b)
Jeddah, Saudi Arabia	20 10 15 10	0.6 (0.1–5.2) µg/m³ 0.4 (0.2–0.6) µg/m³ 0.7 (0.2–1.8) µg/m³ 0.3 (0.2–0.5) µg/m³	NR	GC-MS (NR)	Homes Hotel Kitchen Office Measurement of anthracene in PM_{10}	Ali (2019)
Urban area, Enerhen Warri, Nigeria, 2021	6	0.30 (0.15–0.44) ng/m³	NR	PUF passive samplers, GC-MS (NR)	Indoor smoking, bars in city with high petroleum activities	Adesina et al. (2021)
Urban–suburban, Cordoba, Argentina, winter and summer 2015	12 urban homes	0.31 ± 0.05 (SD) µg/m³ 0.52 ± 0.38 (SD) µg/m³ 0.25 ± 0.08 (SD) µg/m³ 0.63 ± 0.34 (SD) µg/m³	NR	HPLC	Summer, urban, TSP, passive collection for 28 days, non-smokers Winter, urban, TSP Summer, suburban, TSP Winter, suburban, TSP	Florencia et al. (2022)

GC-MS, gas chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; HPLC-FLD, high-performance liquid chromatography method-fluorescence detection; IQR, interquartile range; LC-PAD, pulsed amperometric detection for liquid chromatography; LOD, limit of detection; MS, mass spectrometry; ND, not detected; NR, not reported; $PM_{2.5}$, particulate matter with diameter ≤ 2.5 µm; PM_{10}, particulate matter with diameter ≤ 10 µm; PUF, polyurethane foam; SD, standard deviation; TD-GC-MS, thermal desorption-gas chromatography-mass spectrometry; TSP, total suspended particulate matter.

2023). During 2013–2020, releases to water in the USA were < 1% of total industry releases and disposal of anthracene, except in 2019 (1.96%; 1317 pounds [0.60 tonnes] of the total of 67 215 pounds [30.49 tonnes]) (US EPA, 2023). The Canadian National Pollutant Release Inventory reported releases to water varying from 0.91 kg (2018; 0.009% of the total of 9956 kg released) to 2.39 kg (2019; 0.022% of the total of 10 757 kg released) during 2017–2021 (Government of Canada, 2022b). Besides industrial effluent discharge, other sources that contribute to the transfer of anthracene into water are municipal sewage, atmospheric deposition, surface runoff, and oil spills (IARC, 2010). Anthracene is not expected to hydrolyse in water, but its direct photolytic degradation to anthraquinone can be significant (half-lives in the range of 20 minutes to 125 hours), particularly under sunlight in shallow surface waters (half-lives, < 1 hour) (ECHA, 2008a; NCBI, 2022). Volatilization from water surfaces can also occur, but it is reduced by anthracene sorption to suspended particulate matter from the water column (NCBI, 2022).

Studies have shown the ubiquitous occurrence of anthracene in the aquatic environment worldwide, i.e. in seawater, surface water, groundwater, drinking-water, and wastewater (Table 1.5). Overall, levels for uncontaminated sites and drinking-water are the lowest, within the range of "not detected" picograms per litre to low nanograms per litre (< 10 ng/L). However, the median anthracene concentration in drinking-water was reported to be 28.76 ng/L in Nanjing, China, in 2007–2008 (Wu et al., 2010). Moreover, despite the very low solubility of anthracene in water, levels up to the microgram per litre range and as high as 14.14 µg/L and 14.89 µg/L in Algoa Bay, South Africa (Adeniji et al., 2019a), and 35.5 µg/L in Agbabu, Nigeria (Olajire et al., 2007), were reported for surface water or bottom water from geographical regions strongly affected by anthropogenic activities (Table 1.5). Groundwater is poorly characterized with regard to the presence of anthracene across continents, but the few available data also suggest that there is a high impact of industrial activities on groundwater contamination with anthracene (up to 5 µg/L in Sydney, Australia, and up to 3900 µg/L in Minnesota, USA, at old industrial sites) (Coffey Geotechnics Pty Ltd, 2016; Minnesota Department of Health, 2019). The levels in groundwater in the vicinity of non-industrial areas are typically in the low to tens of nanograms per litre range (e.g. 1.61–58.6 ng/L in the Grand Canal from Hangzhou to Beijing, east China) (Li et al., 2015), although higher concentrations were detected in Nigeria (0.010–2.91 µg/L) (Adekunle et al., 2017). Industrial and municipal wastewater displays the highest levels of anthracene, even after treatment, when compared with river water and other environmental waters from the same geographical area, e.g. not detected in river water and 16.4 ng/L in treated wastewater (Pena et al., 2009; Domínguez et al., 2018).

(c) Soil

Anthracene in soil can come from natural sources, such as oil spills, wildfires, and weathering of rocks, and from anthropogenic sources, including emissions from combustion of fossil fuel and biomass, and coking through atmospheric deposition (Tsibart & Gennadiev, 2013; Schlaback et al., 2016; Government of Canada, 2022b; US EPA, 2022). A large fraction of anthracene in the atmosphere is first accumulated in plants before being introduced into soils (Simonich & Hites, 1994). In soil, anthracene mainly undergoes sorption, leaching, and biodegradation processes and can be re-emitted into the atmosphere, representing part of the dynamic surface–air exchanges. The biodegradation of anthracene is influenced by the soil type and the resident microbial communities; half-lives ranging from 19 to 134 days have been reported (NCBI, 2022). The occurrence and concentrations of anthracene in soil are thus determined by source proximity, vegetation coverage, and

Table 1.5 Occurrence of anthracene in environmental waters

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Africa							
Seawater: Surface microlayer (< 1 mm)	Alexandria coast, Egypt, 2002	21	24 (ND–69) ng/L	NR	GC-MS (0.2 µg/L)	12 sites	El Nemr & Abd-Allah (2003)
Subsurface water (0.5 cm)		51	3.8 (0.3–12.49) ng/L	NR			
Surface water: Raw water	Nile River, Greater Cairo, Egypt, 2018	96	ND–496 (ND–672) ng/L	NR	GC-MS (NR)	8 sites; drinking-water supply	Fouad et al. (2022)
Treated water		96	NR	NR			
Surface water	Estuaries and lagoons, coastal belt, Ghana, 2007	60	ND–1.2 µg/L	NR	GC-FID (0.001 µg/L)	6 sites	Essumang (2010)
Subsurface water (0.5 m)	Agbabu, Nigeria, NR	6	ND–35.5 µg/L	NR	GC-FID (1 ng/L)	Sampling in the vicinity of a bitumen exploration	Olajire et al. (2007)
Lagoon: Surface microlayer (< 1 mm)	Lagos Lagoon, Nigeria, NR	72	ND–0.2 µg/L	NR	GC-ECD (NR)	6 sites	Benson et al. (2014)
Subsurface water (15–20 cm)		72	ND–0.1 µg/L	NR			
Groundwater: Wet season Dry season	Ife North Local Government Area of Osun State, Nigeria, 2014	72	 0.51 (0.01–2.91) µg/L 0.10 (0.01–0.19) µg/L	 NR NR	GC-TOMS (NR)	Non-industrial area site; sampling done in wet and dry season	Adekunle et al. (2017)
Bay: Surface water (10 cm depth)	Algoa Bay, South Africa, 2015–2016	250	5.61 (ND–14.14) µg/L	NR	GC-FID (NR)	5 sites; the bay (only 30 m depths) receives large influx of wastes from Swartkops and Sundays Rivers	Adeniji et al. (2019a)
Bottom water (30 m depth)		250	6.87 (ND–14.89) µg/L	NR			
River Surface water (2.40–6.16 m)	Buffalo River Estuary, East London city, South Africa, 2015–2016	60	1.97 (ND–7.81) µg/L	NR	GC-FID (NR)	5 sites	Adeniji et al. (2019b)
Groundwater (12–30 m)	Bwaise and Wobulenzi, Uganda, 2018–2020	NR	ND–340.0 ng/L	NR	GC-MS (0.9 ng/L)	12 sites	Twinomucunguzi et al. (2021)

Table 1.5 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
North America							
Lake, surface water Urban (basins with > 15% urban land cover)	Minnesota, Wisconsin, Michigan, Indiana, Ohio, and New York, USA, 2010–2013	196	0.016 (ND–0.14) µg/L	NR	GC-MS (0.01 µg/L)	Means computed using left-censored data methods	Baldwin et al. (2016)
Non-urban (samples from basins with < 15% urban land cover)		513	0.005 (ND–0.03) µg/L	NR			
Drinking-water wells	Minnesota, USA, 2018	NR	3900 µg/L	NR	NR	Wells near known contamination sites	Minnesota Department of Health (2019)
Drinking-water	Texas, Rhode Island, USA, 2017–2019	NR	0.00259–0.00407 µg/L	NR	NR		EWG (2022)
River water	South Dakota, USA, 2001–2004	NR	0.082 to < 0.5 µg/L (dissolved) 0.06 to < 0.5 µg/L (whole water)	NR	NR		USGS (2006)
Drinking-water			0.082 µg/L (dissolved) 0.06 to < 0.5 µg/L (whole water)				
Wastewater treatment plant effluent			0.082 to < 0.5 µg/L (dissolved)				
South America							
Surface water	São Paulo, Brazil	6	< 0.036 µg/L	NR	HPLC (NR)		Pereira et al. (2017)
Groundwater		3	< 0.036 µg/L				
Lake (5 m)	North Patagonian lake, Chile, 2017	13	10.1 (0.42–58.3) pg/L	NR	GC-MS (NR)	13 sampling events	Tucca et al. (2020)
River (15–30 cm)	Cauca River, Colombia, 2010–2011	NR	ND–431.1 ng/L	NR	HPLC-UV-FLD, GC-MS (NR)	8 sites; 3 campaigns	Sarria-Villa et al. (2016)
Asia							
Groundwater	Grand Canal from Hangzhou to Beijing, east China, 2014	50	1.61–58.6 ng/L	NR	GC-MS (0.24 ng/L)	8 sites	Li et al. (2015)
Drinking-water	Nanjing China, 2007–2008	32	28.80 (ND–79.82) ng/L	28.76 ng/L	GC-MS (NR)		Wu et al. (2010)

Table 1.5 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Lakes Surface water	Northern China, 2014	66	(0.05–6.82) ng/L	0.95 (1.7) ng/L	GC-MS (0.5 ng/mL)	44 lakes	He et al. (2020)
Lake	China, 2006–2018	NR	ND–1410 ng/L	NR	NR	Data retrieved from literature; 14 lakes	Meng et al. (2019)
Lakes and rivers (1 m)	China, 2013	NR	0.06–0.46 ng/L	NR	GC-MS (NR)	42 sites; the concentration in South Lake (27 ng/L), Wuhan, exceeded the water quality guidelines of the Canadian Council of Ministers of the Environment (12 ng/L)	Yao et al. (2017)
Reservoir Surface water (0.5–1 m)	Jilin, China, 2014	12	0.510 (0.120–0.760) µg/L	NR	GC-FID (NR)	12 sites	Sun et al. (2015)
Rivers, river basin, river estuary, reservoir, water body in city, drinking-water resource Surface water	China, 1999–2009	NR	71.235 (ND–2063.96) ng/L	5.225 (NR) ng/L	NR	Data retrieved from literature	Wu et al. (2011)
River Water	Jilin Province to the Russian Federation along Songhua River, China, 2007–2008	42	16.39 (9.68–70.68) ng/L	NR	GC-MS (NR)		Zhao et al. (2014)
River, surface water	Hun River, Liaoning Province, China, 2009				HPLC-FLD (NR)	14 sites	Zhang et al. (2013)
Dry period (April)		28	3.11 (0.33–8.24) ng/L	NR			
Flood period (July)		28	105.25 (ND–187.99) ng/L	NR			
Level period (November)		28	106.45 (62.38–233.3) ng/L	NR			
River	Tianjin, China, 2014				GC-MS (1 ng/L)	7 sites (surface water)	Cao et al. (2005)
Surface water (0–10 cm)		NR	< 1 ng/L	NR			
Reclaimed water (from secondary treated wastewater)		NR	< 1 ng/L	NR			

Table 1.5 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
River Surface water	Tianjin, China, NR	30	0.006 (1.0–22.2) µg/L GM, 0.0051 µg/L	NR	GC-MS (NR)		Yang et al. (2006)
River, subsurface water (0.5 m)	Shenyang, China, 2015–2016	480	0.01 µg/L [a]	NR	HPLC-UV-FLD (NR)	10 sites	Li et al. (2017)
River (0.50 m)	Xihe River, Shenyang, China, 2005	NR	5–10 ng/L [a]	NR	GC-MS (NR)	7 sites	Guo et al. (2011)
River Surface water (0.5 m)	Middle China, 2005–2006	NR	1.1–128.1 ng/L	NR	GC-MSD (NR)	26 sites	Sun et al. (2009)
Seawater Surface water	Liaodong Bay, China, 2009	5	NR	3 (5) ng/L[a]	GC-MS (0.2 ng/L)		Wang et al. (2016a)
Drinking-water	Southern Jharkhand, east India, 2019	120	1.50–4.83 (ND–9.01) ng/L	NR	GC-FID with MS (0.13 ng/L)	6 districts, 60 locations; hand pumps and groundwater wells	Ambade et al. (2021)
River Surface water	Cuttack city, India, 2019	14	1.77 (ND–3.36) µg/L	1.5 (2) µg/L[a]	GC-FID (NR)	14 sites	Kurwadkar et al. (2022)
River Surface water (30 cm)	Gomti River, India, 2004–2006	48	0.03–0.09 (ND–0.86) µg/L	ND–0.01 µg/L	HPLC-UV-VIS (1 ng/L)	8 sites	Malik et al. (2011)
Drinking-water	Misan Governorate, Iraq, 2015	15	ND–70.79 ng/L	NR	HPLC-FLD (NR)	15 stations, 1 sample per station	Jazza et al. (2016)
Drinking-water	Tehran, Islamic Republic of Iran, 2011–2012	99	NR	NR	GC-MS (NR)	6 districts, 4 samples per district in each season	Karyab et al. (2013)
Tap water	Tehran, Islamic Republic of Iran, 2014	36	ND	NR	GC-MS (NR)	6 regions, 6 samples per region	Sadeghi et al. (2016)
Seawater (1.3–12.2 m)	Tokyo Bay and Suruga Bay, Japan, 2003	8	ND–4.7 ng/L	NR	GC-MS (0.2 ng/L)	8 sites	Kurihara et al. (2005)
Groundwater Surface water	Mongolia	22 11	0.81 ng/L	NR	GC-MS (NR)		Zhang et al. (2022)
River Surface water	Soan River, Kurang River, Ling Stream, Nallah lai Potohar, Pakistan, 2013	30	17.7 (8.7–28.0) ng/L	NR	GC-MS (NR)	10 sites	Aziz et al. (2014)

Table 1.5 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Europe							
River and lake	Strymonas River, Greece, 2013					7 sites	Terzopoulou & Voutsa (2017)
Active sampling		33	ND	ND	GC-MS/MS (NR)		
Passive sampling		12	0.039 µg/sample	ND	SPMD (NR)		
River Surface water	Nestos River, Greece, 2008–2009	NR	ND–0.062 µg/L	NR	GC-MS (NR)	16 sites	Gikas et al. (2020)
River Surface water (0.3 m)	Rackevei-Soroksari Danube Branch, Hungary, 2002–2004	240	13.2 (0.9–96.2) ng/L	6.9 (NR)	HPLC-UV-FLD (0.5 ng/L)	10 sites	Nagy et al. (2007)
River Surface water (30 cm)	Raba River, Hungary, 2008–2012	54	0.50–2.25 (ND–8) ng/L	NR	GC-MS (1 ng/L)	4 sites	Nagy et al. (2013)
Lake (1 m)	Headwater lake catchments, Ireland, 2009–2010	15	7.59 (ND–38.0) pg/L	NR	GC-LRMS (NR)	5 sites	Scott et al. (2012)
Tap	Galicia (north-western Spain), NR	9	ND	NR	HPLC-FLD (0.2 ng/L)		Pena et al. (2009)
Bottled			ND				
Fountain			ND				
Well waters			8.1 ng/L				
Rainwater			ND				
River waters			ND				
Treated wastewater			16.4 ng/L				
Urban wastewater	Sevilla, Spain, 2016–2017	18			GC-HRMS (NR)	18 sites; LOQ, 0.07 ng/L	Domínguez et al. (2018)
Influents			119.89–177.21 ng/L	NR			
Effluents			3.62–158.43 ng/L	NR			
Oceania							
Groundwater	Sydney, Australia, 2016	3	< 1–5 µg/L	NR	NR	3 sites near fuel bunkers	Coffey Geotechnics Pty Ltd (2016)

GC-ECD, gas chromatography-electron capture detection; GC-FID, gas chromatography-flame ionization detection; GC-HRMS, gas chromatography-high-resolution mass spectrometry; GC-LRMS, gas chromatography-low-resolution mass spectrometry; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; GC-TOMS, time-of-flight-mass spectrometry; GM, geometric mean; HPLC, high-performance liquid chromatography; HPLC-FLD, high-performance liquid chromatography method-fluorescence detection; HPLC-UV-FLD, high-performance liquid chromatography-ultraviolet detection-fluorescence detection; HPLC-UV-VIS, high-performance liquid chromatography-ultraviolet detection-visible light detection; IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; ND, not detected; NR, not reported; SPMD, semipermeable membrane device.

[a] Estimated value extracted from a graph.

soil properties, and show large spatial variation. Levels of soil anthracene are reported to be higher in city centres and residential areas than in urban green spaces (Ciarkowska et al., 2019). Levels of anthracene are typically lower in forest soils than in urban soils. Anthracene concentrations are higher in forest soils than in rural agricultural soils in temperate zones, and lower in tropical zones (Amazon basin) (Wilcke, 2000) than in temperate zones. Globally, south and east Asia show relatively high levels of soil anthracene (in the order of 1.0–100 µg/kg) (Table 1.6), primarily due to high levels of anthropogenic emissions (Tao et al., 2004; Liu et al., 2016). For example, anthracene concentrations of up to 448.1 µg/kg have been reported in severely contaminated agricultural soils in Tianjin, China (Tao et al., 2004). High levels of anthracene have been also detected in Europe and North America, especially in urban settings (Mielke et al., 2004; Ciarkowska et al., 2019). For example, it was reported that, in metropolitan New Orleans, USA, the median level of soil anthracene was 42 µg/kg at an inner-city site, whereas anthracene was not detected at a suburban site (Mielke et al., 2004).

(d) Food

Anthracene is detected in foodstuffs because of environmental contamination (via water, soil and/or air) and/or unintended formation during food processing. Smoking, barbecuing, grilling, broiling, roasting, frying, and other high-temperature heating processes are responsible for the highest levels of contamination, e.g. 0.01–0.02 and 13.23 ng/g fresh weight (fw) in raw and smoked meat, respectively (Golzadeh et al., 2021); 9.52 and 12.15–157.41 µg/kg dry weight (dw) in raw and smoked sausage, respectively (Roseiro et al., 2011); 0.2187–4.2340 and 0.9901–9.5054 µg/kg fw in uncooked and grilled meat products, respectively (Samiee et al., 2020) (Table 1.7). Data retrieved from several food surveys show that the lowest anthracene concentrations are always present in the raw food, independently of the category (Table 1.7). Also, in general, higher levels are found in animal-based raw or processed foodstuffs (predominantly in meat and meat products, and fish and shellfish) and the lowest levels are found in fruit and vegetables, e.g. 0.01–1.18 µg/kg fw in fresh fruit; 0.01–2.65 µg/kg fw in fish and fish products; and 0.01–7.84 µg/kg fw in meat and meat products (Cirillo et al., 2010); 0.018 µg/kg fw in fruit; 0.110 µg/kg fw in fish and shellfish; and 0.180 µg/kg fw in meat and meat products (Falcó et al., 2003; Domingo & Nadal, 2015; Aamir et al., 2021) (Table 1.7). [The Working Group noted the variation in precision of the reported values in the literature. Values are stated as reported in the original publications.] Since anthracene has low hydrophilicity, it mainly accumulates in lipophilic matrices such as fat animal or fish tissues and high-fat foodstuffs, such as fish oil (Table 1.7). When applicable, the contamination level of fat or oil introduced into the commercial formulations should be considered as a potential anthracene source in the final foodstuff (Santonicola et al., 2017). Moreover, when released into water, anthracene adsorbs to suspended particle matter and sediments, which can be ingested and promote bioaccumulation in aquatic organisms, particularly predators, in species that occupy higher trophic positions, in bottom-dwelling fish species, and in bivalves (filter feeders). Moderate to very high bioconcentration factors (162–9200) have been reported for anthracene (NCBI, 2022). Bivalves, molluscs, crustaceans, and cephalopods seem to be unable to significantly metabolize PAHs, including anthracene, resulting in higher concentrations in these species than in finfish, when collected from the same polluted site (Perugini et al., 2007; Ramalhosa et al., 2012; Semedo et al., 2014) (Table 1.7).

(e) Consumer products

Anthracene has been mostly detected in the particulate phase emitted from different products or uses such as tobacco

Table 1.6 Occurrence of anthracene in soil

Location and collection date	No. of samples	Mean (range)	Median	Analytical method (LOD)	Comments	Reference
Agra, India, 2003	319	1.29 ± 1.12 µg/g (mean ± SD)	NR	HPLC-UV (NR)	Industrial	Masih & Taneja (2006)
	319	1.02 ± 0.66 µg/g			Roadside	
	319	0.57 ± 0.39 µg/g			Residential	
	319	0.36 ± 0.21 µg/g			Agricultural	
Ulsan, Republic of Korea, July 2010	5	26 (14–52) µg/kg	20 µg/kg	GC-ITMS (1.2 µg/kg)	Rural area	Kwon & Choi (2014)
	10	19 (2.3–61) µg/kg	17 µg/kg		Urban area	
	10	50 (3.0–330) µg/kg	15 µg/kg		Industrial area	
	25	33 (2.3–330) µg/kg	16 µg/kg		Total	
Hong Kong Special Administrative Region, China, 2003	39	13.5 (ND–389) µg/kg	2.5 µg/kg	GC-MS (10 µg/kg)	Urban park	Chung et al. (2007)
	14	4.6 (ND–14.4) µg/kg	3.2 µg/kg		Greening area	
	9	1.4 (ND–4.3) µg/kg	1.0 µg/kg		Country park	
	19	2.4 (ND–10.0) µg/kg	1.0 µg/kg		Rural area	
	11	3.8 (ND–26.3) µg/kg	1.0 µg/kg		Restored landfill	
	9	1.5 (ND–5.08) µg/kg	1.0 µg/kg		Agricultural farmland	
	5	1.9 (ND–5.59) µg/kg	1.0 µg/kg		Orchard farm	
	10	1.0 (ND–ND) µg/kg	1.0 µg/kg		Crematorium	
	18	7.0 (ND–87.8) µg/kg	1.0 µg/kg		Industrial area	
	4	31.1 (2.2–56.1) µg/kg	33.1 µg/kg		Nearby highway	
Krakow, Poland, 2016	4	531 ± 740 (63.2–1628) µg/kg	NR	GC-MS (2 µg/kg)	City central	Ciarkowska et al. (2019)
Zakopane, Poland, 2016	3	45.2 ± 41.1 (ND–84.5) µg/kg			City central	
Krakow, Poland, 2016	3	34.8 ± 7.7 (25.9–40.2) µg/kg			Residential	
Zakopane, Poland, 2016	3	43.7 ± 47 (ND–94.9) µg/kg			Residential	
Krakow, Poland, 2016	3	12.1 ± 6.6 (6.0–19.1) µg/kg			Green area	
Zakopane, Poland, 2016	3	10.2 ± 9.8 (ND–21.2) µg/kg			Green area	
Republic of Korea, 2000	126	8.0 (0.30–43.1) µg/kg		GC-MS (NR)	Paddy soil	Nam et al. (2003)
	100	6.88 (0.30–33.7) µg/kg			Upland soil	
Temperate topsoil	14	2.4 (ND–11) µg/kg	1.6	GC-MS (NR)	Arable, temperate topsoil	Wilcke (2000)
	33	1.6 (ND–4.3) µg/kg	1.5		Grassland, temperate topsoil	
	54	8.6 (ND–75) µg/kg	3.4		Forest, temperate topsoil	
	94	58 (ND–1400) µg/kg	18		Urban, temperate topsoil	
Bangkok, Thailand, 1996	4	1.5 (1.3–1.9) µg/kg	1.4	GC-MS (NR)	Rural agricultural and forest	Wilcke et al. (1999a)
	30	1.2 (0.1–5.0) µg/kg	0.7		Urban	

Table 1.6 (continued)

Location and collection date	No. of samples	Mean (range)	Median	Analytical method (LOD)	Comments	Reference
Uberlândia, Brazil, 1996	18	1.0 (0.0–6.2) µg/kg	0.8	GC-MS (NR)	Urban	Wilcke et al. (1999b)
Amazonian, Brazil, 1997	6	0.8 (0.3–2.6) µg/kg	0.4	GC-MS (NR)	Forest	Wilcke (2000)
Bangkok, Thailand, 1996	4	1.2 (0.1–5.0) µg/kg	0.7	GC-MS (NR)	Rural agricultural and forest	Wilcke et al. (1999a)
Shanghai, China, 2007	36	6.4 ± 6.4 (1.0–36.6) µg/kg		GC-MS (NR)	Agricultural	Jiang et al. (2011)
Tianjin, China, 2006	105	36.2 ± 47.3 (ND–261) µg/kg	21.5	GC-MS (2.9 µg/kg)	Industrial	Jiao et al. (2009)
Shanxi, China, 2014	32	281.68 ± 347.07 (ND–1892.51) µg/kg	165.80	GC-MS (NR)	Agricultural soils in the vicinity of a chemical plant	Liu et al. (2016)
Beijing, China, 2008	127	12.5 ± 17.6 (ND–124.8) µg/kg GM, 6.4 µg/kg	NR	GC-MS (NR)	Urban	Liu et al. (2010)
Xiangfen, Shanxi, China, 2012	128	20.62 ± 29.79 (ND–287.25) µg/kg	14.01	GC-MS (NR)	County average	Pan et al. (2015)
Beijing, China, 2010	162 73	5.7 ± 8.8 (0.5–55.3) µg/kg 3.8 ± 4.6 (0.7–21.8) µg/kg	2.8 2.1	GC-MS (0.10 µg/kg)	Suburban Rural	Peng et al. (2016)
Yangtze River Delta, China, 2004	138	4.7 (ND–34.7) µg/kg	1.6	HPLC-FLD (0.35 µg/kg)		Ping et al. (2007)
Xianyang, China,	59	1.61 ± 4.07 (ND–20.61) µg/kg	NR	HPLC-FLD (NR)	Vegetable soil from suburbs	Wang et al. (2016a)
New Orleans, USA, 2001	38 38	NR (2–163) µg/kg NR (ND–45) µg/kg	42 ND	GC-MS (NR)	Inner city Suburban	Mielke et al. (2004)
Tianjin, China	4 4	42.4 µg/kg 448.1 µg/kg	NR	GC-MS (NR)	Agricultural Agricultural	Tao et al. (2004)

GC, gas chromatography; GC-ITMS, gas chromatography-ion trap-mass spectrometry; GM, geometric mean; HPLC, high-performance liquid chromatography; HPLC-FLD, high-performance liquid chromatography-fluorescence detection; IT-MS, ion trap-mass spectrometry; LOD, limit of detection; ND, not detected; NR, not reported; SD, standard deviation; UV, ultraviolet detection.

Table 1.7 Occurrence of anthracene in food and beverages

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Africa							
Meat	Zagazig city, Egypt, 2017				HPLC-FLD (0.03 ng/g)	Beef	Darwish et al. (2019)
Raw		25	0.19 (0.12–0.28) ng/g fw	0.17 (NR) ng/g fw			
Boiled		25	0.31 (0.08–0.55) ng/g fw	0.29 (NR) ng/g fw			
Pan-fried		25	0.55 (0.35–0.78) ng/g fw	0.53 (NR) ng/g fw			
Grilled		25	1.24 (0.25–2.25) ng/g fw	1.13 (NR) ng/g fw			
Raw cocoa beans	Ghana; Côte d'Ivoire; Dominican Republic; Ecuador; Nicaragua; Venezuela, date of collection, NR	9 of each origin and variety	0.26–1.1 µg/kg dw	NR	HPLC-FLD (0.07 µg/kg)		Ciecierska (2020)
Roasted cocoa beans			0.40–1.48 µg/kg dw				
Cocoa mass			0.62–2.85 µg/kg dw				
Cocoa butter			1.35–5.54 µg/kg dw				
Chocolate			0.29–1.33 µg/kg dw				
North America							
Meat	Chicago, USA	15	2 µg/kg fw	NR	UV-FLD (NR)	Charcoal-broiled steak meat	Lijinsky & Shubik (1964)
Meat	Alberta, Canada, 2015			NR	GC-LRMS (NR)	Raw meat: grouse muscle, moose muscle, bear muscle; Raw fish muscle; Plants: berry, rat root, old man's beard	Golzadeh et al. (2021)
Raw		17	0.01–0.02 ng/g fw				
Smoked		5	13.23 ng/g fw				
Fish		6	0.04 ng/g fw				
Plants		21	0.03–0.67 ng/g fw				
Fish (*Megalops atlanticus*) (raw)	Lagoon of Terminos, Mexico, NR	NR	0.1 (0–1.2) ng/g dw	NR	GC-FID (NR)		Canedo-Lopez et al. (2020)
Fish oil from Menhaden fish	New Jersey, USA, 2010	NR	90–130 ng/g fw	NR	GC-MS (1 ng/g)		Chopra et al. (2019)
South America							
Liquid smoke flavour	São Paulo, Brazil, NR	22	ND–600.4 µg/kg fw	NR	HPLC-FLD- (0.6 µg/kg)	Ox rib, bacon, loin, ham, sausage	Yabiku et al. (1993)
Smoked meat and meat products		88	ND (ham) – 83.6 (ox rib) µg/kg fw				

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Gastropods (*Buccinanops globulosus*)	Patagonia, Argentina, NR	50	ND–174 µg/kg dw	NR	GC-FID, GC-MS (5 µg/kg)	2 locations	Primost et al. (2018)
Hybrid corn grains No drying (no heat) Drying (firewood and direct fire)	Rio Grande do Sul, Brazil, NR	88	 ND 10.90–16.09 µg/kg dw	NR	GC-MS (2.3 µg/kg)		de Lima et al. (2017)
Asia							
Kebabs (grilled foods)	Kermanshah Province, Islamic Republic of Iran	50	NR	15.91 (3.5) µg/kg fw	GC–MS (NR)		Gholizadah et al. (2021)
Meat products Sausage (all cooking methods) Burger (all cooking methods) Uncooked Fried Grilled	Tehran, Islamic Republic of Iran, NR	50	 2.21 (0.56–4.20) µg/kg fw 5.05 (0.21–14.21) µg/kg fw 1.82 (0.219–4.23) µg/kg fw 4.86 (1–14.24) µg/kg fw 4.82 (0.99–9.51) µg/kg fw	NR	GC-MS (NR)	Sausages and burgers; fried in sunflower oil; grilled on charcoal (just burgers)	Samiee et al. (2020)
Fish: mackerel (*Scomber japonicas*); Alaska pollock (*Theragra chalcogrammus*); yellow croaker (*Larimichthys polyactis*); hair tail (*Trichiurus lepturus*); flatfish	Republic of Korea, NR	100	ND (mackerel, Alaska pollock, hair tail, flatfish) – 0.01 (yellow croaker) µg/kg fw	NR	GC-MS (0.01 µg/kg)	7 locations; raw	Hwang et al. (2012)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Shellfish: shortneck clam (*Tapes phillipinarum*); oyster (*Crassostrea gigas*); sea mussel (*Mytilus coruscus*); granular ark clam (*Tegillarca granosa*)		80	ND				Hwang et al. (2012) (cont.)
Cephalopod: cuttlefish (*Todarodes pacificus*); whip-arm octopus (*Octopus variabilis*); common octopus (*Octopus dofleini* [*vulgaris*])		60	ND				
Crustacea: crab (*Portunus trituberculatus*); shrimp (*Exopalaemon orientis Holthuis*)		40	ND (shrimp) –0.1 (crab) µg/kg fw				
Bread	Tehran city, Islamic Republic of Iran, NR	47	14.59 (ND–20.77) ng/g fw	NR	GC-MS (0.561 ng/g)	Iranian traditional Sangak bread	Peiravian et al. (2021)
Wheat grain	Shaanxi and Henan Provinces, China, 2015	51	4.04–5.22 (2.40–9.56) µg/kg fw	4.19–5.26 µg/kg (NR)	HPLC-UV-FLD (NR)	4 locations	Tian et al. (2018)
Coffee beans (*Coffea canephora*)	Chumphon Province, Thailand, NR	NR		NR	GC-MS (NR)		Rattanarat et al. (2021)
Hot air			0.09–0.34 µg/kg dw				
Superheated steam			0.10–0.21 µg/kg dw				

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Natural mineral water	China, NR	3	ND (NR)	NR	HPLC-FLD (liquid, 0.6 ng/L; solid, 0.05 ng/g)	Beverages: natural, litchi juice, white grape juice, jasmine tea Solid: ginseng, milkvetch, Maojian tea, honeysuckle, Anji white tea	Deng et al. (2021)
Beverages		9	ND (NR)	NR			
Honey		3	37.86 (NR) ng/L	NR			
Plants		15	1.24 (ginseng) – 27.39 (Anji white tea) ng/g dw	NR			
Yogurt	Tehran, Islamic Republic of Iran, 2018–2019	48	0.02 (0.02–0.02) µg/kg fw	NR	GC-MS (0.040 µg/kg)	Yogurt	Kiani et al. (2021)
Butter		48	0.02 (0.02–0.02) µg/kg fw				
Europe							
Smoked fish	Denmark, Scotland, Norway, Italy, France	10	25.6 (ND) (salmon, swordfish, eel) to 51.8 (herring) ng/g fw	NR	HPLC-FLD (NR)	Salmon, swordfish, herring, eel, bluefin tuna	Storelli et al. (2003)
Mollusk (*Haliotis tuberculat*)	Italy, 2014	60	0.23–0.86 (ND–2.41) µg/kg fw	ND–0.30 (0.11–1.38) µg/kg fw	HPLC-FLD (0.30 µg/kg fw)	3 locations; raw	Conte et al. (2016)
Smoked dry-cured ham (prosciutto)	Herzegovina, Bosnia and Herzegovina, 2019	34	1.39 (0.50–5.06) µg/kg dw	NR	GC-MS (0.30 µg/kg)		Mastanjević et al. (2020)
Smoked seafood: Automatic smoking kilns	Northern Germany, NR	35	0.4 (salmon cold smoked) to 25 (belly flaps of spurdog) (ND (salmon cold smoked) to 29 (belly flaps of spurdog)) µg/kg fw	0.4 (salmon cold smoked) – 25 (belly flaps of spurdog) (NR) µg/kg fw	HPLC-FLD (1 µg/kg) GC-MS (NR)	8 locations; mackerel fish, eel, belly flaps of spurdog (Schillerlocke), salmon cold smoked, salmon hot smoked, herring, halibut cutlets, red-fish, sprat	Karl & Leinemann (1996)
Tradition smoking kilns		27	14 (eel) – 30 (sprat) (2 (eel) – 60 (sprat)) µg/kg fw	15 (eel) – 29 (sprat) (NR) µg/kg fw	NR		
Frankfurter-type sausages, smoked under different experimental conditions	Germany, NR	50	4.8–36.3 µg/kg dw	NR	GC-HRMS (0.1 µg/kg)		Zastrow et al. (2019)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Sausage (dry-cured fermented), raw	Alentejo, Portugal, NR		9.52 µg/kg dw	NR	HPLC-UV-FLD (NR)		Roseiro et al. (2011)
Sausage (dry-cured fermented), smoked (modern/ industrial)			12.15–157.41 µg/kg dw	NR			
Sausage (dry-cured fermented), smoked (traditional)			46.47–297.32 µg/kg dw	NR			
Smoked sausage	Spain, NR	32	9.96–15.39 µg/kg dw	NR	HPLC-FLD (NR)	16 locations	Lorenzo et al. (2011)
Milk	Naples, Italy, NR	80	(0.04–3.58) µg/kg fw	0.34 µg/kg fw	HPLC-UV-FLD (0.03 ng/g)		Cirillo et al. (2010)
Cakes, biscuits, pastries, etc.		120	0.01–4.00) µg/kg fw	0.21 µg/kg fw			
Cereal (cornflakes)		81	(0.11–2.24) µg/kg fw	0.58 µg/kg fw			
Fruit juices		65	(0.01–1.11) µg/kg fw	0.30 µg/kg fw			
Ham or salami sandwiches		67	(0.01–2.24) µg/kg fw	0.24 µg/kg fw			
Chocolate		67	(0.01–8.29) µg/kg fw	0.46 µg/kg fw			
Candies		28	(0.11–2.25) µg/kg fw	0.22 µg/kg fw			
Pasta/rice with tomatoes sauce/legumes		203	(0.01–4.48) µg/kg fw	0.18 µg/kg fw			
Meat and meat products		126	(0.01–7.84) µg/kg fw	0.26 µg/kg fw			
Fish and fish products		58	(0.01–2.65) µg/kg fw	0.22 µg/kg fw			
Dairy products		75	(0.01–1.66) µg/kg fw	0.21 µg/kg fw			
Egg-based products		79	(0.01–1.68) µg/kg fw	0.25 µg/kg fw			
Pizza		57	(0.01–1.12) µg/kg fw	0.24 µg/kg fw			
Fresh or cooked vegetables		91	(0.01–11.95) µg/kg fw	0.20 µg/kg fw			
Bread, crackers, bread sticks, rusks		42	(0.01–6.15) µg/kg fw	0.22 µg/kg fw			
Fresh fruit		88	(0.01–1.18) µg/kg fw	0.31 µg/kg fw			

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Meat and meat products	Catalonia, Spain, 2000	30	0.180 µg/kg fw	NR	HPLC-UV-FLD (0.2 µg/kg)	7 locations	Falcó et al. (2003)
Fish and shellfish		16	0.110 µg/kg fw				
Vegetables		16	0.015 µg/kg fw				
Tubers		4	0.069 µg/kg fw				
Fruits		12	0.018 µg/kg fw				
Eggs		4	0.023 µg/kg fw				
Milk		4	0.011 µg/kg fw				
Dairy products		4	0.056 µg/kg fw				
Cereals (bread, pasta, rice)		8	0.131 µg/kg fw				
Pulses (lentils, beans)		4	0.045 µg/kg fw				
Oils and fats		6	0.185 µg/kg fw				
Meat and meat products	Catalonia, Spain, 2008	3120	3.38–32.00 µg/kg fw	NR	GC-HRMS (NR)	48 locations	Martorell et al. (2010)
Fish			0.12 µg/kg fw				
Squid			< 0.16 µg/kg fw				
Clam			< 0.16 µg/kg fw				
Mussel			0.42 µg/kg fw				
Shrimp			< 0.16 µg/kg fw				
Vegetables			0.06 µg/kg fw				
Tubers			0.05 µg/kg fw				
Fruits			0.05 µg/kg fw				
Eggs			0.24 µg/kg fw				
Milk			0.03 µg/kg fw				
Dairy product			0.13 µg/kg fw				
Cereals			0.08 µg/kg fw				
Pulses			0.10 µg/kg fw				
Oils and fats			1.23 µg/kg fw				
Industry bakery			0.08 µg/kg fw				
Honey	Serbia, 2017	61	NR (ND–6.51) µg/kg fw	2.11–2.38 µg/kg fw	GC-MS (1.10 µg/kg fw)	4 types	Petrović et al. (2019)
Wheat	Poland, 2017–2018	200	0.32 (NR–0.87) µg/kg fw	0.28 (NR) µg/kg fw	GC-MS (0.015 µg/kg fw)	16 locations	Roszko et al. (2020)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Milk infant formula	Italy, NR	30	32.22 (ND–72.88) µg/kg fw	NR	HPLC-FLD (NR)		Santonicola et al. (2017)
Breast milk		30	39.07 (ND–89.55) µg/kg fw				
Yogurt	Italy, 2014	20		NR	HPLC-FLD (0.01 µg/kg fw)		Battisti et al. (2015)
Low fat			0.08 (ND–0.20) µg/kg fw				
High fat			0.15 (ND–0.30) µg/kg fw				
Cheese	Vitoria, Spain, NR			NR	GC-MS (NR)		Guillén et al. (2011)
Unsmoked		2	0.03 µg/kg fw				
Smoked		24	1.33–7.13 µg/kg fw				
Olive oil	Bari, Italy, NR	NR	ND	NR	GC-MS (0.30 ng/g)		Cotugno et al. (2021)

dw, dry weight; fw, fresh weight; GC-FID, gas chromatography-flame ionization detection; GC-HRMS, gas chromatography-high-resolution mass spectrometry; GC-LRMS, gas chromatography-low-resolution mass spectrometry; GC-MS, gas chromatography-mass spectrometry; HPLC-FLD, high-performance liquid chromatography-fluorescence detection; HPLC-UV-FLD, high-performance liquid chromatography-ultraviolet and fluorescence detection; IQR, interquartile range; LOD, limit of detection; ND, not detected; NR, not reported.

(< 0.5–2.3 µg/mainstream smoke of 100 cigarettes), marijuana (< 0.5–3.3 µg/mainstream smoke of 100 cigarettes) (Graves et al., 2020; NCBI, 2022), incense burning (3.63–11.37 pg/µg particle mass) (Yang et al., 2017), exhausts of automotive diesel fuel and low concentration biodiesel blends (1.93–9.24 µg/km emissions) (Karavalakis et al., 2010) (Table 1.8), and exhausts of wood-, coal-, or other biomass-burning stoves and fireplaces (IARC, 2010). It has also been identified in smokeless tobacco traditionally consumed in north Africa, although no quantitative data were reported (Guezguez et al., 2021). Anthracene has also been detected in herbicides (< 2–26.5 mg/L) (Seralini & Jungers, 2020), wood vinegars (4.5–115.0 µg/L) (Zhang et al., 2021) (prepared from the condensation of biomass pyrolysis, and may be used as fungicides or biocides, among other uses), and in creosote wood preservatives (Table 1.8). Although the latter cannot be sold for domestic uses in Europe, some creosote-treated wood products may be placed on the secondhand market for reuse (building of fences, agricultural stakes, etc.); concentrations have been reported in the range of 4–2573 µg/g wood (Ikarashi et al., 2005). Coal tar can be used at levels of 0.5–5% in the USA and in Japan in over-the-counter products for the treatment of chronic skin diseases, but its use (as crude and refined) in Europe in cosmetic products is prohibited (Cosmetic Ingredient Review Expert Panel, 2008). European cosmetic products may only include pitch/coal tar-petroleum, low/high temperature pitch/coal tar, and residues (coal tar) of creosote oil distillation if these contain < 0.005% w/w benzo[*a*]-pyrene (B[a]P, a surrogate marker of exposure to carcinogenic PAHs) (European Parliament and Council, 2009b, regulation (EC) No. 1223/2009). These ingredients are likely to contain anthracene as part of a complex mixture (Mariani et al., 1997; Wang et al., 2019), predictably at ultra-trace levels, but there is a general lack of data on anthracene concentrations in these consumer products.

1.4.2 Occupational exposure

According to the National Occupational Exposure Survey (NOES) conducted by NIOSH from 1981 to 1983 (NIOSH, 1988), workers potentially exposed to anthracene in the USA were almost exclusively roofers, a few construction workers, and workers from the health services (janitors and cleaners) and business services (physicists, astronomers, and chemists) (NIOSH, 1983). [Considering the 2304 exposed workers that were reported, the Working Group estimated a confidence interval of 1500–3100; however, the Working Group considered the numbers to be underestimated because of the lack of representation of industries with known exposure, such as coking industries and road paving (see *IARC Monographs* Volume 103, IARC, 2013).]

Like other PAHs, occupational exposure to anthracene is likely to occur primarily through inhalation and dermal absorption (IARC, 2010). As anthracene is a low-molecular-weight PAH with three aromatic rings, it is expected to be found predominantly in the gas phase in ambient air (see Section 1.4.1a). Exposure to anthracene occurs in general in combination with exposure to other PAHs, which occurs mainly in the following occupational settings: the production and use of coal tar and coal tar-derived products, coke production and coke ovens, use of asphalt for paving and roofing, carbon-electrode manufacture, aluminium production, creosote use, chimney sweeping, firefighting, and others (IARC, 2010). Consequently, most scientific papers report exposure to the sum of several PAH congeners (usually six carcinogenic PAHs, eight high-molecular-weight PAHs, or other combinations of compounds), and exposure to single PAHs (including anthracene) is seldom reported. A selection of studies reporting occupational exposure to anthracene considered through different exposure assessment methods is shown in Table 1.9.

Table 1.8 Occurrence of anthracene in consumer products

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Fuels							
Biodiesel blends	Greece, date of collection NR	5 fuel blends with 2 samples per blend	NR (1.93–9.24) µg/km emissions	NR	GC-MS (NR)	The methyl esters incorporated (10% v/v) in the automotive diesel fuel (EN590) originated from soybean oil, used frying oil, palm oil, sunflower oil, and rapeseed oil.	Karavalakis et al. (2010)
Incense							
Smoke-free incense Binchotan charcoal incense Traditional incense	Taiwan, China and Japan, date of collection is NR	3 of each type	3.63 pg/µg particle mass (NR) 11.37 pg/µg particle mass (NR) 8.95 pg/µg particle mass (NR)	NR	GC-MS (NR)	The total suspended matter originated from incense burning was analysed.	Yang et al. (2017)
Preservatives and pesticides							
Creosote wood preservatives Creosote-treated woods	Japan (new railway sleepers and stakes) and China (used railway sleepers), date of collection NR	9 6	NR (7168–18 391) µg/g NR (4–2573) µg/g	NR	GC-MS (40 mg/g for paints and 4 mg/g for wood)	Wood placed on the secondhand market for reuse.	Ikarashi et al. (2005)
Herbicides without glyphosate	Commercially available in France, Poland, and Germany, 2019	14	4.7 (< 2–26.5) µg/L	NR	GC-MS		Seralini & Jungers (2020)
Wood vinegars (liquid product obtained from biomass pyrolysis)	Liaoning Province, Heilongjiang Province, China, date of collection NR	9	NR (4.5–115.0) µg/L	NR	GC-MS (0.01 µg/L)	9 different types of biomass were characterized. Wood vinegars are used as biocides, feed additives, and preservatives.	Zhang et al. (2021)
Tobacco and related products							
Smokeless tobacco (neffa)	Sousse, Tunisia, date of collection NR	7	NR	NR	HPLC-FLD (NR)	Thinly sliced tobacco leaves are inhaled by the nose or kept in the mouth. Anthracene was detected but no values are presented.	Guezguez et al. (2021)

Table 1.8 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Particulate phase of mainstream tobacco smoke	Kentucky, USA, 2019	NR	< 0.5 µg (NR)	NR	GC × GC-TOFMS (0.5 µg)	Reference products that represent tobacco products of US consumers were used. Comparison of smoke produced from a filtered tobacco cigarette with a nonfiltered marijuana joint.	Graves et al. (2020)
Particulate phase of marijuana smoke		NR	< 0.5 µg (NR)				
Aerosol of cigarettes	China, 2005–2006	7 brands with 3–4 cigarettes per brand	NR (0.02–0.2 µg)/(m^3 g tobacco)	NR	GC-MS and GC-C-IRMS		Zhang et al. (2009)
Particulate phase of mainstream cigarette smoke	USA, date of collection is NR	3 randomly selected from 5 different packs of each brand	NR (5.8–86.1) ng/cigarette		GC-MS (7 ng)	30 cigarette domestic brands.	Ding et al. (2005)
Tobacco smoke condensate	Tobacco cigarettes commercially available, Mexican marijuana, England, date of collection NR	2000	2.3 µg/100 cigarettes (NR)	NR	GC-MS (NR)		Lee et al. (1976)
Marijuana smoke condensate		2000	3.3 µg/100 cigarettes (NR)				

GC-C-IRMS, gas chromatography-combustion-isotope ratio mass spectrometry; GC × GC-TOFMS, two-dimensional gas chromatography with time-of-flight mass spectrometric detection; GC-MS, gas chromatography-mass spectrometry; HPLC-FLD, high-performance liquid chromatography-fluorescence detection; IQR, interquartile range, LOD, limit of detection; ND, not detected; NR, not reported; v/v, volume per volume.

Table 1.9 Measurement of anthracene in occupational settings

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Stationary air monitoring							
Carbon black-manufacturing plant, Taiwan, China, date NR	Air measurements	16	GC-MS (NR)	NR (1.23–1976.34) ng/m^3	NR		Tsai et al. (2002)
Butchers burning scrap tyres, Nigeria, date NR	Air measurements	3	NR	50 ± 0.00 g/m^3	NR		Okonkwo et al. (2018)
Refractory-brick manufacturing, Italy, date NR	Air measurements	18	HPLC-FLD (0.10 µg/m^3)	Production area, 1.0 ± 0.16 µg/m^3 Packaging area, 0.51 ± 0.33 µg/m^3 External area, 0.65 ± 0.32 µg/m^3	Production area, 1.1 µg/m^3 Packaging area, 0.41 µg/m^3 External area, 0.50 µg/m^3	NIOSH method 5506.	Sartorelli et al. (2020)
Firefighters, Australia, 2017–2018	Air measurements	15	GC-MS/MS (0.050 ng/m^3)	NR	0.81 (0.45–2.3) ng/m^3		Banks et al. (2020)
Firefighters in incident command post, California, USA, 2015	Air measurements	2 (12 days of measurements each)	GC-MS (NR)	1 ng/m^3	1 (< 1–2) ng/m^3 (GM, min. to max.)		Navarro et al. (2019)
Surface contamination							
Refractory-brick manufacturing, Italy, date NR	Wipe test	17	HPLC-FLD (0.006 ng/cm^2)	Production area, clean surfaces, 7.4 ± 8.2 ng/cm^2 Production area, dirty surfaces, 601 ± 296 ng/cm^2 Packaging area, 1.1 ± 1.3 ng/cm^2 External area, 1.5 ± 0.46 ng/cm^2	Production area, clean surfaces, 5.1 ng/cm^2 Production area, dirty surfaces, 589 ng/cm^2 Packaging area, 0.61 ng/cm^2 External area, 1.2 ng/cm^2	Technique complies with the ASTM.	Sartorelli et al. (2020)
Settled dust measurements							
Automobile workshop, Jeddah, Saudi Arabia, 2016	Indoor settled dust	18	GC-MS/MS (10 ng/g)	0.410 ± 0.490 µg/g	0.235 (0.085–2.070) µg/g		Ali et al. (2017)
Firefighters, Australia, 2017–2018	Dust	49	GC-MS/MS (0.012 µg/g)	NR	0.032 (< LOD–0.17) µg/g		Banks et al. (2020)

Table 1.9 (continued)

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Personal monitoring: air measurements							
Hazardous waste disposal facility, Baton Rouge, Louisiana, USA, 1980	Workers' breathing zone	36	HPLC (0.1 µg/sample)[a]	5 (1–18) µg/m³			NIOSH (1982a)
Coal-tar pitch roof tear-off and application of hot asphalt, Lancaster, Ohio, USA, 1981	Workers' breathing zone	16	HPLC (NR)[a]	Tear-off, 0.2–6.7 µg/m³ Application, 0.1–0.6 µg/m³			NIOSH (1982b)
Coal-tar pitch roof tear-off and application of hot asphalt, USA, 1987	Workers' breathing zone	10	HPLC-FLD (NR)	1.5 ± 0.6 µg/m³ (day 1) 0.5 ± 0.2 µg/m³ (day 2)	NR	NIOSH method 5506.	Wolff et al. (1989)
Impregnation and handling of creosote-impregnated wood, Finland [a]	Workers' breathing zone	Impregnation plants, 23 Handling, 11	GC-FID (NR)	Workers of the impregnation plants, 1.0 µg/m³ Openings, 19 µg/m³ Cleaning of chamber, 6.0 µg/m³ Handling during switch element assembly, 0.5 µg/m³ Manual metal-arc welding, 1.8 µg/m³	NR		Heikkilä et al. (1987)
Bitumen paving, Switzerland, 1992	Workers' breathing zone	9	GC-MS (1 ng/m³)	0.073 µg/m³ (GM)	NR		Petry et al. (1996a)
Shipbuilding, steel-pipe manufacturing, and paint-manufacturing workplaces handling coal-tar painting, 2001	Workers' breathing zone	106 workers from 10 workplaces	GC-MS (NR)	GM, 8 µg/m³ (range, 0–8230 µg/m³)	NR		KOSHA (2001)

Table 1.9 (continued)

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Asphalt workers and construction workers, Milan, Italy, 2003	Workers' breathing zone	100 (asphalt) 47 (construction)	HPLC-FLD (0.4 ng/m^3)		Asphalt, 0.7 (< 0.4–97.7) ng/m^3 Construction, 0.4 (< 0.4–2.5) ng/m^3 (median, min. to max.)		Campo et al. (2006a)
Asphalt workers, north Italy, 2014–2015	Workers' breathing zone	7 workers (3 pavers, 3 ground operators and 1 roller)	HPLC-UV (0.10 ng)	20.39 ± 2.69 ng/m^3 (GM ± GSD)	NR	NIOSH method 5506.	Fostinelli et al. (2018)
Carbon anode plant, Switzerland, 1992	Workers' breathing zone	30	GC-MS (1 ng/m^3)	0.894 µg/m^3 (GM)	NR		Petry et al. (1996a)
Carbon anode plant, Switzerland	Workers' breathing zone	6	GC-MS (1 ng/m^3)	Range, 0.420–5.510 µg/m^3	NR		Petry et al. (1996b)
Graphite production, Switzerland, 1992	Workers' breathing zone	16	GC-MS (1 ng/m^3)	GM, 0.042 µg/m^3	NR		Petry et al. (1996a)
Silicon carbide production, Switzerland, 1992	Workers' breathing zone	14	GC-MS (1 ng/m^3)	GM, 0.006 µg/m^3	NR		Petry et al. (1996a)
Metal recycling process, Switzerland, 1992	Workers' breathing zone	5	GC-MS (1 ng/m^3)	GM, 0.04 µg/m^3	NR		Petry et al. (1996a)
Coke-oven workers, Germany, date NR	Workers' breathing zone	11	HPLC-UV (NR)	Topside, 14.34 (1.30–57.38) µg/m^3 Bench-side, 1.10 (< LOD–2.90) µg/m^3	NR	NIOSH method 5506	Strunk et al. (2002)
Coke-oven workers, Taiwan, China, July–November, 2003	Workers' breathing zone	17 (top-oven) 35 (side-oven)	GC-MS (NR)	Top-oven, 31.18 ± 5.72 ng/m^3 Side-oven, 10.35 ± 4.16 ng/m^3	NR		Lin et al. (2006)
Coke-oven workers, southern Taiwan, China, date NR	Workers' breathing zone	17 (top-oven) 25 (side-oven)	GC-MS (NR)	Top-oven, 43.29 ± 64.86 ng/m^3 Side-oven, 5.54 ± 6.81 ng/m^3	NR		Jeng et al. (2011)

Table 1.9 (continued)

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Coke-oven workers, southern Taiwan, China[a]	Workers' breathing zone	31 (top-oven) 23 (side-oven)	GC-MS (NR)	Top-oven, 353.54 ± 93.33 ng/m³ Side-oven, 340.98 ± 66.58 ng/m³	NR		Jeng et al. (2023)
Coke-oven workers, Upper Silesia, Poland, 2005–2010	Workers' breathing zone	162	HPLC-FLD (11–113 ng/m³)		0.042 (0.014–0.653) µg/m³		Bieniek & Łusiak (2012)
Coke-oven workers, Taranto, Italy, 2005	Workers' breathing zone	45	HPLC-FLD (4.3 ng/m³)	3.184 (0.278–34.348) µg/m³	NR	NIOSH method 5506.	Campo et al. (2012)
Coke-oven workers, Anshan, China, 2002	Workers' breathing zone	57	GC-MS (0.05 ng/m³)	NR	0.01 (< LOQ–28.35) µg/m³	US EPA	Yamano et al. (2014)
Fire-proof materials production plants, Germany, 1999–2004	Workers' breathing zone	117	HPLC-DAD (NR)	NR	1.36 (< LOD–69.01) µg/m³	NIOSH method 5506.	Preuss et al. (2006)
Traffic police officers, Beijing, China, winter 2005	Workers' breathing zone	30	GC-MS (NR)	Vapour phase, 18.2 ± 10.6 ng/m³ Particulate phase, 21.0 ± 27.7 ng/m³	NR		Liu et al. (2007)
Firefighters in non-fire work environments (fire stations), northern Portugal, 2014	Workers' breathing zone	54	HPLC-FLD (NR)	0.223–0.330 ng/m³ (mean range in 5 stations) 0.223–0.551 (min. to max. in 5 stations)	NR		Oliveira et al. (2017a)
Firefighters during emergency fire suppression, Canada, 2015	Workers' breathing zone	29	GC-MS (0.71 ng/m³)	50.91 (0.03–746.85) µg/m³	NR		Keir et al. (2020)
Workers from an iron ore mine exposed to diesel and renewable diesel exhaust, northern Sweden, 2019	Workers' breathing zone	12	HRGC/LRMS after passive sampling (NR)	NR	2.78 (8–103) ng/m³		Gren et al. (2022)

Table 1.9 (continued)

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Dermal contamination measurements							
Manufactured-gas plants, Paris, France, 1997	Dermal pads	29	HPLC-FLD (10 ng/cm^2)	NR	Neck, < LOD–13 ng/cm^2 Shoulder, < LOD ng/cm^2 Wrist, < LOD–100 ng/cm^2 Groin, < LOD ng/cm^2 Ankle, < LOD–12 ng/cm^2 (range)	Dermal pad locations: neck and wrist (uncovered), shoulder, groin, and ankle under the clothes.	Dor et al. (2000)
Asphalt workers, Finland, 1999–2000	Dermal pads	22	HRGC-MS (0.01 ng/cm^2)	NR (< 0.01–0.75) ng/cm^2	NR	Wrist contamination.	Väänänen et al. (2005)
Asphalt workers, Milan, Italy, 2003	Dermal pads	24	PTV-GC-MS (0.020 ng/cm^2)	NR	Wrist, 0.385 (< 0.02–6.455) ng/cm^2 Total body, 10.86 (3.86–142.19) µg (min. to max.)	Wrist contamination and total body contamination.	Fustinoni et al. (2010)
Biological monitoring							
Asphalt workers and construction workers, Milan, Italy, 2003	Urine	100 (asphalt) 47 (construction)	HS-SPME-GC-MS (2 ng/L)	NR	Asphalt workers: BS, 2 (< 2–16) ng/L ES, 5 (< 2–28) ng/L Construction workers: BS, < 2 (< 2–15) ng/L ES, 3 (< 2–9) ng/L (median, min. to max.)		Campo et al. (2007)
Coke-oven workers, Poland, 2000	Urine	55 (all smokers)	HS-SPME-GC-MS (2 ng/L)	NR	49 (9–319) ng/L (5th to 95th)		Campo et al. (2010)
Coke-oven workers, Poland, 2006	Urine	49 workers (non-smokers) 49 controls (non-smokers)	SPME-GC-MS (0.8 ng/L)	NR	Workers, 13 (< LOD–69.4) ng/L Controls, 1.3 (< LOD–3.7) ng/L (5th to 95th percentile)		Campo et al. (2014)

Table 1.9 (continued)

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Electric steel-foundry workers, Menzel Bourguiba, Tunisia, 2013	Urine	93	SPME-GC-MS/ MS (0.2 ng/L)	NR	Steel smelter workshop, 1.97 (1.02–6.64) ng/L Rolling mill and galvanization workshop, 2.17 (1.18–6.30) ng/L Engine maintenance, 2.58 (1.17–11.43) ng/L		Campo et al. (2016)
Beauty salons workers, Islamic Republic of Iran[a]	Urine	50 women (workers) 35 women (controls)	SPME-GC-MS (NR)	Workers: BS, 14.51 ± 12.52 ng/L ES, 17.31 ± 15.01 ng/L Controls, 1.57 ± 0.62 ng/L (morning sample)	NR		Arfaeinia et al. (2022)
Firefighters, Korea, 2019	Serum	92 firefighters 70 controls	GC-MS/MS (NR)	Firefighters, 0.675 (< LOD–23.9) ng/g lipid weight Controls, < LOD	NR		Ekpe et al. (2021)
Coal-fired power plant workers, Shandong Province, China, 2021	Serum	125 men 32 women	GC-MS/MS (NR)	Men, 50 ± 48 ng/g lipid weight Women, 37 ± 10 ng/g lipid weight	NR		Zhao et al. (2022)
Sanitation workers, Guangzhou, China, 2020	Serum	115 sanitation workers working on roads 81 office employees and workers working in parks (controls)	GC-MS/MS (NR)	Workers, 2.13 ± 2.38 (< LOD–17.0) ng/mL Controls, 1.64 ± 2.04 (< LOD–10.08) ng/mL	Workers, 2.32 ng/mL Controls, 1.67 ng/mL		Lv et al. (2022)

Table 1.9 (continued)

Occupational group/ job type/industry, location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median (IQR)	Comments	Reference
Firefighters, Spain [a]	Saliva	45 firefighters working in firefighting activities 10 firefighters not working in firefighting activities (controls)	GC-MS (0.091 µg/L)	Exposed, < LOD–0.379 µg/L Controls, < LOD	NR		Santos et al. (2019)

ASTM, American Society for Testing and Materials; BS, before shift; ES, end of shift; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; GM, geometric mean; GM ± GSD, geometric mean ± standard deviation; HPLC, high-performance liquid chromatography; HPLC-DAD, high-performance liquid chromatography-diode array detection; HPLC-FLD, high-performance liquid chromatography-fluorescence detection; HPLC-UV, high-performance liquid chromatography-ultraviolet detection; HRGC/LRMS, high-resolution gas chromatography/low-resolution mass spectrometry; HRGC-MS, high-resolution-gas chromatography-mass spectrometry; HS-SPME-GC-MS, headspace solid-phase microextraction-gas chromatography; IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; min. to max., minimum to maximum; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; PTV-GC-MS, programmable temperature vaporizer-gas chromatography-mass spectrometry; SPME-GC-MS, solid-phase microextraction-gas chromatography-mass spectrometry; SPME-GC-MS/MS, solid-phase microextraction-gas chromatography-tandem mass spectrometry; US EPA, United States Environmental Protection Agency; UV, ultraviolet.

[a] The detector used in this application was not indicated.

(a) Stationary air monitoring

Anthracene has been reported in the air of carbon black-manufacturing plants in Taiwan, China (range, 1.23–1976.34 ng/m^3) (Tsai et al., 2002) and in refractory-brick manufacturing in Italy (Sartorelli et al., 2020). In the latter, higher levels were found in the production area (mean, 1.0 μg/m^3) than in other areas of the plant (mean levels, < 1.0 μg/m^3). The presence of anthracene was reported at very high levels in sporadic measurements taken in the ambient air of an abattoir in Nigeria where burning tyres were used to remove the fur of slaughtered animals (mean, 0.05 μg/cm^3 [50 g/m^3]) (Okonkwo et al., 2018). In studies involving firefighters, anthracene was measured in the ambient air at the incident command post, (mean, 1 ng/m^3) (Navarro et al., 2019), and similar values were measured in the living quarters of fire stations (median levels, 0.81 ng/m^3) (Banks et al., 2020).

[Overall, according to the data reported above, the Working Group considered that the highest exposure to airborne anthracene may be in the industrial setting of carbon black-manufacturing plants.]

(b) Settled dust measurements

Anthracene was measured in dust samples from fire stations in Australia, including samples collected from living quarters, firefighter ensemble storage areas, and fire engine cabins, at levels up to 0.17 μg/g (Banks et al., 2020), and similar values were measured in indoor settled dust from automobile workshops in Saudi Arabia (median levels, 0.235 μg/g) (Ali et al., 2017). [The Working Group noted that the detection of anthracene on surfaces indicates a potential further source of worker exposure via hand-to-mouth involuntary behaviour, even if this possibility has not yet been studied.]

(c) Personal monitoring

Personal exposure to anthracene in the workplace has been studied by means of air samples collected by active samplers placed near the worker's breathing zone. Exposure to anthracene was evaluated for 36 workers from a hazardous waste disposal facility in Louisiana, USA (NIOSH, 1982a). Unit operations at this site included incineration, biological stabilization and treatment, landfilling, and landfarming. Anthracene was detected in samples from five workers (four operation and one maintenance personnel), and mean exposure to anthracene was 5 μg/m^3 (range, 1–18 μg/m^3). This exposure was probably a result of the previous mixing of anthracene-containing waste sludge with soil in the area (NIOSH, 1982a). Two studies evaluated the personal exposure of workers during the tear-off of old coal-tar roofs and the application of hot asphalt in the USA (NIOSH, 1982b; Wolff et al., 1989); mean exposure levels ranged from 0.2 to 6.7 μg/m^3 during tear-off operations, whereas levels were lower during hot asphalt application. Much lower anthracene levels (median, 0.7 ng/m^3) were found in asphalt workers laying asphalt at low temperature in Italy (Campo et al., 2006a), in a small group of asphalt workers laying hot-mix asphalt containing modified bitumen in Italy (mean, 20.39 ng/m^3) (Fostinelli et al., 2018), and in construction workers potentially exposed to diesel exhaust (median, 0.4 ng/m^3) (Campo et al., 2006a). Much higher mean levels (73 μg/m^3) were reported for workers employed in bitumen paving in Switzerland, but no details were given on the bitumen type (Petry et al., 1996a). Exposure to anthracene may occur during the manufacture of creosote or creosote-containing products. Heikkilä et al. reported exposure to creosote in two bulk impregnation plants and during the handling of creosote-treated wood in Finland; mean personal exposure to airborne anthracene ranged from 0.5 μg/m^3 (operators in the switch element assembly) to 19 μg/m^3 (peak values for

operators during the openings of cylinders in the impregnation plant) (Heikkilä et al., 1987). In a plant producing carbon anodes for aluminium electrolysis in Switzerland, airborne anthracene exposure was measured (i) in the personal air of 6 workers employed in different tasks – levels ranged from 420 ng/m^3 (foreman) to 5510 ng/m^3 (floor worker with operating and maintenance functions at the paste plant) (Petry et al., 1996b); and (ii) in the personal air of 30 workers – the mean level was 894 ng/m^3 (Petry et al., 1996a). Several studies assessed anthracene exposure in coke-oven workers; mean levels were up to 14.34 µg/m^3 (Strunk et al., 2002; Lin et al., 2006; Jeng et al., 2011, 2023; Bieniek & Łusiak., 2012; Campo et al., 2012; Yamano et al., 2014) when different sites in the coke plants were sampled; exposure levels were higher in top-side workers than in side-oven workers (Strunk et al., 2002; Lin et al., 2006; Jeng et al., 2011). Personal exposure to anthracene has also been reported for workers from fire-proof material production plants in Germany (median, 1.36 µg/m^3) (Preuss et al., 2006), workers from a graphite production plant (mean, 42 ng/m^3), workers involved in silicon-carbide production (geometric mean, 6 ng/m^3) and metal-recycling processes in Switzerland (geometric mean, 40 ng/m^3) (Petry et al., 1996a), and in workers from an iron ore mine in northern Sweden who were exposed to diesel and renewable diesel exhaust (median, 2.78 ng/m^3) (Gren et al., 2022). In firefighters, mean anthracene levels ranging from 0.223 to 0.330 ng/m^3 were reported for firefighters in non-fire work environments (fire stations) in northern Portugal (Oliveira et al., 2017a), whereas much higher values were found during emergency fire suppression (mean, 50.91 µg/m^3) in Canada (Keir et al., 2020). In a study that assessed anthracene exposure for traffic police officers during winter in Beijing, China, anthracene mean concentrations were reported to be much lower than those reported for industrial settings, and values were similar in the vapour phase and the particulate phase (mean, 18.2 and 21.0 ng/m^3, respectively) (Liu et al., 2007).

In 2001, the Korea Occupational Safety and Health Agency (KOSHA) in the Republic of Korea investigated workplaces with exposure to PAHs, including anthracene. In 106 workers from 10 workplaces (shipbuilding, steel-pipe manufacturing, and paint manufacturing) at which coal-tar paint was handled, mean personal exposure to anthracene was 8 µg/m^3 (assessed according to NIOSH method 5515) (KOSHA, 2001).

The COLCHIC database, which contains workplace exposure results for chemical samples collected by the prevention network in France from 1987 to 2020, identified 662 measures of occupational exposure to anthracene. The median levels for personal exposure were 2740 ng/m^3 before the year 2000 and below the LOD after the year 2000; median levels for ambient measures were below the LOD both before and after the year 2000 (INRS, 2022). Among the three most frequent workplace activities (road construction and highways; collection, treatment, and distribution of water; and manufacture of rubber articles), the highest median levels for personal exposure were found in the sector "collection, treatment, and distribution of water" (6322 ng/m^3, 24 measures). Among the three most frequent occupations, the highest median level for personal exposure was measured for "railway maintenance" (5732 ng/m^3, 26 measures). Among the three most frequent tasks, the highest median level for personal exposure was measured for the job task "machining by mechanical abrasion: cutting, sawing, filing, sharpening" (3438 ng/m^3, 22 measures) (INRS, 2022).

Overall, the highest personal exposure to anthracene via air is likely to be for asphalt workers dealing with the tear-off of old coal-tar roofs, firefighters in emergency situations, coke-oven workers, and workers employed in the production of carbon anodes.

Personal dermal exposure to anthracene has been studied in asphalt workers in Finland (Väänänen et al., 2005) and in Italy (Fustinoni et al., 2010). In the first study, in workers in Finland laying stone mastic asphalt containing coal fly ash or limestone, or using remixed asphalt, dermal contamination (evaluated only at the wrist using polypropylene pads) was between 0.20 and 0.42 ng/cm^2 (Väänänen et al., 2005). Higher concentrations were reported for asphalt workers laying hot asphalt in Italy, for whom a median wrist contamination of 0.385 ng/cm^2 and a median total body contamination of 10.86 µg were reported (Fustinoni et al., 2010). In workers from manufactured-gas plants in France, detectable levels of anthracene, at concentrations of up to 100 ng/cm^2, were found only in 3% of dermal pads (Dor et al., 2000).

(d) Biomonitoring

No biological marker for evaluating the internal dose of anthracene has been validated to date, and biological monitoring of occupational exposure to anthracene has very seldom been performed. The measurement of anthracene metabolites in the urine has not been reported. Anthracene has been quantified in urine samples from asphalt workers and construction workers in Italy (Campo et al., 2007), two groups of coke-oven workers in Poland (Campo et al., 2010, 2014), and in electric steel-foundry workers in Tunisia (Campo et al., 2016); median levels were ≤ 5 ng/L in all settings, except for coke-oven workers (see below). These values were higher than those found in the general population in Italy (median, 2.1 ng/L; 95th percentile, 3.5 ng/L) (Gatti et al., 2017). In asphalt workers, an association between personal exposure to anthracene and urinary levels was reported; levels were higher in end-shift samples than in before-shift samples (Campo et al., 2007). In coke-oven workers, median concentrations were 13.0 ng/L in the group of non-smokers but as high as 49 ng/L in the group of smokers. However, anthracene concentrations in the non-smokers were about 10-fold those in non-smokers from the general population living in the same area as the plant (median, 13 ng/L versus 1.3 ng/L) (Campo et al., 2014). Anthracene in the urine has also been reported for beauticians in the Islamic Republic of Iran, for whom values were 10-fold those for women not working in beauty salons (mean, 17.31 ng/L versus 1.57 ng/L) (Arfaeinia et al., 2022).

Sporadic measurements of anthracene in the serum and saliva have been reported. Anthracene was detected in 5.2% of serum samples from firefighters from the Republic of Korea, with a mean concentration of 0.675 ng/g lipid weight, but was below the LOD in all samples from the general population (Ekpe et al., 2021). In workers from a coal-fired power plant in China, mean concentrations of anthracene in serum were higher in men than in women (50 ng/g lipid weight versus 37 ng/g lipid weight) (Zhao et al., 2022). In sanitation workers from Guangzhou, China, mean concentrations of anthracene in serum samples from workers on busy roads were higher than in office employees or in sanitation workers working in parks (2.13 ng/mL versus 1.61 ng/mL), with the highest concentrations attributed to exposure to exhaust emissions (Lv et al., 2022). In saliva, anthracene was detected in 50% of samples taken from firefighters immediately after firefighting activities ceased (concentrations in the range of < 0.091 to 0.329 µg/L), but was always below the LOD in samples from firefighters not involved in firefighting activities (Santos et al., 2019).

1.4.3 Exposure of the general population

(a) Exposure data

Exposure of the general population to anthracene can occur via multiple routes, i.e. inhalation, ingestion of food and beverages, and skin absorption.

The most significant sources of polluted air for the general population are tobacco smoke

(mainstream and sidestream smoke), wood burning (indoors or outdoors, including forest fires) and high-traffic or highly industrialized urban locations. The average daily intake of anthracene from inhalation has been estimated to be approximately 11 ng in the USA (considering a background environmental level of 0.54 ng/m^3) (US EPA, 1987), and the maximum daily intake in Europe was estimated to be 680 ng (based on the measured maximum of 34 ng/m^3) (ECHA, 2008b).

Ingestion from contaminated food (contaminated as a result of environmental pollution – soil, water or atmospheric deposition – and/or processing) is the major route of anthracene intake by the non-smoking and non-occupationally exposed population. Intakes vary depending on diet (Falcó et al., 2003; Cirillo et al., 2010; Martorell et al., 2010) (see Table 1.2 in Section 1.4.1) but were estimated to be 45 ng/kg bw per day, i.e. 3.1 µg/day, in the United Kingdom; 10 µg/day (maximum conservative estimate) in Europe (ECHA, 2008b); and 0.96 µg/day in autumn to 2.53 µg/day in winter (median values) in China (Duan et al., 2016), based on occurrence in food and beverages. However, higher intakes may be observed for population groups that grill or bake using biomass as fuel, or whose diets are based on smoked products or foodstuff from highly contaminated agricultural lands (Li et al., 2018). Human milk can be an exposure source for infants and young children (Santonicola et al., 2017). Ingestion of soil and dust can be also a potential route of exposure for children because of behavioural differences from adults (e.g. playing on the floor indoors or outdoors, and hand-to-mouth behaviour) (see Table 1.6, Section 1.4.1) (US EPA, 2009, 2014b, 2017; Islam et al., 2018; Gao et al., 2019). Levels of anthracene have been reported in the range of < 0.001–10.0 µg/g in household dust from different countries (Canada, China, Nepal, Portugal, Saudi Arabia, Sweden) (Vicente et al., 2019; Alamri et al., 2021; Lim et al., 2021).

Dermal absorption in non-occupationally exposed individuals may take place mainly through contact with contaminated soils, wood treated with creosote and related products and used in secondhand goods (see Table 1.6 and Table 1.7 in Section 1.4.1), and with coal tar-based pharmaceuticals or cosmetic products (over-the-counter shampoos, skin and hair care products for the treatment of seborrheic dermatitis and psoriasis) (IARC, 2010). Because of their biological and physiological characteristics, as well their different behavioural patterns (e.g. playing on the ground), skin absorption can be also relevant for children (US EPA, 2014b, 2017). The amount of absorption is influenced by the concentration of anthracene, duration of contact, the individual's skin-specific properties (hydration, thickness, and fat), and temperature (US EPA, 2014b).

(b) Biomonitoring

The determination of anthracene in human biological fluids and tissues has been reported in several population groups, mostly in Asia and Europe (Table 1.10).

A survey conducted in Italy (2010; 2012–2013) showed that urinary levels of anthracene in participants living and working within 4 km of a municipal solid-waste incinerator were markedly influenced by the incinerator emissions, even when these complied with the European regulations (Ranzi et al., 2013; Gatti et al., 2017). Exposure of adults with pulmonary ailments, e.g. pulmonary cancer, in Romania (Cioroiu et al., 2013) and chronic obstructive pulmonary disease, in China (Che et al., 2020), was also described. Statistically higher levels of anthracene were observed in lung and in bronchoalveolar lavage fluid from patients living in polluted urban areas (Table 1.10). The same pattern of variation was described for anthracene in hair samples from urban inhabitants and/or smokers when compared with rural inhabitants and/or non-smokers in two regions of China (Palazzi et al., 2018; Wang et al., 2020; Table 1.10).

Table 1.10 Measurement of anthracene in human matrices

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in blood (venous)	Owerri city, Imo State, Nigeria, children (age, 4–14 yr), date NR	36	0.06 (0.051–0.064) µg/dL	NR	NR	GC-MS	Boys (50%) and girls (50%).	Wirnkor et al. (2019)
Blood (venous)	Lucknow, India, children (age, 2–12 yr), 2005–2006	56	NR	3.6 (5.54) ng/mL	25th percentile, 1.45 ng/mL 75th percentile, 6.99 ng/mL	HPLC-UV-FLD (0.015 µg/L)	Average exposure time near kitchen: 31.57 minutes; residence distance from highway/traffic: 805.20 m.	Singh et al. (2008b)
Anthracene in blood serum (venous)	Nantong, China, pregnant women (age, 18–40 yr), 2018–2019	48	ND	NR	25th, 50th and 75th percentiles, ND	HPLC-UV-FLD (0.02 ng/mL)	Healthy pregnant women, non-smokers, no drinking habit, no history of occupational exposure to PAHs, no family genetic history of lung cancer, stomach cancer or asthma.	Guo et al. (2021)
Anthracene in serum	State of Tennessee, autopsied individuals, USA, 2001–2003	650	NR	NR	NR	GC-MS		Ramesh et al. (2014)

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in maternal serum	Asaluyeh port (petrochemical and gas area) and Bushehr port (urban area), Islamic Republic of Iran, pregnant women (37–42 wk gestation; age, 18–37 yr) and fetuses, 2018–2019 Petrochemical and gas area	99	2.12 (ND–23.9) µg/L	ND 1.80 µg/L	25th percentile, ND 75th percentile, 1.75 µg/L	GC-MS (0.76 ng/L)	6 h between the collection of the maternal and cord blood. Pregnant healthy women that lived at least 1 yr in the sampling areas. Smoking/ passive smoking, alcoholism, mothers who had infants with congenital malformations and multiple gestations were excluded.	Khalili Doroodzani et al. (2021)
	Urban area	100	2.90 (ND–18.3) µg/L		25th percentile, 0.10 µg/L 75th percentile, 4.60 µg/L			
Anthracene in urine	Modena, Italy, participants (mean age, 48.1 yr) living and working within and outside 4 km of the solid waste incinerators, 2010					GC-MS (NR; LOQ, 0.5 ng/L)	Spot sampling in the morning. Smokers and non-smokers.	Ranzi et al. (2013)
	Exposed	65	0.9 ng/L	0.8 ng/L	5th percentile, < 0.5 ng/L 95th percentile, 2.3 ng/L			
	Unexposed	103	0.6 ng/L	< 0.5 ng/L	5th percentile, < 0.5 ng/L 95th percentile, 1.9 ng/L			
Anthracene in urine	Owerri city, Imo State, Nigeria, children (age, 4–14 yr), date NR	36	0.55 (0.51–0.62) µg/dL	NR	NR	GC-MS	Boys (50%) and girls (50%).	Wirnkor et al. (2019)

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in urine	Modena, Italy, adults (age, 18–69 yr) living and working within 4 km of a municipal solid waste incinerator, 2012–2013	488	2.2 ng/L	2.1 ng/L	5th percentile, 0.9 ng/L 95th percentile, 3.5 ng/L	GC-MS/MS (NR; LOQ, 0.4 ng/L)	First morning void.	Gatti et al. (2017)
Anthracene in breast milk	Baltimore and North Carolina, nursing mothers (age, 15–25 yr), USA, 2015	12	ND	NR	NR	GC-MS (0.04 ng/g fat; 0.001 ng/mL milk)	Non-smoking women.	Kim et al. (2008)
Anthracene in breast milk	Italy, pregnant (age, 25–35 yr), women, NR	30	39.07 (0.00–89.55) µg/kg	NR	HPLC-FLD (NR)	NR	Non-smokers.	Santonicola et al. (2017)
Anthracene in breast milk	Aveiro, Coimbra, Lisboa, Viseu and Vila Real, Portugal, nursing mothers (age, 21–40 yr), 2019–2020	65	NR (0.044–2.04) ng/mL	0.050 ng/mL	25th percentile, 0.049 ng/mL milk 75th percentile, 0.087 ng/mL milk	HPLC-DAD-FLD (0.07 µg/L)	Healthy and non-smoking mothers.	Oliveira et al. (2020)

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in placental tissue	Agra, India, pregnant women (age, 18–40 yr), 2016–2017			NR	NR	GC-FID (NR)	Tobacco usage: 45.45% in full-term; 58.62% in preterm. Higher values of anthracene in the preterm group but no significant difference ($P < 0.05$) between the control and the study group. High contribution of rural women (62%) in the preterm group that used biomass fuel as a cooking source.	Agarwal et al. (2018)
	Control group (gestational age, > 36 wk (full-term delivery, undergoing spontaneous labour at term)	55	0.027 µg/L					
	Case group (gestational age, < 36 wk (preterm delivery, undergoing preterm labour)	29	0.134 µg/L					
Anthracene in placental tissue	Agra, India, pregnant women (age, 18–32 yr), 2017–2018	110 (14.28% detection)	0.25 (ND–7.87) µg/L	NR	NR	GC-FID (NR)	Healthy pregnant women; smokers, having the previous history of serious chronic disease or pregnancy complications were excluded. Chewing tobacco and alcohol usage, 35.45%.	Agarwal et al. (2022)
Anthracene in placental tissue	Lucknow, India, pregnant women (age, 20–35 yr), 2005–2006			NR	NR	HPLC-FLD (0.03 µg/L)	Healthy, non-smokers.	Singh et al. (2008a)
	Full term (normal deliveries at term)	31	25.81 ppb [ng/g]					
	Preterm labour (gestational age, < 36 wk)	29	33.26 ppb [ng/g]					

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in umbilical cord blood	La Palma, Canary Islands, Spain, pregnant women (age, 16–42 yr), 2015–2016	447	0.181 (ND–0.181) ng/mL	NR	NR	GC-MS (NR)	Smokers, 11.18%. Anthracene was only detected in 1 sample.	Cabrera-Rodríguez et al. (2019)
Anthracene in cord serum	Asaluyeh port (petrochemical and gas area) and Bushehr port (urban area), Islamic Republic of Iran, pregnant women (gestation, 37–42 wk; age, 18–37 yr) and fetuses, 2018–2019					GC-MS (0.76 ng/L)	6 h between the collection of the maternal and cord blood. Pregnant healthy women that lived at least 1 yr in the sampling areas. Smoking/passive smoking, alcoholism, mothers who had infants with congenital malformations and multiple gestations were excluded.	Khalili Doroodzani et al. (2021)
	Petrochemical and gas area	99	2.07 (ND–26.5) µg/L	ND	25th percentile, ND 75th percentile, 1.75 µg/L			
	Urban area	100	3.21 (ND–14.5) µg/L	2.85 µg/L	25th percentile, 0.05 µg/L 75th percentile, 5.25 µg/L			

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in hair	Baoding and Dalian, healthy women (age, 25–45 yr), China, 2016					GC-MS/MS (NR)	Baoding as polluted city and Dalian as less polluted city. Subjects living for at least 15 yr in the same city.	Palazzi et al. (2018)
	Baoding	102	10.31 (0.06–40.9) pg/mL	8.63 pg/mL	25th percentile, 5.13 pg/mL 75th percentile, 12.6 pg/mL			
	Dalian	102	6.20 (0.06–29.7) pg/mL	5.8 pg/mL	25th percentile, 2.27 pg/mL 75th percentile, 8.86 pg/mL			
1-Hydroxy-anthracene in hair	Baoding	102	1.44 (0.24–10.9) pg/mL	0.92	25th percentile, 0.68 pg/mL 75th percentile, 1.48 pg/mL			
	Dalian	102	1.22 (0.24-3.71) pg/mL	1.05	25th percentile, 0.9 pg/mL 75th percentile, 1.47 pg/mL			
Anthracene in hair	Nanjing and Ningbo, general population, China, 2018	NR	NR	NR	NR	GC-MS (NR)	No hair dying in the past 2 yr. The first 12 cm of hair was used.	Wang et al. (2020)
	By region:							
	Nanjing (urban)	33	209 (ND–271) pg/mg	208 pg/mg				
	Ningbo (rural)	33	65.7 (ND–98.6) pg/mg	77.8 pg/mg				
	By region and smoking:							
	Nanjing							
	Smokers	9	270.9 pg/mg					
	Non-smokers	12	48.9 pg/mg					

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in hair	Ningbo							Wang et al. (2020) (cont.)
	Smokers	9	20.6 pg/mg					
	Non-smokers	12	NR					
Anthracene in hair	Kanazawa, Japan, general population (age, 21–47 yr, 6 females and 14 males), date NR	20		NR	NR	HPLC-FLD (1.6 pg/injection)	Statistically significant differences between smokers and non-smokers.	Toriba et al. (2003)
	Smokers		8.2 (2.9–22.6) pg/mg hair					
	Non-smokers		3.5 (0.8–8.7) pg/mg hair					
Anthracene in forensic samples:	Murcia, Spain, cadavers (age, 29–80 yr), date NR			NR	NR	GC-MS (0.050 ng/g)		Pastor-Belda et al. (2019)
Brain		8 (87.5% of detection)	0.256 (0–0.601) ng/g					
Liver		8 (62.5%)	0.145 (0–0.597) ng/g					
Lung		8 (75%)	0.214 (0–0.469) ng/g					
Kidney		8 (62.5%)	0.152 (0–0.491) ng/g					
Heart		8 (75%)	0.178 (0–0.332) ng/g					
Fat		8 (62.5%)	2.774 (0–19.093) ng/g					
Spleen		8 (100%)	0.069 (0–0.176) ng/g					

Table 1.10 (continued)

Compound or metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Analytical method (LOD)	Comments	Reference
Anthracene in lung tissue	Moldavia, Romania, pulmonary cancer patients (mean age, 59.48 yr), 2008–2009	31			NR	HPLC-UV-FLD (NR)	Smokers (90%) and non-smokers (10%), mean level of 30 cigarettes per day.	Cioroiu et al. (2013)
	By location:							
	Urban donors	16	4.83 (0.12–30.59) ng/g wet tissue	2.72 ng/g wet tissue				
	Rural donors	15	1.89 (0.08–15.27) ng/g tissue	0.77 ng/g wet tissue				
	By blood type:							
	Group A	6	3.28 (0.15–12.84) ng/g wet tissue	0.81 ng/g wet tissue				
	Group O	7	4.41 (0.49–15.27) ng/g wet tissue	2.44 ng/g wet tissue				
	Group B	17	3.01 (0.08–30.59) ng/g wet tissue	0.79 ng/g wet tissue				
Anthracene in bronchoalveolar lavage fluid	Harbin, China, patients with chronic obstructive pulmonary disease (age, > 40 yr), 2017–2018			NR	NR	GC-MS (NR)	Non-smoking and non-secondhand smoking. Statistically higher levels of anthracene in the group with high risk of $PM_{2.5}$ inhalation.	Che et al. (2020)
	High-risk group of fine PM inhalation	13	0.78 ng/mL					
	Low-risk group of fine PM inhalation	19	0.35–0.40 ng/mL					

GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; h, hour(s); HPLC-DAD-FLD, high-performance liquid chromatography-diode array-fluorescence detection; HPLC-FLD, high-performance liquid chromatography-fluorescence detection; HPLC-UV-FLD, high-performance liquid chromatography method with ultraviolet and fluorescence detectors; IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; ND, not detected; NR, not reported; $PM_{2.5}$, particulate matter with diameter ≤ 2.5 μm; PAH, polycyclic aromatic hydrocarbon; ppb, parts per billion; wk, week(s); yr, year(s).

1-Hydroxyanthracene in hair samples was additionally analysed to assess total exposure to anthracene in women from areas with different pollution levels and the same trend was observed; there were significantly higher concentrations in the hair samples collected from women living in the most polluted city (Palazzi et al., 2018).

Anthracene was also one of the low-molecular-weight PAHs that predominated in forensic biological samples (brain, liver, lung, kidney, heart, adipose tissue, and spleen) retrieved during autopsies in Spain (Pastor-Belda et al., 2019); mean tissue levels ranged from 0.069 ng/g (range, 0–0.176 ng/g) in the spleen to 2.774 ng/g (range, 0–19.093 ng/g) in fat (Table 1.10).

In one study, urine and blood were simultaneously collected from children. Concentrations of anthracene were 0.55 µg/dL (range, 0.51–0.62 µg/dL) [5.5 ng/mL (range, 5.1–6.2 ng/mL)] in the urine and 0.06 µg/dL (range, 0.051–0.64 µg/dL) [0.6 ng/mL (range, 0.51–6.4 ng/mL)] in the blood (Wirnkor et al., 2019).

Exposure of pregnant women (smokers or non-smokers, living in industrial or urban areas) and their fetuses and infants have been characterized through the analysis of venous maternal blood and serum, placental tissues, cord blood and serum, and breast milk (Table 1.10). Anthracene can cross the human placental barrier. Mean levels detected in placental tissue ranged from 0.027 µg/L (Agarwal et al., 2018) to 33.26 ppb [33.26 µg/kg] (Singh et al., 2008a) in two studies in India. Mean values measured in maternal venous serum ranged from < 0.02 µg/L in China to 2.9 µg/L (range, < 0.76–18.3 µg/L) in the Islamic Republic of Iran. In a study in Spain, anthracene was detected in only 1 out of 447 umbilical cord blood samples (0.181 ng/mL) (Cabrera-Rodríguez et al., 2019) [the LOD was not reported]. In a study from Iran (Khalili Doroodzani et al., 2021), the mean concentration of anthracene in cord serum was 3.21 µg/L (range, < 0.76–14.5 µg/L) (Table 1.10).

Anthracene can be transferred to breast milk. Reported levels of anthracene in human breast milk varied between < 0.04 ng/g fat (< 0.001 ng/mL, the LOD) in the USA (Kim et al., 2008), 0.05 ng/mL (median, range, 0.044–2.04 ng/mL) in Portugal (Oliveira et al., 2020) and 39.07 µg/kg (mean, range, not detected to 89.55 µg/kg) in Italy (Santonicola et al., 2017).

Overall, smoking habits, living or working in industrial or urban polluted areas, and cooking using biomass as fuel were the main exposure determinants of anthracene concentrations measured in the general population.

1.5 Regulations and guidelines

Available regulations and guidelines are reported in Table 1.11.

An occupational limit value specifically aimed to regulate the exposure of workers to anthracene was not available to the Working Group.

The EU Water Environmental Quality Standards Directive 2008/105/EC set an emission limit value of 0.1 µg/L for anthracene emissions into inland surface waters and other surface waters (encompassing rivers and lakes, and related to artificial or heavily modified water bodies) as an annual average (European Parliament and Council, 2008).

The US EPA suggested the reference dose 0.3 mg/kg day as the level not expected to cause adverse effects to human health when drinking-water or eating seafood from contaminated surface water (US EPA, 2015).

In the EU, anthracene is in the list of Hazardous Substances for Purposes of Council Directive 90/385/EEC on active implantable medical devices, 20 July 1990 (European Council, 1990), amended by Directive 2007/47/EC, 21 September 2007 (European Parliament and Council, 2007). This list contains hazardous substances particularly as regards Article 3 and Annex I, concerning essential requirements

Table 1.11 Regulatory and guideline values for anthracene

Regulatory or guideline value	Country, location	Description, applicability	Value and units	Comments	Reference
Environment					
Water	EU	Water environmental quality standards directive for anthracene emission into surface water (encompassing rivers and lakes and related artificial or heavily modified water bodies)	0.1 µg/L		European Parliament and Council (2013)
Soil	Canada	Environmental Quality Guidelines (Soil Quality Guidelines) for anthracene	2.5 µg/kg	Contact exposure – agricultural and residential land use soil.	CCME (2010)
			32 µg/kg	Contact exposure – commercial and industrial land use soil.	
			61.5 µg/kg	Soil and food ingestion for the protection of livestock and wildlife.	
Food and drinking-water					
Food and water	USA	Reference dose for human health when drinking water or eating seafood from contaminated surface water	0.3 mg/kg day	Level not expected to cause adverse effects to human health.	US EPA (2015)

EU, European Union

and the choice of materials used, particularly as regards toxicity aspects (ECHA, 2024).

In the EU and in the scope of the REACH regulations, anthracene is specified in the REACH candidate list of substances of very high concern for authorization (IFA, 2023). Also in the EU, and in the context of Directive 2012/18/EU (Seveso III), the substance is subject to the hazard categories of the Hazardous Incident Ordinance (E1 hazardous to the aquatic environment, category acute 1 or chronic 1).

1.6 Quality of exposure assessment in key mechanistic studies in humans

The Working Group reviewed five cross-sectional studies and one case–control study that contributed to mechanistic evidence related to exposure to anthracene (see Sections 4.2.1 and 4.2.4). Four studies were focused on relatively small groups of workers (roofers, steel-foundry workers, and coke-oven workers in a steel plant; fewer than 100 exposed individuals) and involved exposure to multiple PAHs, including anthracene (Herbert et al., 1990; Hanchi et al., 2017; Jeng et al., 2022, 2023). One cross-sectional study concerned exposure to PAHs from indoor (biofuel cooking, smoking) and environmental (traffic) sources among children aged 2–10 years in India (Singh et al., 2008c). The case–control study, also from India, concerned the reproductive health effects (risk of preterm delivery) of environmental (indoors, outdoors and via food consumption) exposure to PAHs, which were measured in placental tissue (Agarwal et al., 2018).

Details on the exposure assessments employed in the five studies are summarized in

Table S1.12 (see Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/631).

In the three studies performed in occupational settings, the different assessment methods used included air monitoring, skin wipes, and urinary biomonitoring.

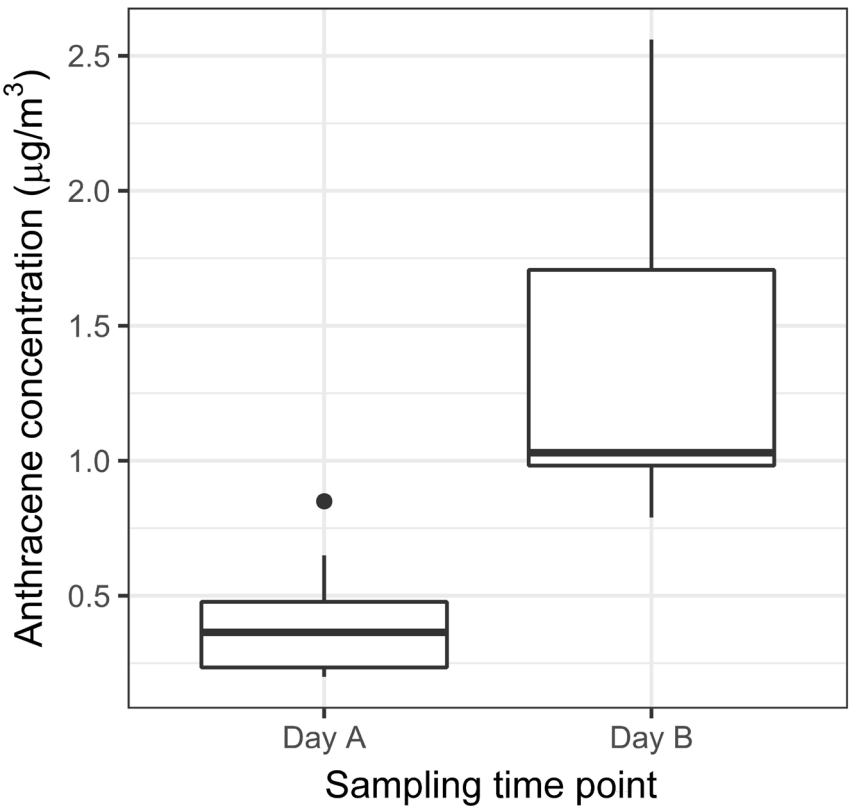
In a small study in the USA in 12 exposed workers involved in the removal of sequential sections of an old (coal-tar) pitch roof followed by replacement with a new asphalt (bitumen) roof (Herbert et al., 1990), inhalation and dermal exposure to anthracene, fluoranthene, pyrene, benzanthracene, B[a]P, benzo[*b*]-fluoranthene, benzo[*ghi*]perylene and benzo[*k*]-fluoranthene was measured on 2 days during 1 week. Inhalation samples were taken on two occasions (Thursday and Monday), and pre- and post-shift dermal wipes were only taken on the second measurement day (Monday). On the subsequent day (Tuesday), blood samples were collected for DNA adduct assessment. The reference group comprised employees of Mount Sinai Medical Center or patients from the Mount Sinai Occupational Health Clinical Center who were matched on age, sex, and smoking status. The inhalation and dermal samples were analysed according to standard NIOSH method 5506 (NIOSH, 1998) and inhalation measurements involved collection of PAHs on a filter and with a sorbent tube (for the more volatile PAHs).

The repeated measurement design and reporting of the results of individual measurements (Table 1 in Herbert et al., 1990) allowed the assessment of exposure variability. As expected among outdoor workers, temporal (day-to-day) variability in exposure outweighed the difference in average exposure for anthracene (see Fig. 1.1) within this group of workers in the same location. Analysis of variance showed that 100% of the variability was due to day-to-day changes in exposure concentrations, and therefore the roofers could be considered a uniformly exposed group. The total variability was relatively low (geometric standard deviation, 1.40 and 1.24 for anthracene and total PAH, respectively). Although not collected repeatedly, the forehead wipe samples showed slightly higher total variability than did the inhalation samples. A statistically significant tenfold difference in total PAH concentrations on forehead skin between wipe samples taken before and after work was apparent; however, anthracene was not detected in the wipe samples, most probably because most of the anthracene would have been in the gaseous phase.

The study among coke-oven workers from a steel plant in Taiwan, China (Jeng et al., 2022, 2023) focused on sperm oxidative DNA damage and semen quality and associations with inhalation exposure to 16 PAHs. One study included 38 coke-oven workers and 24 controls (Jeng et al., 2022), and a second study included 31 topside-oven workers and 23 side-oven workers but no controls (Jeng et al., 2023). PAH samples were collected on filters and XAD-2 sorbent tubes and analysed by GC-MS. No assessment of dermal exposure to PAHs was conducted, although this is considered to be the major route of exposure in coke-oven workers (VanRooij et al., 1993).

Median exposure to anthracene of the workers was reported as 337 (median of log transformed values, 5.82), no units provided, and an interquartile range (IQR) of 296–380. Also, the IQRs for all other PAHs reported were similar and improbably small (see Table 3 in Jeng et al., 2022). [Given that the reported LODs ranged between 6.1 and 9.8 ng for the PAHs analysed, the Working Group assumed that the units were reported in ng/m^3.] In another study by the same authors (Jeng et al., 2023), presumably performed in the same steel plant, similar levels of exposure were reported, but again the reporting was of very poor quality given that the actual statistical parameters were not specified, but simply reported as 353.54 ± 93.33 ng/m^3 and 340.98 ± 66.58 ng/m^3 for top-side oven and

Fig. 1.1 Inhalation exposure to anthracene and total PAHs for 12 roofers on two measurement days



PAH, polycyclic aromatic hydrocarbon.
Created by the Working Group using data from Table 1 in Herbert et al. (1990).

side-oven workers, respectively. [Given that the study results were poorly reported and dermal exposure was not assessed, the Working Group considered that this study could not be confidently interpreted with regard to exposure to anthracene.]

Details of a study among electric steel-foundry workers in Tunisia (Hanchi et al., 2017) were found in a separate paper (Campo et al., 2016). The study focused on biological monitoring of exposure to PAHs via spot urine samples collected at the end of an 8-hour work shift. No repeated samples were collected, and no inhalation or dermal exposure measurements were conducted. The 16 US EPA two- to six–ring unmetabolized PAHs and eight hydroxylated PAH metabolites were analysed by GC-MS/MS and liquid chromatography-triple quadrupole tandem mass spectrometry (LC-MS/MS). Multiple linear regression models showed that job title was a significant determinant for several unmetabolized PAHs but not for anthracene. Urinary levels of unmetabolized anthracene were similar among the three exposure groups, with median values of 1.97 ng/L, 2.17 ng/L, and 2.58 ng/L for workers from the steel smelter workshop ($n = 30$), workers near the fuel furnaces ($n = 43$), and workers involved in a variety of different tasks away from the furnaces ($n = 20$), respectively (Campo et al., 2016). The exposure

assessment in the study resulted in very limited contrasts in exposure to anthracene. In a multivariable analysis of the biomarker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), measuring oxidative DNA damage, the job title grouping and the actual biomonitoring values were used simultaneously as independent variables, but this would most probably have resulted in multicollinearity.

In a case–control study on risk of preterm delivery and exposure to PAHs via the environment and food in India, placental levels of PAHs were measured at the time of delivery (Agarwal et al., 2018). Placental samples were analysed for the 16 US EPA-classified PAHs using gas chromatography-flame ionization detection (GC-FID). For further confirmation, a few samples from each batch were randomly analysed by GC-MS. Levels of individual PAHs and several PAH sum measures were presented for cases and controls, and means and standard deviations were reported. No insight was given into the actual distributions of the individual PAH concentrations.

The cross-sectional study in 50 children (aged 2–10 years) concerned exposure to PAHs, as assessed in blood, and associations with indices of oxidative stress. Concentrations of naphthalene, acenaphthylene, phenanthrene, anthracene, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, and B[a]P were measured in blood samples collected on one occasion at the time of enrolment (Singh et al., 2008c). Blood levels of PAHs were determined by HPLC-FLD-UV. The results of anthracene were not reported, which precludes the assessment of an association between anthracene exposure and the outcome.

[The Working Group noted that all studies dealt with simultaneous exposure to multiple PAHs. Only one of the three industrial studies measured both inhalation and dermal exposure (Herbert et al., 1990). The reporting of exposure results was very poor in the coke-oven workers study (Jeng et al., 2022).]

2. Cancer in Humans

No epidemiological studies were available that investigated the association between exposure to anthracene and cancer in humans. One case report of a cancer of the scrotum after dermal exposure to anthracene oil was considered uninformative by the Working Group, since anthracene oil is a mixture containing anthracene and other two- to four-ring aromatic compounds obtained from coal tar (Weissenbach, 1952).

3. Cancer in Experimental Animals

In previous evaluations, the *IARC Monographs* programme concluded that there was *inadequate evidence* in experimental animals regarding the carcinogenicity of anthracene (IARC, 1987, 2010).

Studies of carcinogenicity with anthracene in experimental animals are summarized in Table 3.1 and Table 3.2.

3.1 Mouse

See Table 3.1.

3.1.1 Oral administration (feed)

In a well-conducted chronic toxicity and carcinogenicity study that complied with Good Laboratory Practice (GLP) (JBRC, 1998; also reported by Takeda et al., 2022), groups of 50 male and 50 female Crj:BDF1 mice (age, 6 weeks) were treated with feed containing anthracene (purity, 99.8–99.9%) at 0, 3200, 8000, or 20 000 ppm (weight per weight, w/w) for males and 0, 8000, 20 000, or 50 000 ppm (w/w) for females, 7 days per week for 104 weeks. On the basis of feed consumption, the estimated doses were 0, 459, 1178, and 3076 mg/kg body weight (bw) per day for male mice at 0, 3200, 8000, and

Table 3.1 Studies of carcinogenicity in mice exposed to anthracene

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 (M) 6 wk 104 wk JBRC (1998)	Oral administration (feed) Purity, 99.8–99.9% Feed 0, 3200, 8000, 20 000 ppm (w/w), continuous dosing 49, 50, 50, 50 41, 41, 37, 42	Any tumour type: no significant increase in incidence		*Principal strengths*: well-conducted GLP study; covered most of the lifespan; multiple dose study; males and females used; adequate number of animals per group; adequate duration of exposure and observation.
Full carcinogenicity Mouse, Crj:BDF_1 (F) 6 wk 104 wk JBRC (1998)	Oral administration (feed) Purity, 99.8–99.9% Feed 0, 8000, 20 000, 50 000 ppm (w/w), continuous dosing 50, 50, 50, 50 35, 31, 34, 34	*Liver* Hepatocellular adenoma 2/50 (4%), 3/50 (6%), 6/50 (12%), 20/50* (40%) Hepatocellular carcinoma 0/50, 2/50 (4%), 5/50* (10%), 12/50** (24%)	$P < 0.0001$, Peto prevalence method test $P < 0.0001$, Cochran–Armitage test; NC, Peto standard method test, Peto combined analysis test *$P = 0.0003$, Fisher exact test $P < 0.0001$, Peto prevalence method test $P < 0.0001$, Peto combined analysis $P < 0.0001$, Cochran–Armitage test; NS, Peto standard method test *$P = 0.036$, Fisher exact test; **$P = 0.0005$, Fisher exact test	*Principal strengths*: well-conducted GLP study; covered most of the lifespan; multiple dose study; males and females used; adequate number of animals per group; adequate duration of exposure and observation. *Historical controls*: hepatocellular adenoma, 45/899 (5.0%); range, 2–10%; hepatocellular carcinoma, 20/899 (2.2%); range, 0–8%; hepatocellular adenoma or carcinoma (combined), 65/899 (7.2%); range, 2–12%; histiocytic sarcoma (all organs), 199/899 (22.1%); range, 12–30%.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 (F) 6 wk 104 wk JBRC (1998) (cont.)		Hepatocellular adenoma or carcinoma (combined) 2/50 (4%), 5/50 (10%), 11/50* (22%), 26/50** (52%)	$P < 0.0001$, Peto prevalence method test $P < 0.0001$, Peto combined analysis test $P < 0.0001$, Cochran–Armitage test; NS, Peto standard method test *$P = 0.0170$, Fisher exact test **$P = 0.0001$, Fisher exact test	
		All organs (uterus, liver, subcutis, salivary gland, urinary bladder) Histiocytic sarcoma 6/50 (12%), 18/50 (36%)*, 11/50 (22%), 11/50 (22%)	*$P = 0.0222$, Fisher exact test	
Initiation–promotion (tested as initiator) Mouse, Crl:CD/1 (ICR)BR (F) 50–55 days 24 wk La Voie et al. (1985)	Skin application Purity, > 99% Acetone 0, 100 µg 100 µg in 100 µL acetone, once every other day for a total of 10 doses, followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, for 20 wk 20, 20 20, 20	*Skin* Tumours 2/20, 3/20	NS	*Principal limitations*: limited reporting; macroscopic evaluation of skin tumours only.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Initiation–promotion (tested as initiator) Mouse, CD-1 (F) 7 wk 32 wk Wislocki et al. (1982)	Skin application Purity, "sufficiently pure for use" Acetone 0, 400, 1000 nmol Single application of 400 or 1000 nmol in 200 µL of acetone, followed 1 wk later by promotion with 10 µg TPA, 2×/wk for 31 wk; mice were examined for skin papillomas, and tumours were counted when they were > 2 mm in diameter and present for 2 wk 30, 30, 30 29, 29, 29	*Skin (site of application)* Papilloma Tumour incidence: 13%, 11%, 21% Tumour multiplicity: 0.23, 0.18, 0.27	[Tumour incidence was reported as percentages. It was unclear how many tumour-bearing mice (numerator) or how many mice in total (denominator) were used for calculation of these percentages.]	*Principal limitations*: limited reporting; uncertainty regarding purity; uncertainty regarding the number of animals examined; macroscopic evaluation of skin tumours only.
Co-carcinogenicity Mouse, Skh: hairless-1 (M) 6 wk 38 wk Forbes et al. (1976)	Skin application Purity, 99% Methanol 4 µg/day, 5 days/wk, for 38 wk Daily skin application (in 40 µL of methanol solution, 0.1 g/L), 5 days/wk (Monday through Friday only), followed by 2 h of UV light after each application 24 NR	Skin: tumours Incidence, NR		*Principal limitations*: use of one sex only; limited reporting of histopathology. *Other comments*: tumour incidence was not reported although, on the basis of the final tumour prevalence, incidence did not seem to differ between the anthracene- and UV-treated group and the control group; the time to 50% prevalence of skin tumours did not differ significantly between anthracene treatment (28.2 wk) and methanol vehicle-control treatment (27.2 wk) by Wilcoxon rank-sum test statistics.

F, female; GLP, Good Laboratory Practice; h, hour(s); M, male; ppm, parts per million; NC, not calculable; NR, not reported; NS, not significant; TPA, 12-*O*-tetra-decanoylphorbol-13-acetate; UV, ultraviolet; wk, week(s); w/w, weight per weight.

20 000 ppm, respectively, and 0, 1459, 3711, and 9725 mg/kg bw per day for female mice at 0, 8000, 20 000, or 50 000 ppm, respectively. The survival rate for all dosed groups of males and females was similar to that of controls. At study termination, survival was 41/49, 41/50, 37/50, and 42/50 in males, for the groups at 0 (control), 3200, 8000, 20 000 ppm, respectively, and 35/50, 31/50, 34/50, and 34/50 in females, for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively. Body weights of the males at 20 000 ppm male and females at 50 000 ppm were significantly decreased throughout the administration period, compared with their respective controls. All mice underwent complete necropsy, and all organs and tissues were sampled for histopathology in all the animals.

In female mice, there was a significant positive trend ($P < 0.0001$, Peto trend test, prevalence method; $P < 0.0001$, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma – 2/50 (4%), 3/50 (6%), 6/50 (12%), and 20/50 (40%) for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – with the incidence being significantly increased at the highest dose ($P = 0.0003$, Fisher exact test), and exceeding the upper bound of the range observed for historical controls from the same laboratory – 45/899 (5.0%); range, 2–10%. [The Working Group noted that several Peto trend tests were conducted in this study, with the Peto test standard method being referred to as "death analysis", the Peto test prevalence method being referred to as "incidental tumour test", and the Peto test combined analysis being referred to as "death analysis plus incidental tumour test". A significant *P* value in any Peto test was considered relevant for the detection of treatment-related increases in tumour incidence.] There was a significant positive trend ($P < 0.0001$, Peto trend test, prevalence method and combined analysis; $P < 0.0001$, Cochran–Armitage trend test) in the incidence of hepatocellular carcinoma – 0/50, 2/50 (4%), 5/50 (10%), and 12/50 (24%) for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – with the incidence being significantly increased at the intermediate and highest dose ($P = 0.036$, Fisher exact test; $P = 0.0005$, Fisher exact test, respectively), and exceeding the upper bound of the range observed for historical controls from the same laboratory – 20/899 (2.2%); range, 0–8%. There was a significant positive trend ($P < 0.0001$, Peto trend test (prevalence method and combined analysis); $P < 0.0001$, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma or carcinoma (combined) – 2/50 (4%), 5/50 (10%), 11/50 (22%), and 26/50 (52%) for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – with the incidence being significantly increased at the intermediate and highest dose ($P = 0.017$, Fisher exact test; $P = 0.0001$, Fisher exact test, respectively), and exceeding the upper bound of the range observed in historical controls from the same laboratory – 65/899 (7.2%); range, 2–12%. The incidence of histiocytic sarcoma of all organs (uterus, liver, subcutis, salivary gland, and urinary bladder) – 6/50 (12%), 18/50 (36%), 11/50 (22%), and 11/50 (22%) for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – was significantly increased ($P = 0.0222$, Fisher exact test) at the lowest dose; and exceeded the upper bound of the range for historical controls from the same laboratory – 199/899 (22.1%); range, 12–30%.

In male mice, dietary administration of anthracene did not cause a significant increase in the incidence of any type of neoplasm.

Regarding the non-neoplastic lesions, anthracene caused hyaline droplet degeneration in superficial cells of the transitional epithelium of the urinary bladder in all treated groups of male and female mice. Significant treatment-related increases in the incidence of clear cell and basophilic cell foci in the liver of female mice were also observed (JBRC, 1998; also reported in Takeda et al., 2022). [The Working Group noted that this was a well-conducted GLP study that

had a duration of exposure and observation of most of the lifespan, used multiple dose groups, both sexes, and an adequate number of animals per group.]

3.1.2 Subcutaneous injection

A group of 40 male and female NMRI mice (age, 2 days) received a single subcutaneous injection of 71.3 µg (400 nmol) of anthracene (purity, 99.9%) dissolved in 50 µL of an aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20) (Platt et al., 1990). A control group of 49 male and female mice was treated with the solvent alone. After 40 weeks, all mice underwent necropsy and the lung tissues were analysed by histopathology. There was no increase in the incidence of lung tumours: 2/17 treated male mice developed lung adenoma compared with 1/14 male mice from the solvent control group, and 1/12 treated female mice developed lung adenoma compared with 1/19 female mice from the solvent control group. [The Working Group noted that this study was limited because the numbers of male mice and female mice at the start of treatment were not reported (40 was reported for males and females combined in the anthracene group and 49 was reported for males and females combined in the control group), the use of a single dose, only lung tissue was examined, necropsy observations were not reported for animals that did not survive to the end of the experiment, and histopathology was not reported. Therefore, this study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of 40–50 male and female C57BL mice [age not reported] were given a single subcutaneous injection of 5 mg of anthracene [purity not reported] dissolved in 0.5 mL of tricaprylin (Steiner, 1955). The number of mice surviving at 4 months was used to calculate tumour incidence. At study termination (between months 22 and 28), none of the anthracene-treated mice (0/26) developed sarcoma at the injection site. [The Working Group noted that the study was limited by the lack of untreated or solvent controls, the use of a single dose, the lack of information on anthracene purity, and the use of males and females in one group. Therefore, the study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.1.3 Intraperitoneal injection

A group of 20 male and female SPF Kun Ming mice [age not reported] were given 10 daily intraperitoneal injections of anthracene [purity not reported] at a dose of 50 mg/kg bw dissolved in dimethyl sulfoxide (DMSO) at 0.1 mL/g bw (Wang & Xue, 2015). Histological examinations were performed on the liver, kidney, stomach, and lung tissues, 3 months after exposure. There was a significant increase in the incidence of hepatocellular carcinoma (control, 0/20; anthracene, 5/18; $P < 0.05$, Fisher exact test). No tumours were observed in the kidney, stomach, or lung tissues in anthracene-treated or control animals. [The Working Group noted that this study was limited by the short duration, the combination of males and females in one group, the lack of information on anthracene purity, and the limited histological examination. Therefore, the study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of five male Swiss mice (age, 2.0–2.5 months) were given a single intraperitoneal injection of 25 mg of anthracene [purity not reported] in ~750 µL olive oil (Shubik & Della Porta, 1957). Five months after the injection, no tumours were observed in the four surviving mice in the group treated with anthracene or the four surviving mice treated with olive oil. [The Working Group noted that this study was limited by the short duration, the small number of animals used, the use of a single dose and a single

sex, the lack of information on anthracene purity, and the limited reporting on histological examination. The study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.1.4 Skin application

A group of 20 male C3H/HeJ mice (age, 8–10 weeks) were given skin applications of 0.1% anthracene (purity, 99.5%) in 50 µL of toluene solution twice per week for 104 weeks (Warshawsky et al., 1993). Fifty male C3H/HeJ mice were given toluene as the solvent control. Only tumours at the application site were examined. No skin tumours were observed in the anthracene-treated or control group (control, 0/39; anthracene, 0/14). [The Working Group noted that this study was limited by the use of a single dose and a single sex, and the limited reporting of the histological examination. The study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of five female Swiss mice [age not reported] received skin applications of anthracene [purity not reported] as a 10% solution in acetone, three times per week for up to 20 months (Wynder & Hoffmann, 1959). By 20 months, all five mice were dead, and no tumours were observed. [The Working Group noted that this study was limited by the lack of information on purity, the small number of animals used, the lack of information on the age of animals, the lack of control, and the use of a single unknown dose. Therefore, the study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of 41 albino mice [strain, sex, and age not specified] were treated with an unknown amount of anthracene [purity not reported], diluted in water, benzene, or sesame oil, by intrascapular skin painting (Pollia, 1939). The average lifespan of all mice in the study (including mice receiving chemicals other than anthracene) was 133 days after skin painting. At 10 months, none of the surviving mice that received anthracene had developed any skin tumours. [The Working Group noted that this study was limited by the lack of information on purity, the lack of information on the strain, sex, and age of animals, the lack of control, and the use of a single unknown dose. The study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

Two groups of 100 mice [strain, sex, and age not specified] were treated via skin application with 40% anthracene (a tar derivative, purity not reported) in either a lanolin suspension or an ether solution (Kennaway, 1924a, b). No tumours developed in the group receiving anthracene in the lanolin suspension, and one mouse from the group receiving anthracene in the ether solution developed a papilloma after 131 days. [The Working Group noted that this study was limited by the lack of information on purity and probable contamination by other PAHs in tar, the lack of information on the strain, sex, and age of the animals, the lack of control, and the use of a single unknown dose. Therefore, the study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.1.5 Initiation–promotion

A group of 20 female Crl:CD-1(ICR)BR mice (age, 50–55 days) received skin applications of 100 µg of anthracene (purity, > 99%) in 100 µL of acetone, once every other day, for a total of 10 applications (total dose, 1 g). Ten days after the last exposure to anthracene, the mice were given skin applications of 2.5 µg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 100 µL of acetone, three times per week, for 20 weeks (La Voie et al., 1985). The control group received acetone during the initiation phase and TPA during the promotion phase. The incidence

of skin tumours was 3/20 in the anthracene-initiated group, 2/20 in the control group, and 1/20 in a repeated control group. [The Working Group noted that this study was limited by the lack of survival data and the macroscopic evaluation of skin tumours only.]

Groups of 30 female CD-1 mice (age, 7 weeks) were given a single skin application of anthracene (purity reported as "sufficiently pure for use in the experiment") at doses of 400 and 1000 nmol in 200 µL acetone. One week after exposure to anthracene, the mice were given 10 µg of TPA in 200 µL of acetone, twice per week for 31 weeks (Wislocki et al., 1982). The control mice received acetone and TPA. The mice were examined for skin papillomas every other week. The incidence of skin papilloma was reported as follows: 13%, 11%, and 21% for the groups at 0 (control), 400, and 1000 nmol, respectively. Tumour multiplicity (number of tumours per mouse) was reported as follows: 0.23, 0.18, and 0.27 for the groups at 0 (control), 400, and 1000 nmol, respectively. [The Working Group noted that this study was limited by uncertainty regarding anthracene purity and the number of animals examined, and the macroscopic evaluation of skin tumours only.]

A group of 30 female CD-1 mice (age, 8 weeks) were given a single skin application of 10 µmol of anthracene (purity reported as "purified by preparative thin-layer chromatography") in benzene. One week later, the mice were treated with 5 µmol of TPA, twice per week for 34 weeks (Scribner, 1973). A control group of 30 mice were treated with 10 µmol of TPA only, twice per week for 34 weeks. [The Working Group noted that it was unclear whether the controls received solvent or no treatment during the initiation phase.] At week 35, 30, and 28 of the animals from the control group and the anthracene-initiated group, respectively, were alive. The incidence of skin papilloma was 0/30 in the control group and 4/28 in the anthracene-initiated group [P = 0.0483, Fisher exact test]. [The Working Group noted that this study was limited by uncertainty regarding anthracene purity, the use of benzene as a solvent, and the use of different doses of the promoter in the controls and the anthracene-treated animals. Therefore, the study was judged to be inadequate for the evaluation of anthracene in experimental animals.]

A group of 20 strain "S" mice [sex and age not reported] were given 20 skin applications of 0.5% anthracene [purity not reported] in acetone solution, twice per day (with a 30-minute interval), 3 days per week, for a total dose of 30 mg (Salaman & Roe, 1956). Starting on day 25 after the first application of anthracene, the mice were treated with 18 applications of 0.3 mL of croton oil in acetone solution (one application of 0.17% solution, two applications of 0.085% solution, and 15 further applications of 0.17% solution at weekly intervals). A group of 20 control mice received only croton oil according to the same protocol. The incidence of skin papilloma was 4/19 in the control and 3/17 in the anthracene-initiated group. [The Working Group noted that this study was limited by the lack of information on purity, and the lack of information on the sex and age of animals. Therefore, the study was judged to be inadequate for the evaluation of anthracene in experimental animals.]

3.1.6 Co-exposures

Two groups of 87 white mice [sex, strain, and age not reported] were given 10% anthracene (reported as "pure anthracene") ointment in olive oil and Vaseline (petroleum jelly) by skin application, immediately followed by ultraviolet (UV) radiation 5 hours per session, three sessions per week (Heller, 1950). One group received anthracene and long-wave UV-A, while the other group received anthracene, long-wave UV-A, and visible light. In the group that received anthracene and long-wave UV-A, carcinomas were first observed after 39 days and reached 100% incidence after 5–6 weeks, whereas no tumours were observed in the control group [group size not reported]

at up to 12 months. In the group that received anthracene, long-wave UV-A, and visible light, 100% of mice developed carcinoma within 7–8 weeks, whereas no tumours were observed in the control group (5 mice) at up to 12 months. [The Working Group noted that this study was limited by the use of a single unknown dose, and uncertainty regarding anthracene purity and the number of animals for each group. Therefore, the study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

Three groups of mice [strain, sex, and age not reported] were given 5% anthracene (reported as "pure anthracene") in a mixture of olive oil and petroleum jelly by skin application to the ears, three times per week for 9–11 months (Miescher, 1942). Two of the groups also received UV light irradiation 2 hours after anthracene treatment (one group received irradiation for 40 or 60 minutes, another group for 90 minutes), three times per week. A fourth group received UV light only, three times per week, for 40 minutes per session during the first 12 treatments then increased to 60 minutes per session afterwards. At the end of the experiments, high mortality rates were reported for all four groups. No skin tumours were observed (anthracene only, 0/44; anthracene and 40 or 60 minutes of UV, 0/44; anthracene and 90 minutes of UV, 0/100; 40 or 60 minutes of UV only, 0/44). [The Working Group noted that this study was limited by the lack of information on strain, the age and sex of the animals, the use of a single unknown dose, and uncertainty regarding purity. Therefore, the study was judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of 24 male Skh: hairless-1 mice (age, 6 weeks) were given 40 µL of anthracene (purity, 99%) dissolved in methanol (concentration, 0.1 g/L; daily dose, 4 µg) by skin application, followed by 2 hours of UV radiation (Forbes et al., 1976). Anthracene and UV exposure were given once daily, 5 days per week for 38 weeks. Control mice received methanol by skin application followed by UV radiation once daily, 5 days per week for 38 weeks. The time to 50% prevalence of skin tumours was not significantly different between the groups treated with anthracene (28.2 weeks) or methanol vehicle control (27.2 weeks), according to Wilcoxon rank-sum test statistics. Tumour incidence was not reported but did not seem to differ between the anthracene- and UV-treated group and the control group on the basis of final tumour prevalence. [The Working Group noted that this study was limited by the use of one sex only and the limited reporting of histopathology.]

3.2 Rat

See Table 3.2.

3.2.1 Oral administration (feed)

In a well-conducted chronic toxicity and carcinogenicity study that complied with GLP (JBRC, 1998; also reported by Takeda et al., 2022), groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were treated with feed containing anthracene (purity, 99.8–99.9%) at 0 (control), 8000, 20 000, or 50 000 ppm (w/w), 7 days per week for 104 weeks. On the basis of feed consumption, the estimated dose for male rats was 0, 377, 957, and 2483 mg/kg bw per day, and for female rats was 0, 468, 1209, and 3122 mg/kg bw per day, for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively. Survival analysis did not show differences between the anthracene-treated groups and the respective control groups. At study termination, survival was: 33/50, 43/50, 43/50, and 38/50 in males, and 40/50, 40/50, 40/50, and 37/50 in females, for the groups at 0 (control), 8000, 20 000, and 50 000 ppm, respectively. Body weights in all groups of treated females were significantly decreased throughout the study, compared with controls. Body weights

Table 3.2 Studies of carcinogenicity in rats and rabbits exposed to anthracene

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (M) 6 wk 104 wk JBRC (1998)	Oral administration (feed) Purity, 99.8–99.9% Feed 0, 8000, 20 000, 50 000 ppm (w/w), continuous dosing 50, 50, 50, 50 33, 43, 43, 38	*Liver* Hepatocellular adenoma 0/50, 4/50 (8%), 9/50* (18%), 9/50* (18%)	$P = 0.0032$, Peto prevalence method test $P = 0.0056$, Cochran–Armitage test; NC, Peto standard method test, Peto combined analysis test *$P = 0.0029$, Fisher exact test	*Principal strengths*: well-conducted GLP study; covered most of the lifespan; multiple dose study; males and females used. *Historical controls*: hepatocellular adenoma, 18/949 (1.9%); range, 0–6%; hepatocellular carcinoma, 3/949 (0.3%); range, 0–2%; hepatocellular adenoma or carcinoma (combined), 21/949 (2.2%); range, 0–6%; transitional cell papilloma or carcinoma (combined) of the urinary bladder, 1/949 (0.1%); range, 0–2%; transitional cell carcinoma of the urinary bladder, 0/949; transitional cell papilloma of the urinary bladder, 1/949 (0.1%); range, 0–2%.
		Hepatocellular carcinoma 0/50, 0/50, 5/50* (10%), 5/50* (10%)	$P = 0.0158$, Peto prevalence method test $P = 0.0056$, Peto combined analysis test $P = 0.0081$, Cochran–Armitage test; NS, Peto standard method test *$P = 0.0360$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined) 0/50, 4/50 (8%), 13/50* (26%), 13/50* (26%)	$P = 0.0003$, Peto prevalence method test $P = 0.0001$, Peto combined analysis test $P = 0.0002$, Cochran–Armitage test; NS, Peto standard method test *$P = 0.0003$, Fisher exact test	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (M) 6 wk 104 wk JBRC (1998) (cont.)		*Urinary bladder* Transitional cell papilloma 0/50, 0/50, 2/50 (4%), 0/50 Transitional cell carcinoma 0/50, 1/50 (2%), 4/50 (8%), 3/50 (6%) Transitional cell papilloma or carcinoma (combined) 0/50, 1/50 (2%), 6/50 (12%)*, 3/50 (6%)	NS NS *P = 0.0190, Fisher exact test	
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 104 wk JBRC (1998)	Oral administration (feed) Purity, 99.8–99.9% Feed 0, 8000, 20 000, 50 000 ppm (w/w), continuous dosing 50, 50, 50, 50 40, 40, 40, 37	*Kidney* Renal cell adenoma 0/50, 3/50 (6%), 6/50 (12%)*, 4/50 (8%) Renal cell carcinoma 0/50, 0/50, 0/50, 1/50 (2%) Renal cell adenoma or carcinoma (combined) 0/50, 3/50, 6/50*, 5/50**	*P = 0.0190, Fisher exact test NS P = 0.0441, Peto combined analysis test; NS, Peto standard method test; NS, Peto prevalence method test *P = 0.0190, Fisher exact test **P = 0.0360, Fisher exact test	*Principal strengths*: well-conducted GLP study; covered most of the lifespan; multiple dose study; males and females used. *Historical controls*: renal cell adenoma or carcinoma (combined), 1/948 (0.1%); range, 0–2%; renal cell carcinoma, 0/948; renal cell adenoma, 1/948 (0.1%); range, 0–2%; transitional cell carcinoma of the urinary bladder, 0/948; endometrial stromal sarcoma of the uterus, 3/948 (0.3%); range, 0–2%; fibroadenoma of the mammary gland, 92/948 (9.7%); range, 0–20%; adenoma of the mammary gland, 44/948 (4.6%); range, 0–18%; adenoma or fibroadenoma (combined) of the mammary gland, 136/948 (14.3%); range, 4–24%; adenocarcinoma of the mammary gland, 14/948 (1.5%); range, 0–6%; adenocarcinoma, adenoma or fibroadenoma (combined) of the mammary gland, 150/948 (15.8%); range, 4–26%.

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 104 wk JBRC (1998) (cont.)		*Urinary bladder* Transitional cell carcinoma 0/50, 2/50, 3/50, 2/50	NS	
		Uterus Endometrial stromal sarcoma 0/50, 0/50, 0/50, 3/50 (6%)	$P = 0.0164$, Peto standard method test $P = 0.0032$, Peto combined analysis test $P = 0.0051$, Cochran–Armitage test; NS, Peto prevalence method test	
		Mammary gland Adenoma 0/50, 2/50 (4%), 0/50, 1/50 (2%)	NS	
		Fibroadenoma 3/50, 2/50, 3/50, 9/50 (18%)	$P = 0.0172$, Peto standard method test $P = 0.0299$, Peto prevalence method test $P = 0.0057$, Peto combined analysis test $P = 0.0094$, Cochran–Armitage test	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 104 wk JBRC (1998) (cont.)		Adenoma or fibroadenoma (combined) 3/50, 4/50, 3/50, 10/50 (20%) Adenocarcinoma 0/50, 0/50, 2/50 (4%), 0/50 Adenocarcinoma, adenoma or fibroadenoma (combined) 3/50, 4/50, 5/50, 10/50 (20%)	$P = 0.0221$, Peto prevalence method test $P = 0.0077$, Peto combined analysis test $P = 0.0122$, Cochran–Armitage test; NS, Peto standard method test NS $P = 0.0242$, Peto prevalence method test $P = 0.0098$, Peto combined analysis test $P = 0.0157$, Cochran–Armitage test; NS, Peto standard method test	
Carcinogenicity with other modifying factor Rat, Sprague-Dawley Hras128 (M) 7 wk 20 wk Ohnishi et al. (2007)	Oral administration (gavage) Purity, > 99.9% Olive oil 0, 200 mg/kg bw 1×/wk for 3 wk 12, 7 12, 7	*Mammary gland* Adenoma or adenocarcinoma (combined) 0/12, 3/7*	*$P < 0.05$, Dunnett *t*-test *[$P = 0.0361$, Fisher exact test]	*Principal limitations*: limited number of dose groups; limited exposure duration; small number of rats per group. *Other comments*: inadequate use of χ^2 test for statistical analysis of tumour incidence.

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Carcinogenicity with other modifying factors Rat, Sprague-Dawley Hras128 (F) 7 wk 12 wk Ohnishi et al. (2007)	Oral administration (gavage) Purity, > 99.9% Olive oil 0, 200 mg/kg bw 1×/wk for 3 wk 11, 7 11, 7	*Mammary gland* Adenoma or adenocarcinoma (combined) 2/11, 4/7	**P* < 0.05, Dunnett *t*-test [NS, Fisher exact test]	*Principal limitations*: limited number of dose groups; limited exposure duration; small number of rats per group. *Other comments*: inadequate use of χ^2 test for statistical analysis of tumour incidence.
Full carcinogenicity Rat, Sprague-Dawley (M) 7 wk 20 wk Ohnishi et al. (2007)	Oral administration (gavage) Purity, > 99.9% Olive oil 0, 200 mg/kg bw 1×/wk for 3 wk 10, 6 10, 6	*Mammary gland* Adenoma or adenocarcinoma (combined) 0/10, 0/6	NA	*Principal limitations*: limited number of dose groups; limited exposure duration; small number of rats per group.
Full carcinogenicity Rat, Sprague-Dawley (F) 7 wk 12 wk Ohnishi et al. (2007)	Oral administration (gavage) Purity, > 99.9% Olive oil 0, 200 mg/kg bw 1×/wk for 3 wk 12, 8 12, 8	*Mammary gland* Adenoma or adenocarcinoma (combined) 0/12, 0/8	NA	*Principal limitations*: limited number of dose groups; limited exposure duration; small number of rats per group.

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NA, not applicable; NC, not calculable; NS, not significant; ppm, parts per million; wk, week(s); w/w, weight per weight.

of males in all treatment groups were significantly decreased compared with controls at certain time points. Food consumption of all treated males and females was similar to that of their respective controls. All rats underwent complete necropsy. All organs and tissues were sampled for histopathology in all the animals.

In male rats, there was a significant positive trend (P = 0.0032, Peto trend test, prevalence method; P = 0.0056, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma – 0/50, 4/50 (8%), 9/50 (18%), 9/50 (18%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – and the incidence was significantly increased at 20 000 and 50 000 ppm (P = 0.0029, Fisher exact test), exceeding the upper bound of the range observed in historical controls from the same laboratory – 18/949 (1.9%); range, 0–6%. [The Working Group noted that several Peto trend tests were conducted in this study, with the Peto test standard method being referred to as "death analysis", the Peto test prevalence method being referred to as "incidental tumour test", and the Peto test combined analysis being referred to as "death analysis plus incidental tumour test". A significant P value in any Peto test was considered relevant for the detection of treatment-related increases in tumour incidence.] There was a significant positive trend (P = 0.0158, Peto trend test, prevalence method; and P = 0.0056, Peto trend test, combined analysis; P = 0.0081, Cochran–Armitage trend test) in the incidence of hepatocellular carcinoma – 0/50, 0/50, 5/50 (10%), and 5/50 (10%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – and the incidence was significantly increased at 20 000 and 50 000 ppm (P = 0.036, Fisher exact test), exceeding the upper bound of the range observed in historical controls from the same laboratory – 3/949 (0.3%); range, 0–2%. There was a significant positive trend (P = 0.0003, Peto trend test, prevalence method; P = 0.0001, Peto trend test, combined analysis; P = 0.0002, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma or carcinoma (combined) – 0/50, 4/50 (8%), 13/50 (26%), and 13/50 (26%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – and the incidence was significantly increased at 20 000 and 50 000 ppm (P = 0.0003, Fisher exact test). The incidence in all treated groups exceeded the upper bound of the range observed in historical controls from the same laboratory – 21/949 (2.2%); range, 0–6%. The incidence of transitional cell papilloma or transitional cell carcinoma (combined) of the urinary bladder – 0/50, 1/50 (2%), 6/50 (12%), and 3/50 (6%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – was significantly increased (P = 0.0190, Fisher exact test) at 20 000 ppm. The incidence at 20 000 and 50 000 ppm exceeded the upper bound of the range observed in historical controls from the same laboratory – 1/949 (0.1%); range 0–2%. The incidence of transitional cell papilloma at 20 000 and 50 000 ppm exceeded the upper bound of the range observed in historical controls from the same laboratory – 1/949 (0.1%); range, 0–2%. The incidence of transitional cell carcinoma at 20 000 ppm exceeded the incidence observed in historical controls from the same laboratory (0/949).

In female rats, the incidence of renal cell adenoma – 0/50, 3/50 (6%), 6/50 (12%), and 4/50 (8%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – was significantly increased (P = 0.0190, Fisher exact test) in the group at the intermediate dose, exceeding the upper bound of the range observed in historical controls from the same laboratory – 1/948 (0.1%); range, 0–2%. There was a significant positive trend (P = 0.0441, Peto trend test, combined analysis) in the incidence of renal cell adenoma or renal cell carcinoma (combined) of the kidney – 0/50, 3/50 (6%), 6/50 (12%), 5/50 (10%)) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – and the incidence was significantly increased at 20 000 (P = 0.0190, Fisher exact test) and 50 000 ppm (P = 0.0360, Fisher exact test). The incidence in all treated groups exceeded the upper bound of

the range observed in historical controls from the same laboratory – 1/948 (0.1%); range, 0–2%. There was a significant positive trend ($P = 0.0164$, Peto trend test, standard method; $P = 0.0032$, Peto trend test, combined analysis; $P = 0.0051$, Cochran–Armitage trend test) in the incidence of endometrial stromal sarcoma of the uterus – 0/50, 0/50, 0/50, and 3/50 (6%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively – and the incidence at the highest dose exceeded the upper bound of the range observed in historical controls from the same laboratory – 3/948 (0.3%); range, 0–2%. There was a significant positive trend ($P = 0.0172$, Peto trend test, standard method; $P = 0.0299$, Peto trend test, prevalence method; $P = 0.0057$, Peto trend test, combined analysis; $P = 0.0094$, Cochran–Armitage trend test) in the incidence of fibroadenoma of the mammary gland – 3/50 (6%), 2/50 (4%), 3/50 (6%), 9/50 (18%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively. There was a significant positive trend ($P = 0.0221$, Peto trend test, prevalence method; $P = 0.0077$, Peto trend test, combined analysis); $P = 0.0122$, Cochran–Armitage trend test) in the incidence of adenoma or fibroadenoma (combined) of the mammary gland – 3/50 (6%), 4/50 (8%), 3/50 (6%), and 10/50 (20%) at 0 (control), 8000, 20 000, and 50 000 ppm, respectively. [The Working Group noted that mammary gland adenoma and mammary gland fibroadenoma should not be combined, because they are thought to arise from different parts of the mammary gland (see Brix et al., 2010). The only exception might occur when an adenoma or carcinoma arises from a fibroadenoma, and then it should be combined with other adenomas and carcinomas of the mammary gland. The conditions for this exception were not reported for the study by JBRC (1998). Therefore, the Working Group did not consider combination of adenomas and fibroadenomas of the mammary gland, or of adenomas, fibroadenomas, and adenocarcinomas of the mammary gland, to be appropriate for the detection of increases in tumour incidence.] The incidence of transitional cell carcinoma of the urinary bladder – 0/50, 2/50 (4%), 3/50 (6%), and 2/50 (4%) – at all dose levels exceeded the incidence observed in historical controls (0/949) in this laboratory.

Regarding the non-neoplastic lesions, in both males and females, anthracene caused an increased incidence of clear cell foci and acidophilic foci in the liver at all dose levels. In the kidney, the incidence of eosinophilic droplets in proximal tubules was increased at all dose levels in both males and females, and the incidence of atypical tubule hyperplasia was increased at all dose levels in females (JBRC, 1998; also reported by Takeda et al., 2022). [The Working Group noted that this was a well-conducted GLP study, with a duration of most of the lifespan, using multiple dose groups, both sexes, an adequate number of animals per group, and an adequate duration of exposure and observation.]

In a study of BDI and BDIII rats [sex and age not reported], anthracene (reported as "pure") was administered orally (in the feed) at a dose of 4.5 g in oil (not otherwise specified) for 91 weeks (5 mg/day then 15 mg/day, 6 days per week) (Druckrey & Schmähl, 1955; Schmähl, 1955). At the end of the study (median survival, 700 days), 1/28 rats had liver sarcoma and 1/28 rats had uterine adenocarcinoma. [The Working Group noted that this study was limited by the incomplete details regarding post mortem examination and histopathology, the lack of information on sex and age, the lack of details on anthracene purity, and the lack of controls. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.2.2 Oral administration (gavage)

Anthracene was evaluated in a medium-term study using transgenic Hras128 rats (human c-Ha-*ras* proto-oncogene as the transgene) and non-transgenic rats (Ohnishi et al., 2007). These

rats were generated by pronucleus injection of Sprague-Dawley female rat embryos and maintained as a heterozygous line by breeding transgenic and non-transgenic offspring to produce transgenic and non-transgenic (wildtype) littermates, as described by Asamoto et al. (2000) and cited by Ohnishi et al. (2007). Groups of male and female, transgenic and non-transgenic littermates (age, 7 weeks) (number at start not reported) were treated with three consecutive weekly intragastric doses of anthracene (purity, 99.9%) at a dose of 200 mg/kg bw, dissolved in olive oil. Control rats were treated with olive oil alone (Ohnishi et al., 2007). After 12 weeks (females) or 20 weeks (males), all transgenic and non-transgenic rats underwent necropsy to determine the presence of mammary tumours, which were analysed by histopathology.

There was a significant increase ($P < 0.05$, Dunnett *t*-test; [$P < 0.05$, Fisher exact test]) in the incidence of mammary adenoma or adenocarcinoma (combined) in male transgenic rats (controls, 0/12; anthracene-treated, 3/7). In female transgenic rats, the incidence of mammary adenoma or adenocarcinoma (combined) was 2/11 for controls, and 4/7 for the anthracene-treated group. [The Working Group noted that the effect of anthracene treatment on tumour incidence in male rats was statistically significant according to the Fisher exact test, and reportedly statistically significant according to the chi-squared test. However, it was not statistically significant in female rats according to either the chi-squared or the Fisher exact test, although it was reported in the publication as statistically significant by the chi-squared test.] Anthracene administered by oral gavage did not cause any mammary tumours (adenomas or adenocarcinomas, combined) in non-transgenic male or female rats. [The Working Group noted the use of a transgenic rat model carrying the c-Ha-*ras* proto-oncogene as the transgene, the small and unbalanced group sizes used, the lack of survival and body-weight data, and the use of a single dose level. The Working Group also noted that the Fisher exact test was more relevant than the chi-squared test for tumour incidence. The Working Group further noted that a comparison of the group means for mammary tumour multiplicity (tumours/rat) and incidence data indicated that each of the rats in the anthracene-treated and control groups had ≤ 1 tumour, therefore analysis of multiplicity data was inappropriate for these data.]

3.2.3 Subcutaneous injection

A group of 10 rats [strain and sex not reported] were treated with 1 mg of anthracene in 2 mL of aqueous colloidal solution [purity not reported], injected into alternating subcutaneous and intraperitoneal sites, once per week for 103 weeks (Boyland & Burrows, 1935). No control group was reported. Sites of injection were evaluated for tumours at necropsy. Only 2/10 of the treated rats were alive at 18 months. No tumours were observed. [The Working Group noted the small group size, lack of controls, lack of information on anthracene purity, age, and sex, and incomplete experimental details. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of five Wistar rats [sex not reported] (age, 6–8 weeks) were treated with 5 mg of anthracene (purity not reported) in sesame oil by subcutaneous injection of 0.5 mL in the right flank once per week for 6–7 weeks (Pollia, 1941). No tumours were observed at the injection site in 4 of 5 treated mice surviving for 10 months. [The Working Group noted the small number of treated animals, the lack of a control group, the lack of information on anthracene purity, the lack of histopathological examination, and the limited duration because of the poor condition of the treated animals. Therefore, the Working Group judged this study to be inadequate for the

evaluation of the carcinogenicity of anthracene in experimental animals.]

In a study of 10 BDI and BDIII rats [sex and age not reported], anthracene [purity not reported] was administered by subcutaneous injection at a dose of 20 mg in oil (not otherwise specified), once per week for 33 weeks (Druckrey & Schmähl, 1955; Schmähl, 1955). After being followed for a lifetime, 1/9 rats had myxosarcoma and 4/9 rats had fibroma at the site of injection. [The Working Group noted the small number of animals and the lack of information on age and sex, the lack of controls and the lack of information on anthracene purity. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.2.4 Intraperitoneal injection

In a study of 10 BDI and BDIII rats (sex and age not reported), anthracene (purity not reported) was administered by intraperitoneal injection at a dose of 20 mg in oil, once per week for 33 weeks (Schmähl, 1955). After being followed for a lifetime, 1/10 rats had a spindle cell sarcoma at the site of injection. [The Working Group noted the small number of animals and the lack of information on age and sex, the lack of controls, and the lack of information on anthracene purity. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.2.5 Implantation in the liver and lung

A group of 60 female Osborne-Mendel rats (age, 3–6 months) were treated with anthracene [purity not reported] at a dose of 0.5 mg, formulated in pellets each composed of 0.05 mL of bees wax:tricaprylin (1:1), and administered by one-time surgical implantation in the lower left lung (Stanton et al., 1972). Controls were implanted with pellets that were identical except that they lacked anthracene. No lung tumours were observed at necropsy in 28 rats examined at 43–55 weeks post-implantation (23 additional rats were still alive at 120 weeks and were not examined). [The Working Group noted the lack of necropsy data for nearly half of the treated animals, and the lack of information on anthracene purity. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

A group of 11 male rats of the Holtzman strain (age reported as "weanling") were treated with anthracene [purity not reported] at a dose of 61–78 mg administered as a single pellet by surgical implantation in the liver (Aterman, 1987). Control rats received a similar pellet composed of cholesterol. Survival was similar in anthracene-treated rats (490–631 days) and control rats (496–563 days). In anthracene-treated rats, 1/11 developed a fibrosarcoma that was found in a different non-implanted lobe of the liver. In control rats, 0/11 developed any tumour of the liver. [The Working Group noted the lack of information on anthracene purity, the use of one sex only, and the small number of animals used. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.3 Rabbit

See Table 3.2.

A group of nine rabbits [strain, sex, and age not reported] were given a single injection of anthracene pellets [purity not reported] at a dose of 10, 12, or 20 mg into the brain (cerebrum or cerebellum), or 4 or 5 mg into the eye (Russell, 1947). Survival of 20–54 months (after brain injection) or 54 months (after eye injection) was reported. No tumours were observed by histological examination of the injection site

(brain, 0/7; eye, 0/2). [The Working Group noted the small number of animals used, the lack of controls, the lack of information on age, sex and strain, and the lack of information on anthracene purity. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.]

3.4 Evidence synthesis for cancer in experimental animals

The carcinogenicity of anthracene has been assessed in one well-conducted GLP study in male and female Crj:BDF_1 mice treated by oral administration (in the feed) (JBRC, 1998; also reported in Takeda et al., 2022), and in one well-conducted GLP study in male and female F344/DuCrj rats (JBRC, 1998; also reported in Takeda et al., 2022) treated by oral administration (in the feed). The carcinogenicity of anthracene has also been evaluated in studies that did not comply with GLP. Specifically, there were studies of oral administration (feed) in BDI and BDIII rats [sex not reported] (Druckrey & Schmähl, 1955; Schmähl, 1955), oral administration (gavage) in male and female transgenic Hras128 and non-transgenic Sprague-Dawley rats (Ohnishi et al., 2007); in male and female C57BL mice (Steiner, 1955), in male and female NMRI mice (Platt et al., 1990), in rats [strain and sex not reported] (Boyland & Burrows, 1935), in Wistar rats [sex not reported] (Pollia, 1941), and in BDI and BDIII rats [sex not reported] (Druckrey & Schmähl, 1955; Schmähl, 1955) treated by subcutaneous injection; in male and female SPF Kun Ming mice (Wang & Xue, 2015), male Swiss mice (Shubik & Della Porta, 1957), and BDI and BDIII rats [sex not reported] (Schmähl, 1955) treated by intraperitoneal injection; in male C3H/HeJ mice (Warshawsky et al., 1993), female Swiss mice (Wynder & Hoffmann, 1959), albino mice [sex and strain not reported] (Pollia, 1939), and mice [strain and sex not reported] (Kennaway, 1924a, b) treated by skin application; in female Osborne-Mendel rats treated by implantation in the lung (Stanton et al., 1972); in male rats of the Holtzman strain treated by implantation in the liver (Aterman, 1987); and in rabbits [strain and sex not reported] treated by injection of anthracene pellets into the brain or the eye (Russell, 1947). In addition, four initiation–promotion studies in female Crl:CD/1 (ICR) BR mice (La Voie et al., 1985), female CD-1 mice (Scribner, 1973; Wislocki et al., 1982), and strain "S" mice [sex not reported] (Salaman & Roe, 1956); and co-exposure studies in mice [sex and strain not reported] (Miescher, 1942; Heller, 1950), and in male Skh: hairless-1 mice (Forbes et al., 1976) were available.

In the dietary study that complied with GLP in male and female Crj:BDF1 mice (JBRC, 1998; also reported in Takeda et al., 2022), a significant positive trend in the incidence of hepatocellular adenoma was observed in females, and the incidence was significantly increased at the highest dose. There was a significant positive trend in the incidence of hepatocellular carcinoma, and the incidence was significantly increased at the intermediate and highest dose. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined), and the incidence was significantly increased at the intermediate and highest dose. The incidence of histiocytic sarcoma of all organs was significantly increased at the lowest dose. In male mice, dietary administration of anthracene did not cause a significant increase in the incidence of any type of neoplasm (JBRC, 1998; also reported in Takeda et al., 2022).

In the dietary study that complied with GLP in male and female F344/DuCrj rats (JBRC, 1998; also reported in Takeda et al., 2022), there was a significant positive trend in the incidence of hepatocellular adenoma in males, and the incidence was significantly increased at the intermediate and highest dose. There was a significant positive trend in the incidence of hepatocellular

carcinoma, and the incidence was significantly increased at the intermediate and highest dose. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined), and the incidence was significantly increased at the intermediate and highest dose. The incidence of transitional cell papilloma or transitional cell carcinoma (combined) of the urinary bladder was significantly increased at the intermediate dose. In female rats, the incidence of renal cell adenoma was significantly increased at the intermediate dose. A significant positive trend in the incidence of renal cell adenoma or renal cell carcinoma (combined) of the kidney was observed, and the incidence was significantly increased at the intermediate and highest dose. There was a significant positive trend in the incidence of endometrial stromal sarcoma of the uterus. There was a significant positive trend in the incidence of fibroadenoma of the mammary gland (JBRC, 1998; also reported in Takeda et al., 2022).

In the study in male and female transgenic Hras128 and non-transgenic Sprague-Dawley rats treated by oral administration (gavage) (Ohnishi et al., 2007), there was a significant increase in the incidence of mammary adenoma or adenocarcinoma (combined) in male but not female transgenic rats. No mammary tumours (adenoma or adenocarcinoma) were observed in non-transgenic male or female rats.

Studies in BDI and BDIII rats [sex not reported] treated by oral administration (feed) (Druckrey & Schmähl, 1955; Schmähl, 1955); in male and female C57BL mice (Steiner, 1955), male and female NMRI mice (Platt et al., 1990), rats [sex not reported] (Boyland & Burrows, 1935), Wistar rats [sex not reported] (Pollia, 1941), and BDI and BDIII rats [sex not reported] (Druckrey & Schmähl, 1955; Schmähl, 1955) treated by subcutaneous injection; in male and female SPF Kun Ming mice (Wang & Xue, 2015), male Swiss mice (Shubik & Della Porta, 1957), and BDI and BDIII rats [sex not reported] (Schmähl, 1955) treated by intraperitoneal injection; in male C3H/HeJ mice (Warshawsky et al., 1993), female Swiss mice (Wynder & Hoffmann, 1959), albino mice [sex not reported] (Pollia, 1939), and mice [sex not reported] (Kennaway, 1924a, b) treated by skin application; in female Osborne-Mendel rats treated by implantation in the lung (Stanton et al., 1972); in male rats of the Holtzman strain treated by implantation in the liver (Aterman, 1987); and in rabbits [sex not reported] treated by injection of anthracene pellets into the brain or eye (Russell, 1947), the two initiation–promotion studies in female CD-1 mice (Scribner, 1973) and in strain "S" mice (Salaman & Roe, 1956); and the two co-exposure studies in mice [sex not reported] treated by skin application (Miescher, 1942; Heller, 1950) were judged to be inadequate for the evaluation of the carcinogenicity of anthracene in experimental animals.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption, distribution, and excretion

(a) Humans

The absorption, distribution, and excretion of anthracene administered via different routes of exposure were reported in several studies in humans.

Storer et al. (1984) demonstrated that anthracene can penetrate the skin. Five non-smoking adult volunteers, presenting no skin alterations or diseases, were exposed topically to a petrolatum solution of coal tar (85 mL) containing anthracene (190 µg/mL) for two periods of 8 hours. In the blood samples collected, no anthracene was detected before exposure, but anthracene levels were 4.7, 0.1, 0.0, 0.5, and 1.6 ng/mL (mean,

1.4 ng/mL) in each of the five volunteers after the second period of exposure (Storer et al., 1984).

Indirect evidence for the absorption and distribution of anthracene in the human body was provided by measuring levels of PAHs in the urine. In several groups of road pavers and construction workers, anthracene concentrations in the urine were between < 2 and 30 ng/L (medians, 2–9 ng/L) (Campo et al., 2006b). In a similar study, urinary concentrations of anthracene in 55 coke-oven workers in Poland were between 7 and 899 ng/mL (median, 49 ng/mL) (Rossella et al., 2009). More data on urinary concentrations of anthracene assessed in various groups of workers are presented in Section 1.4.2. Anthracene was also determined in several human tissues, e.g. blood (Singh et al., 2008b; Drwal et al., 2017; Wirnkor et al., 2019), serum (Al-Daghri et al., 2014), placenta (Singh et al., 2008a; Drwal et al., 2017; Agarwal et al., 2018), maternal milk (Oliveira et al., 2020), hair (Palazzi et al., 2018; Wang et al., 2020), cord blood (Cabrera-Rodríguez et al., 2019), bronchoalveolar lavage fluid (BALF) (Che et al., 2020), lung cancer tissue (Cioroiu et al., 2013), and brain, liver and spleen (Pastor-Belda et al., 2019). [The Working Group considered the above evidence as proof of the wide distribution of anthracene within the human body.]

(b) Experimental systems

The absorption of anthracene was also assessed in the skin, lungs, and gastrointestinal tract in various experimental systems in various species.

Percutaneous absorption of [^{14}C]-anthracene was investigated in female Sprague-Dawley rats (Yang et al., 1986). In the in vivo study, a single topical dose of 9.3 µg/cm^2 was applied on the rat dorsal area and radioactivity was measured in the urine, faeces, and tissues. In vitro absorption was assessed by measuring radioactivity penetration of a similar dose of anthracene through excised dorsal skin preparations (consisting of stratum corneum, epidermis, and the top portion of the dermis, total thickness of 350 µm) into the receptor fluid of the diffusion cell. Within 6 days after application, 52.3% and 55.9% of the radioactivity administered in vivo and in vitro, respectively, was absorbed, demonstrating the penetration of anthracene through rat skin. Nevertheless, the increase in accumulated radioactivity in the rat excreta proceeded notably slower than did the in vitro penetration of anthracene through excised skin into the receptor fluid. The delay was caused by distribution, metabolism, and elimination in the rat body. Of the anthracene applied in vivo, 29.1% and 21.9% was recovered in the urine and faeces, respectively, during 6 days. At termination of the experiment, 1.3% of anthracene remained in the tissues, mainly the liver and kidney.

Percutaneous absorption of anthracene was also assessed in blood-infused pig ears onto which coal tar containing anthracene (3.7%) was applied at a dose of 11 mg/cm^2 per 24 cm^2. The mean cumulative absorption of anthracene was 138 pmol/cm^2 as measured for 200 minutes after the application of coal tar. [The Working Group noted that this amount accounted for 0.006% of the applied amount of anthracene.] Mean absorption flux at 200 minutes was 110 pmol/hour per cm^2 (VanRooij et al., 1995).

The absorption of anthracene after the administration by gavage of contaminated soil (about 0.5 g) or a solution of the pure compound in sunflower oil (2 mL, containing 2.1 µg of anthracene, 17.5 µg of pyrene, and 7.6 µg of B[a]P) was studied in male Lewis rats. In whole blood, two maximum concentrations (at 1–2 hours and at 3–4 hours) were observed, perhaps because of enterohepatic recycling. Plasma concentration–time curves (area under the curve, AUC) for anthracene during the first 7 hours after administration of the soil sample or of the pure anthracene solution differed in a ratio of 3:1, respectively. No significant difference was observed in the total amount of anthracene excreted in the faeces after treatment with soil

(about 0.5% of the administered dose) or the pure anthracene solution (about 0.4% of the administered dose) (van Schooten et al., 1997).

The bioavailability of anthracene after oral administration was assessed in female Landrace cross pigs treated daily, for 7 days, with artificial soil, solid food (a dough ball), or corn oil, all spiked with anthracene, or with a certified reference material (CRM) soil (natural clay soil collected from a PAH-contaminated area in the USA) (Peters et al., 2015). Several blood samples were collected within 1–24 hours post-exposure on days 1 and 7. For the CRM soil, peak serum concentrations of anthracene occurred at 2 hours post-exposure, followed by a second peak at 8 hours, accounted for by enterohepatic cycling. By far the highest bioavailability of anthracene was observed after ingestion of CRM soil, and then corn oil; no absorption was detected from spiked food and soil. In a separate group of pigs, uptake of anthracene to the stomach, jejunum, ileum, proximal colon, and liver was studied after ingestion of CRM soil. Anthracene was detected in these tissues 4 hours post-exposure at concentrations that continued to increase until 12 hours post-exposure, indicating that these tissues were acting as a repository for anthracene after systemic circulation in the blood and before elimination.

Elimination of [^{14}C]-anthracene from the lung was reported in female F344/Crl rats treated with [^{14}C]-anthracene (1 nmol; in 10% DMSO) by intratracheal instillation. Biphasic clearance was observed: a rapid component with a half-time of 0.1 hour, resulting in removal of 99.7% of the radiolabel, followed by a slower component with a half-time of 25.6 hours (Bond et al., 1985). [The Working Group noted that the 10% DMSO concentration used was too high and was potentially cytotoxic.]

In an in vitro model using full-thickness monkey skin (abdomen of *Cercopithecus aetiops*), percutaneous absorption of anthracene was characterized by a permeability constant (K_p) of $3.44 \pm 3.09 \times 10^{-3}$ cm/hour (Sartorelli et al., 1998).

The uptake of [^{14}C]-anthracene was also measured in a rabbit ocular lens model in vitro by using direct incubation in glutathione-buffered Ringer medium. A concentration ratio of 10:1 (lens:medium) was observed after a 24-hour incubation (Tang-Liu et al., 1992).

4.1.2 Metabolism

(a) Humans

Data on the metabolism of anthracene in humans were sparse. In the majority of studies assessing levels of urinary metabolites after environmental or occupational exposures to various PAH mixtures, anthracene metabolites were neither measured nor reviewed. In one study assessing concentrations of PAHs and a wide array of their metabolites in hair samples from women living in urban environments, 1-hydroxyanthracene concentrations were determined (mean, 1.33 pg/mg), but no correlation was observed with anthracene concentrations (mean, 8.26 ng/g) (Palazzi et al., 2018).

(b) Experimental systems

(i) Non-human mammals in vivo

In urine from rats or rabbits fed a diet containing 5% anthracene, two isomers of free dihydroxydihydroanthracene were found (Boyland & Levi, 1935). The corresponding glucuronic acid conjugates were identified as (+)-1,2-dihydro-1,2-dihydroxy-1-anthracene-glucuronic acid in the rabbit urine and its (–)-analogue in the rat urine. Whereas the concentration of the glucuronic acid conjugate predominated over that of the free compound in the rabbit urine, a much lower concentration of the conjugate was excreted in the rat urine (Boyland & Levi, 1936). [The Working Group noted that the rat and rabbit strains were not reported.]

A systematic analysis of the metabolism of anthracene was carried out in male Chester Beatty rats fed a diet containing 5% anthracene (Sims, 1964). Urine was fractionated into four main fractions that were further analysed to identify metabolic products as follows: (±)-*trans*-1,2-dihydro-1,2-dihydroxyanthracene (major product), 1,2-dihydroxyanthracene (partly conjugated with sulfuric and glucuronic acids), *trans*-9,10-dihydro-9,10-dihydroxyanthracene, and 9,10-dihydroxyanthracene. The latter is further hydroxylated to 2,9,10-trihydroxyanthracene, anthrone, and 1,2-dihydro-2-hydroxy-1-anthrylmercapturic acid (Fig. 4.1). [The Working Group noted that none of the above compound structures was assigned rigorously.]

Three monohydroxy metabolites of anthracene (isomerism not assigned) were tentatively identified for the first time in the urine of female Long-Evans rats treated by gavage with a mixture of PAHs at repeated doses equal to or greater than 0.01–0.20 mg/kg bw. The highest rate of urinary excretion occurred at 6–8 hours post-exposure. Metabolite concentrations strongly correlated (R^2, 0.86–0.95) with the level of exposure (Grova et al., 2017b). Moreover, three tetrahydroxyanthracenes (isomerism not assigned) were found in rat hair from the same experiment (Grova et al., 2017a).

Methylation of anthracene at positions 9 and 10 and subsequent oxidation of these methyl groups was investigated in male Sprague-Dawley rats dosed subcutaneously with anthracene (0.4 µmol in 200 µL of sesame oil) (Myers et al., 1988). The animals were killed 24 hours after dosing, and tissues in contact with anthracene were removed and further extracted with ethyl acetate. HPLC analysis of the dorsal subcutaneous tissue extract revealed the presence of the metabolites 9-methylanthracene (30%), 9,10-dimethylanthracene (7.2%), 9-formylanthracene (36.7%), 9-hydroxymethylanthracene (9.6%), 9-hydroxymethyl-10-methylanthracene (12.2%), and 9,10-dihydroxymethylanthracene (4.2%).

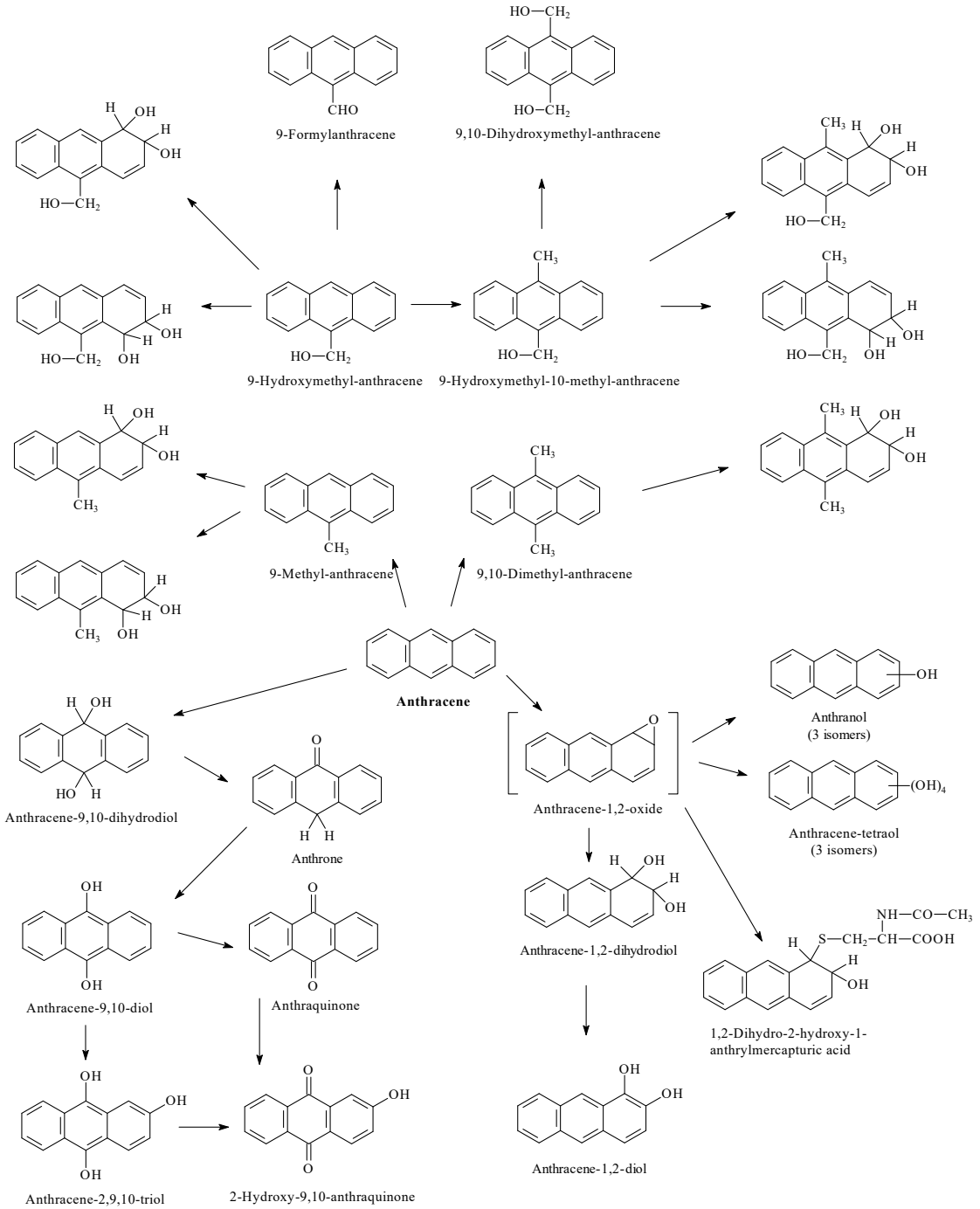
(ii) Non-human mammalian cells in vivo

In vitro metabolism of [^{14}C]-anthracene with liver microsomes from untreated and phenobarbital- or 3-methylcholanthrene (3-MC)-treated Sprague-Dawley rats resulted in retention of > 95% of radioactivity as *trans*-1,2-dihydro-1,2-dihydroxyanthracene; metabolites at the 9,10-position of anthracene were not detected (Akhtar et al., 1979). [The Working Group noted that if metabolites at the 9,10-position of anthracene were to be found in vivo, they might not be of hepatic origin.] The absence of anthrols in the incubate was accounted for by the high activity of epoxide hydrolase in hydrating anthracene 1,2-oxide, combined with the unusual stability of this arene oxide towards isomerization to phenols.

As the formation of 1,2-dihydroxyanthracene metabolites proceeds via the respective anthracene 1,2-oxides, the absolute configuration of the latter has been studied using [^{3}H]-anthracene in the liver monooxygenase system containing cytochrome P450c (CYPc) (current name, CYP1A1) obtained from immature Long-Evans rats treated with Aroclor 1254. In this system, the (+)-(1*R*,2*S*)-oxide form was found to predominate (van Bladeren et al., 1984). On the other hand, oxidation of anthracene in a reconstituted system containing cytochrome P450b (CYPb) (current name, CYP2B1) resulted in predominant formation of (–)-(1*S*,2*R*)-oxide (van Bladeren et al., 1985). In contrast to in rats, incubation of [^{14}C]-anthracene with microsomal fractions from the liver and aural epidermis of male New Zealand White rabbits resulted predominantly in the formation of the anthracene dihydrodiol 1*S*,2*S* enantiomer (Hall & Grover, 1987).

In addition to the studies with rats in vivo (Myers et al., 1988), methylation of anthracene at positions 9 and 10 and subsequent oxidation of these methyl groups was also investigated

Fig. 4.1 Metabolic scheme for anthracene



The scheme is based on studies in rats in vivo and rat liver microsomes in vitro. In this scheme, the enantiomeric composition of the metabolic products (although available for several) has been disregarded. Some hydroxyderivatives are excreted in the urine as glucuronic acid and sulfuric acid conjugates (not shown).
Compiled by the Working Group from Sims (1964), Akhtar et al. (1979), Lamparczyk et al. (1984), La Voie et al. (1985), Myers et al. (1988), and Grova et al. (2017a, b).

in rat liver cytosol preparations fortified with *S*-adenosyl-L-methionine. Identical metabolite patterns in both types of study were observed. The sum of the above metabolites produced in vitro for 1 hour accounted for 10–20% of the initial amount of anthracene. [The Working Group noted that the presence of methyl substituents at positions 9 and 10 of anthracene is associated with mutagenic potency and tumour-initiating activity, which is strongest in 9,10-dimethylanthracene (La Voie et al., 1985).] The biotransformation of anthracene itself and its 9-methyl and 9,10-dimethyl derivatives in rat liver microsomes was studied by La Voie et al. (1985). In this study, the major metabolites of anthracene were 1,2-dihydro-1,2-dihydroxydiol and anthraquinone. The metabolites of 9-methylanthracene were identified as *trans*-1,2-dihydro-1,2-dihydroxy- and *trans*-3,4-dihydro-3,4-dihydroxy-9-methylanthracenes, and 9-hydroxymethylanthracene and its 1,2- or 3,4-dihydrodiols. The microsomal metabolism of 9,10-dimethylanthracene resulted in *trans*-1,2-dihydro-1,2-dihydroxy-9,10-dimethylanthracene (major product), 9-hydroxymethyl-10-methylanthracene, and its 1,2- or 3,4-dihydrodiols. In a similar type of study with rat liver microsomes, 9,10-dihydroxymethylanthracene was identified in addition to the above metabolites (Lamparczyk et al., 1984).

Considering that numerous mouse pulmonary toxicants (including naphthalene) are metabolized by CYP2F2, the potential involvement of this isozyme in the metabolism of anthracene was investigated by Shultz and co-workers (Shultz et al., 2001). Incubation of mouse liver microsomes or recombinant mouse CYP2F2 in presence of glutathione/glutathione transferase with anthracene or [^{14}C]-anthracene, respectively, resulted in both cases in the formation of a tentative glutathione conjugate, most likely hydroxy-glutathionyl-1,2-dihydroanthracene, thus confirming formation of the reactive anthracene-1,2-epoxide as mediated by CYP2F2 (Shultz et al., 2001). [The Working Group, however, noted that metabolic formation of diol epoxides, known to be associated with mutagenicity and carcinogenicity of some PAHs, was not reported for anthracene.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether anthracene is electrophilic or can be metabolically activated to an electrophile; is genotoxic; induces epigenetic alterations; induces oxidative stress; induces chronic inflammation; is immunosuppressive; modulates receptor-mediated effects; causes immortalization; or alters cell proliferation, cell death, or nutrient supply. No data were available for the evaluation of whether anthracene alters DNA or causes genomic instability.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

One study in exposed workers was available to the Working Group. The ^{32}P-postlabelling assay was used to measure adducts in DNA isolated from peripheral leukocytes from roofers exposed to a mixture of PAHs and from non-occupationally exposed participants matched on age, sex, and smoking status (Herbert et al., 1990). [The Working Group considered this study to be of little relevance and excluded it because of the small sample size and the lack of analysis of anthracene-induced DNA adducts.]

(ii) Human primary cells

Only one study examined the potential for anthracene to form DNA adducts in human cells. Exposure of freshly isolated human lymphocytes from healthy volunteers to anthracene at 30 µM

did not result in the formation of DNA adducts (Gupta et al., 1988). Numerous carcinogenic PAHs were assessed in this study and compared with anthracene. No metabolic activation was used. [The Working Group noted that the lack of a metabolic activation system might result in underestimation of the formation of DNA adducts. Only one dose was tested, and the replicates were not listed.]

(b) Experimental systems

(i) Non-human mammals in vivo

Tetra-hydroxylated anthracene (0.01–0.8 mg/kg, three times per week for 90 days) released by the hydrolysis of DNA adducts was used as an indirect measure of DNA adduct formation and as a biomarker of exposure in a study that measured tetra-hydroxylated anthracene in the hair of Long-Evans rats exposed orally to a mixture of PAHs including anthracene (Grova et al., 2017a) (see also Section 4.1). Tetra-hydroxylated PAH metabolites, but not anthracene-specific metabolites, were identified by GC-MS/MS. [A reference standard for anthracene was not included in the study, and the exact dose that led to a detectable level of tetra-hydroxylated anthracene metabolites was unclear (between 0.01 and 0.8 mg/kg, orally, for 90 days); thus, the Working Group considered the results as weak evidence for electrophilic activity of anthracene and only an indirect measure of DNA adduct formation.]

(ii) Acellular systems

See Table 4.1.

PAHs are normally metabolized (e.g. by CYPs and/or epoxide hydrolases), and the resulting oxy-derivative products can actively bind to different biomolecules. [Since PAH–DNA adducts and PAH–protein adducts directly affect cellular functions, the Working Group also considered studies investigating anthracene–DNA or anthracene–protein adduct formation in acellular systems.] Two studies measured the formation of human serum albumin adducts after exposure to anthracene, with conflicting results. At a single concentration (60 μM) of anthracene, UV irradiation induced the formation of human serum albumin adducts via covalent crosslinking (Sinha & Chignell, 1983). By contrast, in a more recent study, no significant adduct formation was observed at low concentrations of anthracene (highest concentration used, 2.8 μM) by albumin fluorescence quenching, with sufficient replicates (Skupińska et al., 2006). [The Working Group noted that some PAHs could bind to specific tryptophan residues on albumin, which could result in the quenching of the albumin fluorescence, and that some oxy-derivatives of anthracene have quenching effects, suggesting that the specific location of residues on the anthracene structure is critical. The Working Group also noted that numerous studies (e.g. Kochevar et al., 1982; Oris et al., 1984) have demonstrated the phototoxic potential of anthracene in various species, for example, erythema in the skin of guinea-pigs, and toxicity in daphnia (*Daphnia pulex*) and mosquito larvae (*Aedes aegypti*), supporting the results observed by Sinha & Chignell (1983).] In another study (Sun et al., 2020), a change in DNA structure caused by groove binding by anthracene was demonstrated at a large range of anthracene concentrations (0–10 μM) and validated using molecular modelling. The Working Group considered that the available evidence for electrophilicity was inconclusive.]

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human primary cells

See Table 4.2.

In a study in lymphocytes, anthracene caused a significant increase in DNA strand breaks at several time points, as measured by the phosphorylated histone γH2AX test (Bhargava et al.,

Table 4.1 Reactions of anthracene with proteins or DNA in acellular systems

End-point	Detection method	Results[a]	Concentration (LEC or HIC)	Comments	Reference
HSA protein concentration	Albumin fluorescence quenching	–	Dose range, 0.003–2.793 µM; 5 measurements		Skupińska et al. (2006)
HSA-crosslinking adducts	Binding of [^{14}C]-anthracene to HSA in the presence of light	(+)	60 µM	No justification of dose or dose–response relation.	Sinha & Chignell (1983)
DNA adduct, ct-DNA	UV-induced covalent DNA binding	(+)	56 µM	No justification of dose or dose–response relation.	Sinha & Chignell (1983)
Change in DNA structure by groove binding, ct-DNA	Resonance light scattering spectra	+	Large dose range, 0–10 µM	Validated using molecular modelling.	Sun et al. (2020)

ct-DNA, calf thymus DNA; HIC, highest ineffective concentration; HSA, human serum albumin; LEC, lowest effective concentration; UV, ultraviolet.

[a] +, positive; –, negative; (+) positive in a study of limited quality.

Table 4.2 Genetic and related effects of anthracene in human cells in vitro

End-point	Assay	Tissue, cells	Results[a] Without metabolic activation	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks	γH2AX	Primary peripheral blood lymphocytes	(+)	10 µM [1.78 µg/mL]	Study quality was poor; only one dose; cell species not reported but assumed to be human. Significant at 30 minutes, 3 h and 6 h; comparable to B[a]P; $n = 3$ but not clear whether replicated more than once.	Bhargava et al. (2020)
DNA strand breaks	Comet assay	Human keratinocyte (HaCaT) cells Human lymphocyte (A3) cells	– –	10 µM [1.78 µg/mL]	Study quality was good; doses, 0–10 µM; no detail about method or replicates.	Hu et al. (2009)
DNA repair	Unscheduled DNA synthesis	Human cervical cancer (HeLa S3) cells in the presence or absence of a rat liver mixed-function oxidase preparation	–	100 µg/mL	Study quality was good; dose range, 0.1–100 µg/mL, for 2.5 h. No response in presence or absence of metabolic activation.	Martin et al. (1978); Martin & McDermid (1981)
Micronucleus formation	Cytokinesis blocked cells (binucleated cells)	Human lymphoblastoid $TK^{+/-}$ (MCL-5) cells	–	10 µg/mL	Study quality: good; 0–10 µg/mL doses for 24 h followed by cytochalasin B; micronuclei/500 cells; 2 replicates; vehicle control.	Crofton-Sleigh et al. (1993)
Mutagenesis	Selection of DT-resistant mutants	Human embryo skin and muscle explant epithelial-like (EUE) cells	–	10 µM [0.178 µg/mL]	Study quality: poor; only one dose for chronic toxicity, 10^{-7} M (continuous exposure); acute dose, 10^{-6} M (24 h); few DT mutants ($< 7 \times 10^{-6}$); duration of chronic exposure not reported.	Rocchi et al. (1980)
DNA damage	ADP ribosyl transferase-mediated decrease in cellular NAD content	Human amnion FL cells	–	NR	Study quality: good; 24-h exposure at 4.58×10^{-3} to 10^{-7} mol/L; no clear information on number of replicates.	Yu et al. (1990)

B[a]P, benzo[*a*]pyrene; DT, diphtheria toxin; h, hour(s); γH2AX, phosphorylated histone 2AX; HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; TK, thymidine kinase.

[a]–, negative; (+), positive in a study of limited quality. None of these studies used metabolic activation.

2020). Phosphorylation of γH2AX with anthracene at 10 μM was higher than with B[a]P at an equimolar concentration, although no statistical analysis was conducted (Bhargava et al., 2020). [The Working Group considered that the evidence was weak because of various study limitations: only one dose was tested, and there was no clear description of the biological replicates and of the species origin of the lymphocytes, presumably human.]

(iii) Human cell lines

DNA damage after exposure to anthracene was assessed in human keratinocyte (HaCaT) and human lymphocyte (A3) cell lines by the comet assay (Hu et al., 2009). Anthracene (up to 10 μM) did not elicit any increase in comet tail moment in either cell type, compared with controls (Hu et al., 2009). [The Working Group noted that no details regarding methodology or replicates were provided.]

An assay for unscheduled DNA synthesis (UDS) was used to detect excision or removal of damaged DNA in a human cervical cancer cell line (HeLa S3) exposed to anthracene. No changes in UDS were observed with anthracene at any concentration tested (range, 0.1–100 μg/mL) (Martin et al., 1978; Martin & McDermid, 1981).

The frequency of micronuclei was measured in human lymphoblastoid $TK^{+/-}$ (MCL-5) cells blocked in cytokinesis by cytochalasin B and exposed to anthracene at several concentrations for 24 hours. The average number of micronuclei was not increased above that in the vehicle (DMSO) controls in replicate analyses (Crofton-Sleigh et al., 1993).

In a quantitative mutagenesis study in human embryo skin and muscle explant epithelial-like cells (EUE) that are sensitive to diphtheria toxin, the number of mutant cells that were resistant to diphtheria was measured after either acute or chronic exposure to anthracene (Rocchi et al., 1980). The maximum recovery of mutants was observed after an expression time of 3 weeks, corresponding to 10 cell generations. Anthracene did not induce any increase in the number of mutant cells (mutation frequency, $< 7 \times 10^{-6}$) after 10 or 20 cell doublings, in comparison with controls or other known carcinogens (Rocchi et al., 1980).

Lastly, in human amnion cells (FL), exposure to anthracene at concentrations of up to 10^{-3} mol/L for 24 hours did not induce an ADP ribosyl transferase (ADPRT)-mediated decrease in cellular nicotinamide adenine dinucleotide (NAD) content, whereas exposure to B[a]P, the positive control, at concentrations of 10^{-3} to 10^{-5} mol/L, resulted in a significant reduction in NAD content (Yu et al., 1990).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

The genotoxic potential of anthracene was investigated in several in vivo studies in rodent tissues.

When transgenic Sprague-Dawley rats carrying the human c-Ha-*RAS* proto-oncogene Hras128 (which are prone to spontaneous tumours of the mammary gland) were treated with anthracene at 200 mg/kg in oil administered orally by gavage once per week for 7–9 weeks, mutations of transgene *Hras128* were found in 2 of 3 tumours in females and in 2 of 3 tumours in males (Ohnishi et al., 2007). [The Working Group noted that these data were uninformative since no tumours were identified in the control group, thus there was no estimation of mutation frequency in the negative controls.]

In a study in female and male Chinese hamsters exposed to anthracene at a dose of 450 mg/kg as two intraperitoneal injections, the number of sister-chromatid exchanges (SCEs) per metaphase was not increased, compared with controls, in contrast to the results observed for similar experiments with B[a]P or benzanthracene (Roszinsky-Köcher et al., 1979).

Table 4.3 Genetic and related effects of anthracene in non-human mammals in vivo

End-point	Assay	Tissue, cell Species, strain (sex)	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mutation analysis of Ha-*Ras* codons 12 and 61	PCR-RFLP	Mammary tumours Transgenic Sprague-Dawley rats with human c-Ha-*RAS* (Hras128)	+	200 mg/kg bw	Gastric intubation (in oil) to Hras128 rats (age, 7 wk), 1×/wk, for 3 wk	Study was inadequate – there was no negative control group for mutation frequency estimation.	Ohnishi et al. (2007)
Micronucleus formation	In vivo micronucleus assay	Bone marrow, PCE Mouse, B6C3F_1	–	Phase 1: 80% of LD_{50} Phase 2: 40% and 80% of LD_{50}	Phase 1: double i.p. injection at 0 h and 24 h, and analysis at 48 h, 72 h, and 96 h Phase 2 (if the result of phase 1 was negative): single i.p. injection, analysis at 30 h, 72 h, and 96 h	10 mice per group; 500 PCE from each animal; positive controls, B[a]P, DMBA.	Salamone et al. (1981)
Micronucleus formation	In vivo micronucleus assay	Bone marrow, PCE Mouse, C57BL/6 or BALB/c, DBA/2, and BDF_1 and CDF_1	–	100 mg/kg bw	Single i.p. injection	Analysis was performed at 48 h, 72 h and 96 h; 3 mice per group, 1000 erythrocytes from each animal; positive controls, B[a]P, DMBA.	Sato et al. (1987)
Sister-chromatid exchange	In vivo sister-chromatid exchange	Bone marrow Chinese hamsters, (M, F), age 8–12 wk	–	450 mg/kg bw	Two i.p. injections at 0 h and 24 h	Analysis at 48 h after first injection, 8 animals per group; 50 well-stained metaphases were analysed from each animal; positive controls, B[a]P and benzanthracene.	Roszinsky-Köcher et al. (1979)
Inhibition of DNA synthesis	[^{3}H]thymidine uptake into DNA	Testes Swiss mice (M), (25–35 g)	–	125 mg/kg bw	Single i.p. injection of anthracene and i.p. injection of [^{3}H]thymidine 3 h later	Positive controls, 3-methylcholanthrene or diethylnitrosamine. Analysis was performed 30 minutes after [^{3}H]thymidine injection, 3 or 4 mice per group.	Friedman & Staub (1976)

Table 4.3 (continued)

End-point	Assay	Tissue, cell Species, strain (sex)	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mutagenicity of urine	Ames test	Urine Mouse, ICR (M), (age, 5 wk) Tested in *Salmonella typhimurium* TA100, TA98	– +	400 mg/kg bw alone 400 mg/kg bw with NO_2	Single i.p. injection dissolved in tri-*n*-caprylin were used alone or with 20 ppm NO_2; at a flow rate of 350 mL/min	Number of animals per group was not reported. Treatment with anthracene alone or with NO_2 alone did not have an effect. Positive controls, fluoranthene and the combination of fluoranthene with NO_2. Urine samples were treated with β-glucuronidase and arylsulfatase. Ames test performed with and without S9 fraction from rats treated with phenobarbital or 5,6-benzoflavone.	Miyanishi et al. (1996)

B[a]P, benzo[*a*]pyrene; bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; h, hour(s); HID, highest ineffective dose; i.p. intraperitoneal; LD_{50}, median lethal dose; LED, lowest effective dose, M, male; NO_2, nitrogen dioxide; PCE, polychromatic erythrocyte; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; wk, week(s).
[a] +, positive; –, negative.

No change in DNA synthesis, as measured by [^{3}H]thymidine uptake into testicular DNA, was observed in male Swiss mice exposed to anthracene at a dose of 125 mg/kg bw by intraperitoneal injection, in contrast to mice exposed to the known carcinogenic PAHs 3-MC and diethylnitrosamine (Friedman & Staub, 1976). Likewise, anthracene at 100 mg/kg bw, when injected intraperitoneally, did not induce micronuclei in the bone marrow erythrocytes of B6C3F$_1$ mice (Salamone et al., 1981), or in C57BL/6, BALB/c, DBA/2, C57BL/6 × DBA/2, or BALB/c × DBA/2 hybrid mice (Sato et al., 1987).

Elevated mutagenicity was shown in urine samples from ICR mice treated with anthracene (400 mg/kg bw, by intraperitoneal injection) in combination with nitrogen dioxide (NO_2; 20 ppm) according to the Ames test in *Salmonella typhimurium* strains TA100 and TA98, (Miyanishi et al., 1996). [The Working Group noted that this finding showed that anthracene, when co-administered with inhaled NO_2, might be nitrated in vivo and possibly converted to mutagenic compounds.]

(ii) Non-human mammalian cells in vitro

See Table 4.4.

Genotoxicity was not observed in in vitro studies in non-human mammalian cells.

No DNA damage, as analysed by the comet assay, was observed in V79 Chinese hamster lung fibroblasts exposed to anthracene (1–50 μM) both with and without the presence of S9 (9000 × *g* supernatant) from the liver of Aroclor-induced rats. However, DNA damage was observed when anthracene-treated cells were also exposed to white fluorescent lamps (Platt et al., 2008).

In the pseudodiploid Chinese hamster cell line D-6, SCE and chromosomal aberrations were not induced after treatment with anthracene (1 mM) (Abe & Sasaki, 1977).

Genotoxic activity was not observed in anthracene-treated rat liver epithelial cells ARL 18, which have an intrinsic capacity for the metabolic activation of a broad spectrum of mutagens and carcinogens. Sister-chromatid exchange was not induced by anthracene in ARL 18 cells, although it was induced in cells treated with the positive control, B[a]P (Tong et al., 1981a).

Anthracene did not induce UDS in primary cultures of hepatocytes from adult male Fischer 344 rats (Williams, 1977; Tong et al., 1981b), although the positive controls 7,12-dimethylbenz[*a*]anthracene (DMBA) and B[a]P did so efficiently. Similarly, anthracene did not affect UDS in primary cultures of adult hepatocytes from male Fischer 344 rats, either without metabolic activation or with S9 derived from the livers of Aroclor 1254-treated rats, whereas the positive control, 2-acetylaminofluorene (2-AAF), demonstrated a strong increase in UDS in these cells (Probst et al., 1981).

Anthracene (20–60 μM), when activated with S9 from C57BL/6J mice, clearly increased the frequency of gene mutation in L5178Y/*Tk*$^{+/-}$ cells, as did the promutagenic positive controls, 2-AAF and B[a]P. Anthracene was marginally mutagenic when S9 from Aroclor-induced rats was used (Amacher & Turner, 1980). In the presence of Aroclor-induced rat S9, mutants were revealed with anthracene only at a high toxic concentration, 71.2 μM (Amacher et al., 1980).

In contrast to 2-AAF and B[a]P, anthracene (3–20 μg/mL) did not induce *Hprt* gene mutations in Chinese hamster ovary (CHO) cells in the presence of rat S9 microsome mix; only low levels of sporadic mutation (1/10 replicates) occurred, in a non-dose-dependent manner (Oshiro et al., 1988). Higher concentrations of anthracene (50 and 125 μg/mL) induced gene mutagenicity, as measured by the *Hprt* mutation assay, in V79 Chinese hamster lung fibroblasts pre-treated with methylazoxymethanol acetate, but not without pre-treatment (Knaap et al., 1985).

Table 4.4 Genetic and related effects of anthracene in non-human mammalian cells in vitro

End-point	Species Tissue, cells	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks Comet assay	Chinese hamster Lung (V79) fibroblasts	– +	– NT	50 µM 50 µM + white light	Positive control, DMBA. Cells treated with anthracene were also exposed to white fluorescent lamps exhibiting emission maxima at 334.1, 365.0, 404.7, and 435.8 nm.	Platt et al. (2008)
Unscheduled DNA synthesis	Rat Primary (HPC) hepatocytes	–	NT	1 mM	Positive control, DMBA.	Williams (1977)
Unscheduled DNA synthesis	Rat, Fischer 344 (M) Primary hepatocytes	–	NT	1 mM	Positive controls, DMBA and B[a]P. Source of the chemical was not reported.	Tong et al. (1981b)
Unscheduled DNA synthesis	Rat, Fischer 344 (M) Primary hepatocytes	–	–	100 nmol/mL (100 µM)	Positive controls: 2-AAF and MNNG. S9 fraction, derived from the livers of rats pre-treated with Aroclor 1254, was used.	Probst et al. (1981)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO) cells	–	+/–	LEC, 3 µg/mL (+S9) Range, 3–20 µg/mL	Positive controls, 2-AAF and B[a]P. Source of the chemical was not reported; some low-frequency mutations (1/10 experimental samples) were found when S9 activation was used with anthracene at concentrations of 3, 4, 5 µg/mL, but without dose-dependency.	Oshiro et al. (1988)
Gene mutation (*Hprt*)	Chinese hamster lung (V79) fibroblasts	–	–	125 µg/mL	Positive control, methylazoxymethanolacetate. Source of the chemical was not reported.	Knaap et al. (1985)
Gene mutation (*Tk* locus)	Mouse L5178Y/*Tk*$^{+/-}$ lymphoma cells	–	+	LEC, 20 µM Range, 0–60 µM	Positive control: B[a]P and 2-AAF. S9 from C57BL/6J mice clearly activated anthracene to a mutagenic substance; Aroclor-induced rat S9 produced marginal activation.	Amacher & Turner (1980)
Gene mutation (*Tk* locus)	Mouse L5178Y/*Tk*$^{+/-}$ lymphoma cells	–	+/–	LEC, 0.5 µM Range, 0–127 µM	Positive controls, B[a]P and 2-AAF. Mutants appeared only at a highly toxic concentration.	Amacher et al. (1980)
Chromosomal aberrations	Chinese hamster (D-6) cells	–	NT	1 mM	Positive control, DMBA.	Abe & Sasaki (1977)

Table 4.4 (continued)

End-point	Species Tissue, cells	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Chinese hamster (D-6) cells	–	NT	1 mM	Positive control, DMBA.	Abe & Sasaki (1977)
Sister-chromatid exchange	Rat, adult Liver, epithelial (ARL 18) cells	–	NT	1 mM	Positive control, B[a]P.	Tong et al. (1981a)

2-AAF, 2-acetylaminofluorene; B[a]P, benzo[*a*]pyrene; CHO, Chinese hamster ovary; DMBA, 7,12-dimethylbenz[*a*]thracene; HIC, highest ineffective concentration; HPRT, hypoxanthine-guanine phosphoribosyl transferase; LEC, lowest effective concentration; M, male; MNNG, methylnitronitrosoguanidine; NT, not tested; S9, 9000 × *g* supernatant; Tk, thymidine kinase.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study).

(iii) Non-mammalian test systems

See Table 4.5.

Exposure to anthracene (20 ppm) for 12 days did not induce any increase in micronuclei frequency in the erythrocytes of *Pleurodeles waltl* larvae (1000 cells were analysed for every larva and every group contained 15 larvae). Micronuclei were clearly observed in B[a]P-treated cells (Djomo et al., 1995).

In contrast to the mutagenic effects observed with B[a]P, anthracene did not induce chromosomal aberrations in rainbow trout gonads (RTG-2) and bluegill fry (BF-2) (Kocan et al., 1982). Anthracene (50 ppb) caused DNA damage, as demonstrated by the comet assay, in blood cells of the flounder *Paralichthys olivaceus*; B[a]P also demonstrated a significant effect (Woo et al., 2006).

Anthracene was mutagenic in the *Drosophila melanogaster* wing spot test both in the standard cross (at two concentrations, 1 and 10 mM), and in the high bioactivation cross (at four concentrations, 1, 5, 10, and 20 mM). DMBA was also mutagenic in this test system (Delgado-Rodriguez et al., 1995). However, there was no mutagenic effect with anthracene at 2 mM in the *D. melanogaster* eye mosaic spot test, although the positive control represented by DMBA was very efficient (Vogel & Nivard, 1993).

An increase in DNA damage of > 2-fold induced by anthracene (3 µg/L) when activated by sunlight was also demonstrated, by the comet assay, in grass shrimp (*Palaemonetes pugio*) embryos (Lee & Kim, 2002).

In the mussel, *Mytilus galloprovincialis*, the frequency of micronuclei per 1000 cells (gills or erythrocytes) increased significantly after treatment for 7 days with anthracene (0.1 µg/mL) in sterile seawater with a 14:10 hour light:dark photoperiod ratio (Giannapas et al., 2012; Grintzalis et al., 2012). [The Working Group noted that, according to OECD guidelines, these studies analysed insufficient numbers of gills and erythrocytes.]

Treatment with anthracene at 0.6–1.0 µM induced significant DNA damage, measured by the comet assay, in coelomocytes of the earthworm *Eisenia fetida* (Sun et al., 2020).

In vitro assays with *Saccharomyces cerevisiae* D3 did not reveal any recombinogenic activity for anthracene, although such activity was shown for *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (Simmon, 1979b). Reverse mutations were not detected when *Cunninghamella elegans* cultures incubated with anthracene for 48 hours were tested in *S. typhimurium* strains TA98 and TA100, with and without S9 fraction from the liver of Aroclor 1254-treated rats (Cerniglia et al., 1985).

Anthracene (450 µg/plate) induced mutagenicity in *S. typhimurium* strain TA100 in the presence of the hamster metabolic activation system (S9) for procarcinogen activation (Carver et al., 1986). However, anthracene was not mutagenic, compared with the relevant positive controls, in other studies in the presence of S9 liver fraction from Aroclor 1254-induced rats, in *S. typhimurium* strains TA100 and TA98 (La Voie et al., 1985); TA100 and TA98 (La Voie et al., 1979); TA100-lux and TA98-lux (Ackerman et al., 2009); TA1535 and TA1538 (Rosenkranz & Poirier, 1979); TA100, TA1535, TA98, TA1538, TA1537 (Liberman et al., 1982); TA100, TA1535, TA98, TA1538, TA1537 (Ho et al., 1981); TA100, TA1535, TA98, TA1538, TA1536, TA1537 (McCann et al., 1975; Simmon, 1979a); and TM677 (Kaden et al., 1979). Anthracene was also not mutagenic in *S. typhimurium* strains TA100, TA1535, TA98, TA1538, and TA1537 in the presence of S9 liver fraction from 3-MC-induced guinea-pigs (Baker et al., 1980). In a collaborative validation study, which used *S. typhimurium* strain TA98 and TAMIX (a mixture of strains TA7001, TA7002, TA7003, TA7004, TA7005, and TA7006), anthracene was mutagenic in results from 2 out of 15 independent laboratories in the presence of S9

Table 4.5 Genetic and related effects of anthracene in non-mammalian experimental systems in vivo and in vitro

Test system (species, strain)	End-point	Results[a]		Concentration (LEC, HIC) or dose (LED or HID)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Pleurodeles waltl (Amphibia, Salamandridae)	Micronucleus formation in larvae erythrocytes	–	NA	20 ppm (minimum toxic concentration, 20 µg per 100 g of the larvae mixture)	Positive control, B[a]P. Pleurodeles larvae were treated with anthracene for 12 days. Every group contained 15 larvae and 1000 erythrocytes were analysed for every larva.	Djomo at al. (1995)
Rainbow trout gonad cells (RTG-2) and bluegill fry cells (BF-2)	Chromosomal aberrations	–	NA	5–20 µg/mL	Positive control, B[a]P. At each time and concentration, ≥ 200 anaphases were analysed.	Kocan et al. (1982)
Flounder (*Paralichthys olivaceus*)	DNA strand breaks, comet assay, blood cells	+	NT	LED, 50 ppb (range, 0–100 ppb)	Positive control, B[a]P. After treatment for 2 h with anthracene at 50 ppb, DNA damage as measured by tail length in the comet assay increased from 58 µm to 90 µm.	Woo et al. (2006)
Drosophila melanogaster, *flr*^{3}/*In(3LR)TM3*, *ri p*^{p} *sep l(3)89Aa bx*^{34e} *e Bd*^{s} females mated to *mwh* males	SMART, wing spots	+/–	NA	1, 5, 10, 20 mM	Positive control, DMBA. Clone formation frequency per 10^5 cells was calculated.	Delgado-Rodriguez et al. (1995)
Drosophila melanogaster, *ORR/ORR*; *flr*^{3}/*In(3LR) TM3*, *ri p*^{p} *sep l(3)89Aa bx*^{34e} *e Bd*^{s} females mated to *mwh/mwh* males.	SMART, with high bioactivation cross	+	NA	1, 5, 10, 20 mM		
Drosophila melanogaster: *y* (*yellow*) females × *w* (*white*) males, Leiden Standard (LS)	Interchromosomal mitotic recombination, eye mosaic spots	–	NA	2 mM	Positive control, DMBA. Source of anthracene was not provided.	Vogel & Nivard (1993)
Palaeomonetes pugio (grass shrimp) embryos	DNA strand breaks (comet assay)	+	NA	3 µg/L	A positive control was not included in the study. Source of anthracene was not provided. DNA damage increased more than twice when anthracene treatment was combined with sunlight, whereas sunlight alone did not cause a significant effect.	Lee & Kim (2002)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC, HIC) or dose (LED or HID)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Mussel (*Mytilus galloprovincialis*), gills	Micronucleus formation	+	NA	0.1 µg/mL	A positive control was not included in the study. After treatment for 7 days with anthracene at 0.1 µg/mL in sterile sea water with a 14 h:10 h light:dark photoperiod, the frequency of micronucleus abnormalities per 1000 cells increased from 4.3 to 8.4 (this < 2-fold change and use of only 1000 cells were limitations of the experiment).	Grintzalis et al. (2012)
Mussel (*Mytilus galloprovincialis*), haemocytes	Micronucleus formation	+	NA	0.1 µg/mL	A positive control was not included in the study. After treatment for 7 days with anthracene at 0.1 µg/mL in sterile sea water with 14 h:10 h light: dark photoperiod, the relative increase in micronucleus frequency was 2.8.	Giannapas et al. (2012)
Earthworm (*Eisenia fetida)*, coelomocytes	DNA strand breaks, comet assay	+	NT	0.6 µM Range, 0–1 µM	No positive control was included in the study. Increase of 10-fold in DNA damage after cell treatment for 24 h.	Sun et al. (2020)
Saccharomyces cerevisiae D3	Mitotic recombinants	–	–	5% (w/v)	Positive control, MNNG. S9 obtained from rats induced with Aroclor 1254.	Simmon (1979b)
Culture medium from *Cunninghamella elegans* tested in *Salmonella typhimurium* TA98, TA100	Reverse mutation	–	–	125 µg/plate	Positive control, B[a]P.	Cerniglia et al. (1985)
Salmonella typhimurium, TA100	Reverse mutation	–	+/–	450 µg/plate	Positive control, B[a]P. Anthracene was genotoxic only with S9 from hamster liver.	Carver et al. (1986)
Salmonella typhimurium, TA98 and TA100	Reverse mutation	–	–	100 µg/plate	Positive control, 1,9-dimethylfluorene. Source of anthracene was not reported; S9 from the livers of rats pre-treated with Aroclor 1254.	La Voie et al. (1979)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC, HIC) or dose (LED or HID)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA98 and TA100	Reverse mutation	–	–	200 µg/plate Range, 5–200 µg/plate	Positive control, 2,9-dimethylanthracene. S9 from the livers of rats pre-treated with Aroclor 1254.	La Voie et al. (1985)
Salmonella typhimurium, TA98-lux and TA100-lux	Reverse mutation	–	–	10 mg/plate	Positive control, B[a]P. Negative results were obtained by bioluminescent *Salmonella* reverse mutation assay performed in five independent laboratories.	Ackerman et al. (2009)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	–	–	20 µg/plate	Positive control, B[a]P.	Liberman et al. (1982)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	–	–	1000 µg/plate	Positive control, B[a]P. S9 from methylcholanthrene-induced guinea-pigs.	Baker et al. (1980)
Salmonella typhimurium, TA1535, TA1537, TA1538, TA98 and TA100	Reverse mutation	–	–	500 µg/plate Range, 1–500 µg/plate	Positive control, B[a]P. Purity was checked by thin-layer chromatography, gas chromatography, and mass spectrometry; S9 from the livers of rats pre-treated with Aroclor 1254.	Ho et al. (1981)
Salmonella typhimurium, TA98, TA100, TA1535, TA1536, TA1537, and TA1538	Reverse mutation	–	–	250 µg/plate	Positive control, B[a]P. S9 from Sprague-Dawley rats pre-treated with Aroclor 1254.	Simmon (1979a)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	–	–	HIC, 1000 µg/plate Range, 10–1000 µg/plate	Positive control, B[a]P. S9 from livers of rats pre-treated with Aroclor 1254.	McCann et al. (1975)
Salmonella typhimurium, TM677	Reverse mutation	–	–	225 µM [40 µg/mL]	Positive control, B[a]P. S9 from livers of rats pre-treated with phenobarbital and Aroclor 1254.	Kaden et al. (1979)
Salmonella typhimurium, TA98 and TAMix (TA7001–7006)	Reverse mutation	NT	+/–	4–5000 µg/mL	Positive control, B[a]P. Positive results at 100 µg/mL in 2 out of 15 laboratories.	Flückiger-Isler et al. (2004)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC, HIC) or dose (LED or HID)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA98	Reverse mutation	NT NT	– +	50 µg/plate 20 µg/plate + 1 µg/plate B[a]P	Anthracene significantly increased the genotoxic effect of B[a]P at 1 µg/plate; S9 from rat liver.	Hermann (1981)
Salmonella typhimurium, TA102	Reverse mutation	– ++	– –	5 nmol/plate 0.11 nmol/plate under light	Under light exposure (1.1 J/cm² UVA + 2.1 J/cm² visible) –S9, anthracene increased its photomutagenic effect in a dose-dependent manner over a range of 0.11–0.54 nmol/plate.	Yan et al. (2004)
Salmonella typhimurium, TA1535/ pSK1002	umuC test; expression of the reporter transgene of β-galactosidase activated by umu-related proteins	+ (for photoproducts only)	–	20 µg/mL, anthracene and its photoderivatives	Analysis was performed +S9 and –S9, according to International Organization for Standardization (ISO) 13 829. S9 removed genotoxic activity; 1-hydroxyanthracene-9,10-dione and 1,4-dihydroxyanthracene-9,10-dione were identified and confirmed as genotoxic photoderivatives.	Brack et al. (2003)
Salmonella typhimurium, TA1535, TA1538	Reverse mutation	–	–	250 µg/plate	Positive control, MNNG. S9 from livers of rats induced with Aroclor 1254.	Rosenkranz & Poirier (1979)
Escherichia coli, pol A⁺ and pol A⁻ strains	DNA-modifying capacity	NT	–	250 µg/plate	Positive control, B[a]P.	
Escherichia coli, RT7h-RT18h with the reversible his-4 locus	Reverse mutation	–	NT	10 µg/mL + NUV 0–100 kJ m^{-2}	Genotoxicity occurred in the presence of cytotoxicity and cell membrane damage.	Tuveson et al. (1990)
Haemophilus influenzae	Decrease in DNA transforming activity	+	NT	10 µg/mL + NUV 0–100 kJ m^{-2}		
Plasmid pBR322 supercoiled DNA	Nicking of DNA	+	NT	10 µg/mL		
Bacillus subtilis, H17 and M45	Reverse mutation	–	–	62 µg/plate	Positive control, MNNG. S9 (crude extract, ISO 13 829) was obtained from rats after induction with Aroclor 1254.	McCarroll et al. (1981)

B[a]P, benzo[*a*]pyrene; DMBA, 7,12-dimethylbenz[*a*]anthracene; h, hour(s); HIC, highest ineffective concentration; HID, highest ineffective dose; LEC, lowest effective concentration; LED, lowest effective dose; MNNG, methylnitronitrosoguanidine; NA, not applicable; NT, not tested; NUV, near-ultraviolet light, 320–400 nm; ppb, parts per billion; ppm, parts per million; S9, 9000 × *g* supernatant; SMART, somatic mutation and recombination test; UV, ultraviolet; w/v, weight/volume.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study).

liver fraction from rats induced with Aroclor 1254 (Flückiger-Isler et al., 2004).

Anthracene increased the mutagenicity of B[a]P in *S. typhimurium* strain TA98 (Hermann, 1981). Also, anthracene (≥ 0.11 nmol/plate) demonstrated strong photomutagenicity in *S. typhimurium* strain TA102, which is auxotrophic for histidine, under UV-A and light exposure (1.1 J/cm^2 UV-A + 2.1 J/cm^2 visible light) and without S9 activation. No mutagenicity was observed when UV-A was not applied, either with or without S9 activation (Yan et al., 2004). The *umuC* test for genotoxicity in *S. typhimurium* strain TA1535/pSK1002 revealed significant activity for anthracene and its photoderivatives, which decreased in the presence of Aroclor-induced rat S9 (Brack et al., 2003).

Anthracene did not possess a DNA-modifying capacity in normal and DNA polymerase-deficient *Escherichia coli* strains *polA*$^+$ and *polA*$^-$ (Rosenkranz & Poirier, 1979).

Anthracene was inert in the micro-suspension assay for reverse mutations in *Bacillus subtilis* strains H17 and M45, with and without metabolic activation by S9 (McCarroll et al., 1981). An *E. coli* strain deficient in *katF* (which is involved in catalase synthesis) was sensitive to its inactivation by anthracene plus near-visible ultraviolet irradiation, but when histidine independence was used as the end-point, no mutations were detected in experiments with *E. coli* strains RT7h, RT8h, RT10h, RT13h, or RT15h over the complete range of survival levels investigated. Anthracene plus near-visible ultraviolet irradiation inactivated *Haemophilus influenza* transforming DNA, leading to the nicking of supercoiled plasmid pBR322 DNA in vitro (Tuveson et al., 1990).

[The Working Group noted that the genotoxicity of anthracene was shown in several studies performed in mammalian cells and in several non-mammalian systems only in the presence of metabolic activation by mouse or hamster microsomal monooxygenases, photoactivation, or structure modification through interaction with NO_2. DNA damage was not shown in studies performed without photoactivation or in the presence of rat liver microsomal monooxygenases. The need for pre-activation, i.e. photoactivation, provided some explanation for the mixed results across studies of different end-points and in different experimental systems.]

4.2.3 Induces epigenetic alterations

The evidence on whether anthracene might exhibit the key characteristic of "induces epigenetic alterations" was scarce. No data in exposed humans were available to the Working Group. In addition, anthracene, either in a single study in isolated lymphocytes or in yeast, engineered to express human DNMT-1 and DNMT-3B genes, induced no alterations in target genes (Sugiyama et al., 2016; Bhargava et al., 2020). Bhargava et al. (2020) examined the epigenetic effects of treatment with the PAHs, anthracene and B[a]P (10 μM) in isolated lymphocytes [The Working Group noted that the cells appeared to be of human origin but this was not clearly stated in the manuscript.] Anthracene failed to modulate the expression levels of three of four microRNAs (miR-24, miR-34a, miR-150), known to be associated with carcinogenesis, as compared with B[a]P. [The Working Group noted that no statistics were provided for these changes.] In addition, anthracene did not alter the miRNA-related target genes (*MYC*, *P53*, *NFKB*) that were down-regulated by B[a]P or the epigenetic markers *DNMT1*, *HDAC1*, *HDAC7*, *KDM3a*, *EZH2*, and *P300*, which were also significantly altered by B[a]P at up to 72 hours of treatment. Finally, anthracene did not affect mitochondrial DNA methylation.

A yeast engineered to express human DNMT-1 and DNMT-3B genes was shown to respond to DNA methyltransferase inhibitors or the histone deacetylase inhibitor trichostatin A with increased flocculation behaviour (Sugiyama

et al., 2016). Trichostatin A also increased expression of the flocculin-encoding gene *FLO1*, a gene linked to nonsexual flocculation in the yeast. The natural product alizarin, derived from anthracene and considered to be a carcinogenesis promoter, promoted flocculation in this assay and enhanced *FLO1* mRNA expression, but anthracene (4.0–400 μM) was inactive.

4.2.4 Induces oxidative stress

Alterations in the generation of reactive oxygen species (ROS) or reactive nitrogen species and their interactions with biological macromolecules (i.e. lipids, DNA, RNA, and proteins), and alterations in the antioxidant defence capacity can both have a relevant role in neoplastic development (Klaunig et al., 2011; Smith et al., 2016; Suman et al., 2016). Studies investigating the formation of DNA oxidative bioproducts, i.e. 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG); the reaction of ROS combined with polyunsaturated fatty acids (PUFA) in the lipid membranes to generate malondialdehyde (MDA) through lipid peroxidation (Klaunig et al., 2011); the downregulation of antioxidant pathways, such as those downstream of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) (Klaunig et al., 2011), including glutathione reductase (GSR), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx); or other signalling pathways, such as AP-1, which are downstream of MAP kinases (JNK, p38) and are implicated in the regulation of pro-oxidative stress responses (Klaunig et al., 2011) were reviewed here (see details in Table 4.6, Table 4.7, Table 4.8, and Table 4.9).

(a) Humans

(i) Exposed humans

See Table 4.6.

Five studies investigating the potential association of anthracene with oxidative stress in exposed humans were available to the Working Group (Singh et al., 2008b; Hanchi et al., 2017; Agarwal et al., 2018; Jeng et al., 2022, 2023). However, in Agarwal et al. and Singh et al., the contribution of anthracene to the overall effects of the PAH mixture on oxidative stress was not clearly assessed and these studies were excluded.

Among coke-oven workers from a steel factory in Taiwan, China, the association between exposure to individual PAHs (including anthracene) and oxidative stress end-points was evaluated in a dose–response analysis. PAHs were measured in personal breathing-zone air samples, and levels were quantified by GC-MS; time-weighted concentrations of each PAH were calculated and used to estimate exposure. Compared with the controls, the coke-oven workers had significantly higher levels of sperm 8-oxodG, seminal MDA, and seminal ROS. Individual PAH associations with 8-oxodG, ROS, and malondialdehyde were determined in sperm from the same individuals (Jeng et al., 2022). Thirty-eight workers (18 top-side oven workers and 20 side-oven workers) and 22 office workers (reference controls) were evaluated. Levels of 8-oxodG were measured by LC-MS with an electrospray ion source (ESI) using established procedures. An increase in anthracene exposure was not associated with oxidative damage to DNA as assessed by 8-oxodG (Jeng et al., 2022). In addition, there were no positive associations between anthracene and ROS levels. However, increases in MDA levels were positively associated with increases in exposure to all the PAHs evaluated, including anthracene (Jeng et al., 2022), but not anthracene individually.

In a follow-up study from the same group (Jeng et al., 2023), oxidative damage to DNA was assessed in 54 of the workers (31 top-side oven workers and 23 side-oven workers) from the same factory in Taiwan, China. Levels of 8-oxodG were assessed as described above. However, no office worker controls were included in the study. Anthracene exposure did not correlate with oxidative damage to DNA as measured by 8-oxodG. [The Working Group considered

Table 4.6 End-points relevant to oxidative stress with anthracene in exposed humans

End-point	Assay Biospecimen	Location Setting, study design	Exposure level and no. of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
8-oxodG	GC-MS/MS and LC-MS/MS Urine	Tunisia Workers at an electric steel foundry, cross-sectional study	2.86 ng/L; 93 healthy male workers; 3 categories: SSW, $n = 30$; RGC, $n = 43$; MIX, $n = 20$	(↑), $r = 0.357$	Smoking	Limitations: PAH measurements including anthracene; a linear calibration curve was done for 8-oxodG and cotinine; small sample size. The exposure assessment was appropriate.	Hanchi et al. (2017)
8-oxodG	Triple quadrupole mass spectrometer Sperm DNA	Taiwan, China Workers ($n = 38$) from a coke-oven mill, 22 office workers, cross-sectional study	Median, 347 ng; log = 5.82	No alteration	Adjusted for age, education, smoking, drinking, BMI, and job site	Not very informative. Limitations: small sample size; no units provided for the PAHs measured, only log transformed; dermal exposure was not assessed; low confidence in the interpretation of anthracene exposure.	Jeng et al. (2022)
Malondialdehyde	Thiobarbituric acid Seminal plasma			(↑), association with PAHs only			
ROS	Chemiluminescence (by luminol) Seminal plasma			No alteration			
8-oxodG	Triple quadrupole mass spectrometer Sperm	Taiwan, China Workers from a coke-oven mill, cross-sectional study	54 exposed participants (31 topside-oven workers and 23 side-oven workers)	No alteration	Adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants	Not very informative. Limitations: small sample size; the exposure assessment was appropriate, even though the description was not complete.	Jeng et al. (2023)

BMI, body mass index; GC-MS/MS, gas chromatography-tandem mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MIX, maintenance and quality control workers; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; RGC, rolling, galvanization, and cable fibre workers; ROS, reactive oxygen species; SSW, steel smelter workers.
(↑), increase in a study of limited quality.

Table 4.7 End-points relevant to oxidative stress with anthracene in human cells in vitro

End-points	Assay	Tissue, cells	Results	Concentration (LEC or HIC)	Comments	Reference
Primary cells						
ROS	CellROX Deep Red Flow Cytometry assay	Human primary peripheral blood lymphocytes	↑	10 µM, 30 min to 6 h	Limitations: only one concentration; cell species not noted but assumed to be human, measured with ELISA; $n = 3$; unclear if technical or biological replicates.	Bhargava et al. (2020)
NRF2 activity	ELISA	Human primary peripheral blood lymphocytes	↑	10 µM, 1–24 h	Limitations: only one concentration;, measured with ELISA; $n = 3$, unclear if technical or biological replicates.	
$O_2^{\bullet}$	Photosensitized reduction of NBT to NBF, measured spectrophotometrically	Human peripheral blood leukocytes	↑, $r^2 = 0.83$; $P < 0.05$	0.05–0.25 µM for 24 h	Informative: correlation analysis determined that the association was significant; dose range not clear.	Uribe-Hernández et al. (2008)
$O_2^{\bullet}$	Superoxide dismutase-inhibitable cytochrome c reduction	Human peripheral blood monocytes	No change	10 µg/mL for 24 h	Limitations: one concentration tested.	Fabiani et al. (1999)
Human cell lines						
ROS	DCFH-DA	Human alveolar basal epithelial (A549) cells	↑, association with $PM_{2.5}$ anthracene and ROS; $r = 0.81$	Mixture of emission factors; dose not clear (100 or 200 µg/mL); 24 h exposure	Limitations: only association measured; not individual PAHs tested; $PM_{2.5}$ extracts may include other agents.	Sun et al. (2018)
ROS	DCFH-DA	Human keratinocyte (HaCaT) cells	↑ with UV only	0.01–0.5 µg/mL	Informative; sunlight, UV-A and UV-B were used to activate anthracene; no anthracene only or UV only controls; tested with replicates.	Mujtaba et al. (2011)
Radicals $O_2^{\bullet}$ and $^{\bullet}OH$	$O_2^{\bullet}$: photosensitized reduction of NBT to NBF, measured spectrophotometrically; $^{\bullet}OH$: measured by ascorbic acid–iron-EDTA system.	Human keratinocyte (HaCaT) cells	↑ with UV only	0.01–0.5 µg/mL		

DCFH-DA, 2′,7′-dichlorofluorescein diacetate; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; h, hour(s); HIC, highest ineffective concentration; NBF, nitro-blue diformazan; NBT, nitro-blue tetrazolium; NRF2, nuclear factor erythroid 2–related factor 2; $O_2^{\bullet}$, superoxide anion radical; $^{\bullet}OH$, hydroxyl radical; LEC, lowest effective concentration; min, minute; $PM_{2.5}$, particulate matter with diameter ≤ 2.5 µm; ROS, reactive oxygen species; UV, ultraviolet.
↑, increase.

Table 4.8 End-points relevant to oxidative stress with anthracene in non-human mammalian systems in vitro

End-point	Assay	Species, strain (sex), cell line	Tissue	Results[a]	Concentration (LEC or HIC)	Route, duration, dosing regimen	Comments	Reference
ROS	DCFH-DA	Rat, Sprague-Dawley, primary cardiomyocytes	Heart	(↑)	0–10 µM	Treated after cell isolation; dose–response relation.	Semi-quantitative study; not clear if exposure was 1 h.	Ju et al. (2020)
Protein carbonyls	Protein carbonylation colourimetric assay	Rat, Sprague-Dawley, primary cardiomyocytes	Heart	↑	0–10 µM for 24 h	Treated after cell isolation; $n = 4$, repeated 3 times.		
Malondialdehyde	Thiobarbituric acid	Rat, Sprague-Dawley, primary cardiomyocytes	Heart	↑	10 µM, significant response	Treated after cell isolation, dose–response relation over 0–10 µM for 24 h time point; $n = 4$, repeated 3 times.	Significant increases in phosphorylated ERK1/2 and AKT.	
ROS	DCFH-DA	Rat, Sprague-Dawley, vascular smooth muscle cells	Aorta	(↑)	0–10 µM for 1 h	Treated after cell isolation; $n = 3$.	Semi-quantitative study; only 1 experiment; MMP2 also significant; reversed with NAC, but no information about concentration. An increase in MMP2 was also observed.	Ju et al. (2022)
ROS	DCFH-DA	Mouse, hippocampal neuronal cells (HT-22)	Brain	↑	125 µM	Dose–response relation over 0–125 µM for 48 h; repeated 3 times.		Olasehinde & Olaniran (2022)
Antioxidants	CAT activity	Mouse, hippocampal neuronal cells (HT-22)	Brain	↑	25 µM	Dose–response relation over 0–125 µM for 48 h; repeated 3 times.		
Antioxidants	GST activity	Mouse, hippocampal neuronal cells (HT-22)	Brain	↑	25 µM	Dose–response relation over 0–125 µM for 48 h; repeated 3 times.		
Antioxidants	GSH	Mouse, hippocampal neuronal cells (HT-22)	Brain	↑	25 µM	Dose–response relation over 0–125 µM for 48 h; repeated 3 times.		

AKT, protein kinase B; CAT, catalase; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; ERK1/2, extracellular signal-regulated kinase 1/2; GSH, glutathione; GST, glutathione-*S*-transferase; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; MMP2, matrix metalloproteinase 2; NAC, *N*-acetyl cysteine; ROS, reactive oxygen species.

[a] ↑, increase; (↑) increase in a study of limited quality.

Table 4.9 End-points relevant to oxidative stress with anthracene in non-mammalian systems in vivo

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
DTT (oxidative potential)	*Danio rerio*, zebrafish larvae	Whole fish	No change	20 µg/L (HID)	1, 2, 4, 6 h time points; exposure group, *n* = 15; repeated 3 times	In the article text, it was suggested that UV increased oxidative potential but not significantly; fish were laboratory-raised.	St Mary et al. (2021)
GST activity[d]	*Pomatoschistus microps*, common goby, juveniles	Liver	↓	0.5 µg/L	27 animals/dose; exposed for 96 h to 500 mL of anthracene (0.25–4 µg/L)	Naturally caught.	Vieira et al. (2008)
CAT activity	*Pomatoschistus microps*, common goby, juveniles	Liver	↑	2 µg/L	27 animals/dose; exposed for 96 h to 500 mL of anthracene (0.25–4 µg/L)	Naturally caught.	
SOD activity	*Pomatoschistus microps*, common goby, juveniles	Liver	↑	1 µg/L	27 animals/dose; exposed for 96 h to 500 mL of anthracene (0.25–4 µg/L)	Naturally caught.	
LPO (TBARs)	*Chanos chanos*, milkfish	Head, gill, and dorsal fin muscles	↑, all sites	0.011 mg/L	*n* = 3 per treatment; dose–response relation over 0–0.176 mg/L	Naturally caught.	Palanikumar et al. (2012)
CAT activity	*Chanos chanos*, milkfish	Head, gill, and dorsal fin muscles	↑, all sites	0.011 mg/L	*n* = 3 per treatment; dose–response relation over 0–0.176 mg/L	Naturally caught.	
GST activity	*Chanos chanos*, milkfish	Head, gill, and dorsal fin muscles	↑, all sites	0.011 mg/L (head only); 0.022 mg/L for others	*n* = 3 per treatment; dose–response relation over 0–0.176 mg/L	Naturally caught.	
LPO (malonaldehyde)	*Lepomis macrochirus*, bluegill sunfish	Liver microsomes	No change unless +UV light (2 h)	3.015 µg/mL	Exposed the microsomes to anthracene (3 h at 30 °C) and UV (20 min at 37 °C); *n* = 3, no clarity on number of replicate experiments; anthracene alone and UV alone controls were carried out	From hatchery.	Choi & Oris (2000b)
$O_2^{\bullet}$	*Lepomis macrochirus*, bluegill sunfish	Liver microsomes	No change unless +UV light (2 h)	3.015 µg/mL			

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
LPO (malonaldehyde)	*Poeciliopsis lucida*, top minnow hepatoma cell line (PLHC-1)	Cell line	No change unless +UV light (2 h)	Up to 5 mg/L; +UV light ~2.5 mg/L	$n = 4$ per treatment/dose; dose–response over 0–5 mg/L; 3 h with anthracene.		Choi & Oris (2000a)
LPO (TBARS)	*Palaemon serratus*, common prawn	Digestive gland	↑	32 µg/L	Dose range, 16–1024 µg/L in seawater for 96 h; $n = 9$ per treatment		Gravato et al. (2014)
GST activity			No change	1024 µg/L (HID)			
CAT activity			↑	1024 µg/L			
GPx activity			↑	256 µg/L			
GST activity	*Daphnia magna*, water flea	Whole animal	↓	0.25 µM	10 animals/treatment; 96 h exposure to anthracene; dose range, 0.0625–5 µM; repeated 3 times		Feldmannová et al. (2006)
GPx activity			↓	0.5 µM			
SOD activity	*Ruditapes decussatus*, Mediterranean clam	Gill and digestive gland	↑, gill; no change in digestive gland	100 µg/L	5 animals/treatment for 48 h exposure	No replicates.	Sellami et al. (2015a)
CAT activity			↑, gill; no change in digestive gland	100 µg/L			
GPx activity			↑, gill; no change in digestive gland	100 µg/L			
GST activity			↑, gill; no change in digestive gland	100 µg/L			
GSR activity			↑, gill; no change in digestive gland	100 µg/L			

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
Protein carbonyls			↑ observed; probably actin	100 µg/L			Sellami et al. (2015a) (cont.)
SOD activity	*Venerupis decus*[e], Mediterranean clam	Gill and digestive gland	↑, gill; no change in digestive gland	100 µg/L	5 pooled animals/replicate, $n = 3$; sea water used as medium; 48 h exposure	Appropriate replicates run.	Sellami et al. (2015b)
CAT activity			↑, gill; no change in digestive gland	100 µg/L			
GST activity			↑, gill; no change in digestive gland	100 µg/L			
LPO (malonaldehyde)	*Mytilus galloprovincialis*, mussel	Isolated haemocytes (immune system cells)	↑	100 µg/L	7-day exposure; 3 replicates/group		Giannapas et al. (2012)
$O_2^{\bullet}$	*Mytilus galloprovincialis*, mussel	Isolated haemocytes (immune system cells)	↑	100 µg/L	7-day exposure; 3 replicates/group	Response correlated to micronuclei frequency increase	
GSH content	*Mytilus galloprovincialis*, mussel	Digestive gland	↓	0.15 µg/L, 2 days 0.05 µg/L, 4 days 0.05 µg/L, 8 days	Dose range, 0.05–0.4 µg/L, for exposures of 2, 4, or 8 days; 39 mussels/group		Badreddine et al. (2017)
LPO (malonaldehyde)	*Mytilus galloprovincialis*, mussel	Digestive gland	↑	0.15 µg/L, 2 days 0.05 µg/L, 4 days 0.05 µg/L, 8 days	Dose range, 0.05–0.4 µg/L, for exposure of 2, 4, or 8 days; 39 mussels/group		

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
LPO (malonaldehyde)	*Mytilus edulis*, blue mussel	Gills and digestive glands	↑, gills; 18, 21 days ↑, digestive gland; 3, 6 days	0.25 µg/L	0.25 and 2.5 µg/L, 21-day exposures; 0–21 days, time course done; *n* = 3; replicated 3 times		Mengqi et al. (2017)
$O_2^{\bullet}$	*Mytilus edulis*, blue mussel	Gills and digestive glands	↑, gills; several days ↑, digestive gland; a few days	0.25 µg/L	0.25 and 2.5 µg/L, 21-day exposures; 0–21 days, time course done; *n* = 3; replicated 3 times	Lower increases for this end-point in the digestive gland.	
GST activity	*Mytilus edulis*, blue mussel	Gills and digestive glands	↑, in both tissues	0.25 µg/L for several days	0.25 and 2.5 µg/L, 21-day exposures; 0–21-day time course done; *n* = 3; replicated 3 times; days 3 and 9, some decreases		
GSH content	*Mytilus edulis*, blue mussel	Gills and digestive glands	↓, in gills ↑, digestive gland	0.25 µg/L	0.25 and 2.5 µg/L, 21-day exposures; 0–21-day time course done; *n* = 3; replicated 3 times		
GPx activity	*Mytilus edulis*, blue mussel	Gills and digestive glands	↑, in both tissues	0.25 µg/L, for several days	0.25 and 2.5 µg/L, 21-day exposures; 0–21-day time course done; *n* = 3; replicated 3 times; some reductions in the gills at 12 and 15 days		
GSR activity	*Mytilus edulis*, blue mussel	Gills and digestive glands	↑ in both tissues	0.25 µg/L for gills several days; 2.5 µg/L for digestive gland	0.25 and 2.5 µg/L, 21-day exposures; 0–21-day time course done; *n* = 3; replicated 3 times; some reductions at 3 and 9 days		

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
SOD activity	*Acropora tenuis*, scleractinian coral	Larvae	↓	4 µg/L	4 and 17 µg/L; 200 larvae per dose; *n* = 4 per control and treatment; *n* = 2 solvent controls	This study also assessed phototoxicity, which was found not to be a major influence on any oxidative stress biomarkers.	Overmans et al. (2018)
CAT mRNA expression	*Acropora tenuis*, scleractinian coral	Larvae	↑	17 µg/L	4 and 17 µg/L; 200 larvae per dose; *n* = 4 per control and treatment; *n* = 2 solvent controls	4 µg/L was variable for gene expression.	
MnSOD mRNA expression	*Acropora tenuis*, scleractinian coral	Larvae	No change	17 µg/L	4 and 17 µg/L; 200 larvae per dose; *n* = 4 per control and treatment; *n* = 2 solvent controls	4 µg/L was variable for gene expression.	
Hsp70 mRNA expression	*Acropora tenuis*, scleractinian coral	Larvae	↑	17 µg/L	4 and 17 µg/L; 200 larvae per dose; *n* = 4 per control and treatment; *n* = 2 solvent controls	4 µg/L was variable for gene expression.	
Hsp90 mRNA expression	*Acropora tenuis*, scleractinian coral	Larvae	↑	17 µg/L	4 and 17 µg/L; 200 larvae per dose; *n* = 4 per control and treatment; *n* = 2 solvent controls	4 µg/L was variable for gene expression.	
DCFH-DA	*Caenorhabditis elegans*	Whole body	↑ at 12 h	8 µg/L	Exposures, 6–12 h; dose range, 4–8 µg/L	No. of replicates was unclear.	Roh et al. (2018)
SOD activity	*Caenorhabditis elegans*	Whole body	↑ at 12 h	8 µg/L	Exposures, 6–12 h; dose range, 4–8 µg/L		
LPO (TBARS)	*Caenorhabditis elegans*	Whole body	No change	8 µg/L	Exposures, 6–12 h; dose range, 4–8 µg/L		
GSH content	*Caenorhabditis elegans*	Whole body	↑ at 3 h	400 µg/L	Exposures, 3 h		
Sod1 mRNA expression	*Caenorhabditis elegans*	Whole body	↑ at 6 h	8 µg/L	Exposures, 6–12 h; dose range, 4–8 µg/L		
Sod2 mRNA expression	*Caenorhabditis elegans*	Whole body	No change	8 µg/L	Exposures, 6–12 h; dose range, 4–8 µg/L		

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
Sod3 mRNA expression	*Caenorhabditis elegans*	Worm	No change	8 µg/L	Exposures, 6–12 h; dose range, 4–8 µg/L		Roh et al. (2018) (cont.)
DCFH-DA	*Eisenia fetida*, earthworm	Coelomocytes	↑	1 nM	Dose range tested, 0, 1, 300, 600, 1000 nM; significant result for all doses tested		Sun et al. (2020)
GSH activity	*Eisenia fetida*, earthworm	Coelomocytes	No change	60 nM	Dose range tested, 0–60 nM		
CAT activity	*Eisenia fetida*, earthworm	Coelomocytes	↑	6 nM	Dose range tested, 0–300 nM		
SOD activity	*Eisenia fetida*, earthworm	Coelomocytes	↑	3 nM	Dose range tested, 0–1000 nM		
LPO (malonaldehyde)	*Eisenia fetida*, earthworm	Coelomocytes	Upward trend; not significant	100 nM	Dose range tested, 0–100 nM		
TAOC	*Eisenia fetida*, earthworm	Coelomocytes	↑	3 nM	Dose range tested, 0–300 nM; only significant at 3 nM		
DCFH-DA staining	*Marchantia polymorpha* L., liverwort	Thallus	↑	280 µM	Exposure, 30 days; $n = 4$; unclear if there were replicates	Concentrations of anthracene resembled those observed in soil.	Spinedi et al. (2021)
LPO (malonaldehyde)		Whole plant	↑	280 µM			
SOD activity		Whole plant	No change	280 µM			
CAT activity		Whole plant	No change	280 µM			
POD activity		Whole plant	↑	50 µM			
APx activity		Whole plant	↑	280 µM			

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
LPO (TBARS)	*Sinapis alba*, *Triticum aestivum*, *Phaseolus vulgaris*	Whole plant	No change	2 µM	5–7 seeds/Petri dish; 6 Petri dishes/concentration; exposure, 96 h.		Paková et al. (2006)
GPx activity	*Sinapis alba*, *Triticum aestivum*, *Phaseolus vulgaris*	Whole plant	No change	2 µM			
GSH activity	*Sinapis alba*	Whole plant	No change	0.2 µM, 0.02 µM			
	Triticum aestivum		↑				
	Phaseolus vulgaris		↑				
GST activity	*Sinapis alba*	Whole plant	No change	0.2 µM			
	Triticum aestivum		No change				
	Phaseolus vulgaris		↑				
GSR activity	*Sinapis alba*	Whole plant	No change	0.2 µM			
	Triticum aestivum		↑				
	Phaseolus vulgaris		No change				
LPO[f]	*Glomus irregulare*, arbuscular mycorrhizal fungi	Extraradical hyphae	↑	280 µM	Two doses based on previous study in 2009; 6-week exposure to anthracene; 5 replicates.		Debiane et al. (2011)
SOD activity	*Desmodesmus obliquus*	Algal cells	↑	250 µg/L	Exposure, 1–24 h; dose was EC_{50} for growth; $n = 4$, unclear if replicated.		Pokora & Tukaj (2010)
	D. microspina		No change				
	D. subspicatus		No change				
SOD activity	*Scenedesmus armatus*, green alga	Algal cells	↑	0.5 mg/L	Exposure, 1–24 h; 3 replicates.		Aksmann & Tukaj (2004)
mRNA expression: Fds1 (FeSOD isoform)	*Chlamydomonas reinhardtii*, green alga	Algal cells	↑ at 12 and 24 h	5 µM	Exposures, 3–24 h; $n = 4$; unclear if replicated.		Aksmann et al. (2014)
Msd3 (MnSOD isoform)			↓ at 24 h				
Msd5 (MnSOD isoform)			↑ at 12 and 24 h				

Table 4.9 (continued)

Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose (LED, HID) or concentration (LEC, HIC)[b]	Route[c], duration, dosing regimen	Comments	Reference
CAT activity			↑ only at 24 h	5 µM			Aksmann et al. (2014) (cont.)
Cat1 mRNA expression			↑ only at 24 h				
APx activity			↓	5 µM			
Apx1 mRNA expression			No change				
H_2O_2			↑	5 µM			
H_2O_2			↑	5 µM	Exposures, 0–72 h; repeated 3 times.		González et al. (2021)
$O_2^{\bullet}$			↑	5 µM			
LPO			↑	5 µM			
SOD activity			↑	5 µM			
CAT activity			↑	5 µM			
GSR activity			↑	5 µM			
GPx activity			↑	5 µM			
APx activity			↑	5 µM			
DHAR			No change	5 µM			

APx, ascorbate peroxidase; DHAR, dehydroascorbate reductase; CAT, catalase; DCFH-DA, 2,7-dichlorofluorescein diacetate, DHAR, dehydroascorbate reductase; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; EC_{50}, half-maximal effective concentration; GST, glutathione-*S*-transferase; GPx, glutathione peroxidase; GSH, glutathione; GSR, glutathione reductase; h, hour(s); HIC, highest ineffective concentration; HID, highest ineffective dose; H_2O_2, hydrogen peroxide; Hsp, heat-shock protein; LEC, lowest effective concentration; LED, lowest effective dose; LPO, lipid peroxidation; min, minute(s); MnSOD, manganese superoxide dismutase; NAC, *N*-acetyl cysteine; $O_2^{\bullet}$, superoxide anion radical; POD, peroxidase; SOD, superoxide dismutase; TAOC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; UV, ultraviolet.

[a] ↑, increase; ↓, decrease. An arrow (↑ or ↓) indicates a significant difference compared with controls.

[b] If there was a response, the LEC or LED is not listed; if there were no changes, the HIC or HID is listed.

[c] Route for all marine or fresh-water animals was either in seawater or in fresh water. Anthracene was diluted in DMSO or acetone and then in the medium (water/solvent) used.

[d] For CAT, GPx, SOD, GST, and GSR, total protein was used for normalization in all studies and determined using routine assays, such as the Bradford protein assay.

[e] Same species of clam as *Ruditapes decussatus*.

[f] Malondialdehyde–thiobarbituric acid adducts measured; LPO was measured by TBARS.

that these two studies were not informative due to various limitations: small sample size, poor exposure assessment to anthracene, and no adequate adjustments for co-exposures (see also Section 1.6).]

Urinary concentrations of 8-oxodG and several PAHs, including anthracene, were measured in 93 healthy male workers from an electric steel foundry in Tunisia (Hanchi et al., 2017). Cotinine was also measured as a biomarker for smoking and used as an additional predictor variable alongside job title, body mass index, age, and creatinine. Measurement of 8-oxodG and cotinine was performed using LC-MS. Three PAHs were predictors of 8-oxodG: anthracene, phenanthrene, and naphthalene. For anthracene, it was estimated that for each 10-fold increase in urinary anthracene excreted, there was an approximately 2-fold (186%) increase in 8-oxodG excretion (Hanchi et al., 2017). [The Working Group deemed the study of low informativeness because of the lack of longitudinal exposure measurements.]

(ii) Human primary cells

See Table 4.7.

In one study in human primary peripheral blood lymphocytes exposed to increasing concentrations of anthracene (0.05–0.25 μM) for 24 hours, superoxide anion radicals significantly correlated with exposure (r^2 = 0.83, P < 0.05) (Uribe-Hernández et al., 2008). Bhargava et al. (2020), in addition to epigenetic alterations (as reported in Section 4.2.3), also examined the potential of 10 μM anthracene to induce oxidative stress in mitochondria of isolated lymphocytes. Anthracene induced a significant increase in ROS production with a time–response relation from 30 minutes to 3 hours, that levelled off at 6 hours, as measured by CellROX assay. It also induced an increase in NRF2 protein levels (pg/mL; measured by ELISA) and a significant alteration in mitochondrial integrity, with a maximum at 6 hours, attested by an increase in mitochondrial membrane potential (measured with MitoProbe DilC1) (Bhargava et al., 2020).

Conversely, there was no production of superoxide anion radicals in human primary peripheral blood monocytes exposed to anthracene at 10 μg/mL for 24 hours (Fabiani et al., 1999).

[The Working Group considered the study from Uribe-Hernández et al. (2008) to be the most informative of those investigating the effects of anthracene in human primary cells, as it examined the effects of multiple concentrations and made use of the most relevant assays.]

(iii) Human cell lines

The potential effects on oxidative stress of particulate matter with diameter of ≤ 2.5 μm ($PM_{2.5}$) extracts from stoves in the rural Guanzhong Plain, China, were investigated. Levels of individual PAHs (including anthracene) were measured by GC-MS, and $PM_{2.5}$ extracts were used to treat A549 cells (human alveolar basal epithelial cells). ROS were measured by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) assay. The levels of anthracene measured in different samples of $PM_{2.5}$ ranged from 0.01 to 1.6 mg/kg. Associations between ROS and PAHs were determined using Pearson correlation coefficients. Exposure to $PM_{2.5}$ extracts caused a concentration-dependent decline in cell viability but an increase in ROS. The correlation values (R) for pyrene, anthracene, and benzo[*a*]anthracene exceeded 0.80 (R = 0.85, 0.81, and 0.80, respectively). Inflammatory tumour necrosis factor alpha (TNFα) and interleukin 6 (IL-6) also exhibited better correlations with these three species than with other PAH species (P < 0.05 for acenaphthylene and acenaphthene, P > 0.05 for the others), demonstrating that the inflammatory response was induced by oxidative stress (Sun et al., 2018). [The Working Group noted that, although $PM_{2.5}$ extracts include several compounds, there was a positive association between anthracene and ROS increase.]

As observed with genotoxicity end-points, anthracene can be activated and modified by sunlight or UV-A or UV-B exposure to two photoproducts, anthrone and 9,10-anthracenedione, leading to phototoxicity (Mujtaba et al., 2011). Significant levels of oxidative stress, measured by DCFH-DA assay, were observed in a human skin epidermal cell line (HaCaT) after exposure to anthracene at all doses tested (0.01–0.5 μg/mL) followed by sunlight or UV-A exposure (Mujtaba et al., 2011). Also, superoxide anion ($O_2^{\bullet}$) and hydroxyl ($^{\bullet}OH$) radical generation were both significantly increased in these cells after anthracene exposure (0.1–1 μg/mL) combined with sunlight, UV-A, or UV-B, further supporting the phototoxicity of anthracene and its potential to elicit significant oxidative stress. Phototoxicity was also observed in non-mammalian models, as described below (Choi & Oris, 2000a, b). [The Working Group considered that the study from Mujtaba et al. (2011) was relevant, based on the evidence for increased oxidative stress induction.]

(b) Experimental systems

(i) Non-human mammalian cells in vitro

See Table 4.8.

The effects of anthracene exposure on several oxidative stress-associated end-points, including DCFH-DA, protein carbonyls, and MDA were assessed in rat cardiomyocytes in vitro. Treatment with anthracene at 10 μM induced a significant increase in protein carbonyls and MDA when compared with controls (Ju et al., 2020). Ju et al. also observed a significant increase in ROS, as measured with DCFH-DA staining, in rat aortic vascular smooth muscle cells exposed to anthracene at 10 μM (Ju et al., 2022).

In another study, significant increases in DCFH-DA, catalase activity, glutathione *S*-transferase (GST) activity, and total glutathione (GSH) were observed after exposure to anthracene at concentrations ranging from 25 to 125 μM in a murine hippocampal neuronal cell line (HT-22) (Olasehinde & Olaniran, 2022).

(ii) Non-mammalian test systems

See Table 4.9.

Several studies investigating the association between anthracene and oxidative stress biomarkers in non-mammalian species were available to the Working Group.

In the common goby, *Pomatoschistus microps*, anthracene (at concentrations ranging from 0.5 μg/L to 4 μg/L) induced significant increases in antioxidant enzyme activity, namely CAT, SOD, and phase II biotransformation markers GPx and glutathione reductase (GSR) in the liver, and decreases in GST (Vieira et al., 2008). In the milkfish, *Chanos chanos* (Forsskal), lipid peroxidation markers (LPO, MDA, and CAT) and phase II biotransformation markers (GST and GSH) were also significantly altered in a dose- and time-dependent manner in various tissues (e.g. gill, head, and dorsal fin) (Palanikumar et al., 2012). Conversely, anthracene treatment did not induce lipid peroxidation in a topminnow (*Poeciliopsis lucida*) hepatoma cell line (PLHC-1) and in a bluegill sunfish (*Lepomis machrochirus*) liver microsome model (Choi & Oris, 2000a, b), except in the presence of UV light. In addition, anthracene exposure did not elicit oxidative stress in a zebrafish (*Danio rerio*) model (St Mary et al., 2021).

Anthracene (at doses ranging from 256 to 1024 μg/L, or 1.44–5.75 μM) induced significant increases in the antioxidant enzymes SOD, CAT, and GPx but not GST in a prawn (*Palaemon serratus*) model; LPO was also significantly increased at 32 μg/L (Gravato et al., 2014). Similarly, anthracene exposure induced significant alterations in numerous antioxidant enzyme activities, as well as superoxide production and LPO markers such as protein carbonyls, in the gills, digestive glands, and haemocytes of molluscs (various Bivalvia). Species differences were observed in the degree of the responses

(dose and time) and tissue sensitivity, with the gill representing the most sensitive tissue tested (Giannapas et al., 2012; Sellami, et al., 2015a, b; Badreddine et al., 2017; Mengqi et al., 2017). Increased gene expression of several antioxidant and heat-shock stress biomarkers (*Cat*, *Hsp70*, *Hsp90*, *MnSod*) was observed in response to anthracene treatment in coral larvae. However, this was followed by a decrease in SOD activity (Overmans et al., 2018). In the microcrustacean water flea (*Daphnia magna*), GST and GPx activities were decreased after anthracene treatment (Feldmannová et al., 2006). Overall increases in ROS, SOD activity, CAT activity, and total antioxidant capacity were also reported in *C. elegans* and *Eisenia fetida* (earthworm) models (Roh et al., 2018; Sun et al., 2020). [The Working Group considered that the doses for these studies in worms to be low but relevant (8–400 µg/L for *C. elegans*; 1–1000 nM for *Eisenia fetida*).]

In several plants (*Marchantia polymorpha* L.*; Sinapis alba*, *Triticum aestivum*, and *Phaseolus vulgaris*), oxidative stress end-points (such as ROS, LPO, peroxidase, and ascorbate peroxidase) were altered, at least one of them significantly, after anthracene exposure (Paková et al., 2006; Spinedi et al., 2021).

Exposure to anthracene at a high concentration (280 µM) also increased LPO in the arbuscular mycorrhizal fungi *Glomus irregulare* (Debiane et al., 2011). Numerous biomarkers were also altered in several algae species, including *Desmodesmus obliquus*, *D. microspina*, *D. subspicatus*, *Scenedesmus armatus*, *Chlamydomonas reinhardtii*, and *Ulva lactuca* (a marine macroalga) (Aksmann & Tukaj, 2004; Pokora & Tukaj, 2010; Aksmann et al., 2014; González et al., 2021).

[The Working Group considered that all the non-mammalian test systems reported above, despite representing different species, environments, or dose regimens, were relevant bioindicators for toxicities that may elicit adverse human health effects. The doses used (especially in studies in molluscs and crustaceans) were highly relevant to human exposures of 0.05–1024 µg/L. The Working Group also considered that phototoxicity is a concern for exposure to anthracene, given that common exposures are from air pollution outdoors and in the sunlight, therefore anthracene photo-modifications should not be overlooked with regard to the carcinogenic potential of anthracene when combined with sunlight.]

4.2.5 Induces chronic inflammation

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human primary cells and cell lines

Two in vitro studies in human cells were available to the Working Group (Lin et al., 2012; Oostingh et al., 2015). In the first study on endothelial dysfunction (Lin et al., 2012), the effects of particles from incense burning in temples were investigated in normal human coronary artery endothelial cells (HCAEC). Cells were treated with extracts of particulate matter (PM) in three size ranges – $PM_{0.1}$ (diameter < 0.1 µm), $PM_{1.0–0.1}$ (diameter between 1.0 and 0.1 µm), and $PM_{10–1.0}$ (diameter between 10 and 1.0 µm) – at a concentration of 50 µg/mL for 4 hours, and concentrations of IL-6, endothelin-1 (ET-1), and nitric oxide (NO) in the medium were measured. Depending on the particle size and parameter analysed, different effects were observed. $PM_{1.0–0.1}$ stimulation resulted in significantly higher IL-6 and ET-1 production than did $PM_{0.1}$ or $PM_{10–1.0}$. Exposure of cells to $PM_{1.0–0.1}$ markedly reduced NO formation, whereas $PM_{10–1.0}$ and $PM_{0.1}$ activated cells to synthesize higher levels of NO than did the controls. Anthracene was more abundant in $PM_{1.0–0.1}$ and $PM_{10–1.0}$ than in $PM_{0.1}$ (ultrafine particles). This study found that the size and composition of these particles were both important factors in inducing cytokine production and reducing NO formation in HCAEC cultures. In

the correlation of PAHs in $PM_{1.0–0.1}$ with NO, a statistically significant inverse correlation (–0.97) was found for anthracene. Anthracene, naphthalene, acenaphthylene, and acenaphthene in $PM_{1.0–0.1}$ were all highly correlated with NO reduction. No significant correlation was observed between PAHs and the other biological end-points. [The Working Group considered that, although the study was well-conducted, the individual components of the PM extracts were not tested alone, therefore, the contribution of anthracene is unknown.]

In the other study (Oostingh et al., 2015), the immunomodulatory effects of nine different PAHs at aqueous solubility in human alveolar basal epithelial cells (A549 cell line) were determined by analysing the cytokine promoter expression of three different inflammatory cytokines (IL-8, TNFα, IL-6) and NF-κB in stably transfected recombinant A549 cell lines. Anthracene did not affect TNFα or IL-6, and caused only a moderate non-statistically significant increase in IL-8 promoter induction. [The Working Group considered that the study was well conducted; however, release of the selected pro-inflammatory cytokines was not measured.] Anthracene did not induce pro-inflammatory cytokine (IL-8, TNF-α, IL-6) transcription activity in A549 cells. [The Working Group considered that, on the basis of these in vitro studies, it is not clear whether anthracene has inflammatory potential in vitro.]

(b) Experimental systems

Six studies in experimental systems were available to the Working Group (Forbes et al., 1976; Brune et al., 1978; JBRC, 1994a, b, c, d).

In the study by Forbes et al. (1976), anthracene (0.1 g/L, diluted in methanol, 40 µL) did not induce any alterations when applied to the skin (20 cm^2) of Skh: hairless-1 outbred mice. When the same mice were irradiated with solar-simulator radiation after exposure to anthracene (0.1 g/L in methanol; 40 µL per 20 cm^2) (see Section 3, Cancer in Experimental Animals), there was a more severe skin response than in mice whose skin had been pre-treated with the irradiated vehicle; inflammatory changes (oedema and redness) were visible by 6 hours, but no longer visible after 48 hours. Under the above experimental conditions, anthracene in the absence of solar irradiation did not induce skin inflammation (Forbes et al., 1976).

Brune et al. (1978) compared the inflammatory, tumour-initiating, and tumour-promoting activities of several compounds, including anthracene, applied to the NMRI mouse ear. The ID_{50} (irritant dose required to produce a discernible irritant reaction in 50% of the population; as assessed by standard methods at 24 hours after the administration of anthracene) was 6.6×10^{-4} mmol/ear, that is, anthracene was 10 times less potent than the other aromatic hydrocarbons tested (DMBA and B[a]P) and did not have an irritant effect. There was no detectable production of prostaglandin E_2 (PGE_2) or initiation–promotion activity (in a standard experiment with TPA as promoter). [The Working Group considered this study to be of low informativeness since many details were missing, including the supplier and the purity of the chemicals used (which were obtained from commercial sources and purified by recrystallization), and the sex and number of animals tested.]

Two dose-finding studies for carcinogenicity tests that complied with GLP were conducted by the Japan Bioassay Research Center (JBRC, 1994a, b, c, d). Groups of 5 (2-week study) or 10 (13-week study) male and female Crj:BDF_1 mice or F344/DuCrj rats (age, 5 weeks) were treated with feed containing anthracene (purity, ≥ 97.9%) at a dose of 0, 80, 400, 2000, 10 000, or 50 000 ppm for 2 or 13 weeks (JBRC, 1994a, b, c, d). In both studies, there was no significant increase in histopathology findings suggesting chronic inflammation. [The Working Group considered that the results from these studies were not sufficient

to show a chronic inflammatory potential for anthracene in experimental systems.]

4.2.6 Is immunosuppressive

(a) Humans

(i) Exposed humans

No data in exposed humans were available to the Working Group.

(ii) Human cell lines

Two in vitro studies using human cells were available to the Working Group (Zhao et al., 1996; Oostingh et al., 2015). Zhao et al. (1996) investigated the effect of several PAHs, including anthracene, on cloned Ca^{2+}-ATPases (SERCA1, SERCA2a, and SERCA3, which are involved in Ca^{2+}-dependent pathways of T-cell and B-cell activation) that were transiently expressed in human embryonic kidney (HEK) cells. The purpose of the study was to determine whether PAHs directly inhibited cloned SERCA enzymes and whether there was any selectivity for certain isoforms. All PAHs tested, including anthracene, had little inhibitory effect on any of the SERCA enzymes tested, indicating that metabolism might be required for PAH-induced inhibition, or that other cellular elements not present in the HEK transfection model might be required for activity. Davila et al. (1996) and Krieger et al. (1994) had previously published a study on the immunotoxicity of several PAHs (7,12-dimethylbenz[*a*]anthracene, B[a]P, dibenz-[*a*,*h*]anthracene, and 9,10-dimethylanthracene) in murine and human lymphocytes as well as in B- and T-cell lines. In all studies, anthracene gave negative results.

In a study by Oostingh et al. (2015), anthracene showed either no effects or a moderate, not statistically significant increase in IL-8 promoter induction in A549 cells. [The Working Group considered that this study was well conducted; however, it was noted that A549 cells are not immune system cells; also, the model was considered not relevant to address immunosuppression. The Working Group considered that the in vitro studies did not show immunosuppressive effects with anthracene.]

(b) Experimental systems

(i) Non-human mammals in vivo

White et al. (1985) investigated the immunotoxic potential of several PAHs, including anthracene (160 µmol/kg per day by subcutaneous injection, in corn oil), in female B6C3F_1 mice, using a well-established protocol (14-day exposure followed by injection of sheep erythrocytes and assessment of splenic antibody-forming cells at day 4). Anthracene did not reduce the antibody response; on the contrary, an increase of 37% was observed. No changes in thymus weight or body weight were observed. [The Working Group considered this study to be informative; however, no signs of immunosuppression were observed.]

Silkworth et al. (1995) investigated the immunotoxic potential of 15 PAHs by assessing their ability to suppress the antibody response to sheep erythrocytes. In C57BL/6 ($Ah^{+/+}$) mice immunized 12 hours after a single oral dose of anthracene at 0.1, 1, 10, or 100 mg/kg, anthracene had no effect on the immune response to sheep erythrocytes. [The Working Group considered this study to be informative, and the end-point (antibody response to T-cell-dependent antigen) to be very relevant for the measurement of immunotoxicity, specifically immunosuppression. However, the results did not support an immunosuppressive effect of anthracene.]

The study by Wang & Xue (2015) (also reviewed in Section 3, Cancer in Experimental Animals) investigated the ability of several PAHs, including anthracene, to induce solid tumours (e.g. in the liver, stomach, and kidney) and the roles of these PAHs in immune response regulation via the assessment of serum IL-2 and IL-6 levels. These two cytokines were selected

because the effect of PAHs on their production is largely unknown. IL-2 is a T-cell growth factor that enhances the cytotoxic activity of T-cells, and IL-6 is a multi-effect cytokine produced by endothelial cells, monocytes/macrophages, and lymphoid cells. In cancer, the predominant role of IL-6 is the promotion of tumour growth. SPF Kun Ming mice were randomly divided into groups of 10 males and 10 females and intraperitoneally injected with 10 daily doses of DMSO (control) or anthracene (50 mg/kg). The mice were examined once daily for 3 months. There were no changes in serum IL-6 levels, and a decrease in serum IL-2 levels was not statistically significant – control group ($n = 20$), 360 ± 16 ng/L; anthracene-treated group ($n = 18$), 154 ± 5 ng/L. [The Working Group considered that this study showed a slight reduction in serum IL-2, which may be supportive of immunosuppressive effects. However, the study had several drawbacks that limited the relevance of the findings, including that there was no blind assessment of the slides, the qualifications of the pathologist were not mentioned, the results were not separated according to sex, and it was not specified whether results were expressed as mean ± standard deviation or standard error.]

In the dose-finding studies for a carcinogenicity test reported in the previous section, few changes in immune status parameters were observed (JBRC, 1994a, b, c, d).

In the 2-week study in male mice, there was a significant decrease in erythrocyte count and an increase in platelet count after exposure to anthracene at concentrations of ≥ 10 000 ppm (approximately equal to 1823 mg/kg bw), and there were decreases in haemoglobin and haematocrit at 50 000 ppm (approximately equal to 9725 mg/kg bw). In female mice, there was a decrease in leukocyte count after exposure to anthracene at 50 000 ppm (approximately equal to 7690 mg/kg bw), an increase in platelet count at ≥ 10 000 ppm (approximately equal to 1472 mg/kg bw), and decreases in haemoglobin and haematocrit.

In the 13-week study in mice, no changes in leukocyte count were found in males, but there were significant decreases in erythrocyte count, haemoglobin, and haematocrit, and increases in mean corpuscular volume and platelets in males exposed to anthracene at concentrations of ≥ 10 000 ppm. In females, there was a decrease in leukocyte count at 10 000 ppm, a significant decrease in erythrocytes at ≥ 2000 ppm (approximately equal to 287 mg/kg bw), an increase in platelets at ≥ 10 000 ppm, and decreases in haemoglobin and haematocrit. In addition, there was a significant increase in absolute and relative weights of the spleen in males at 50 000 ppm. There was also an increase in the incidence of extramedullary haematopoiesis in the spleen of males at ≥ 400 ppm (approximately equal to 73 mg/kg bw) and females at ≥ 10 000 ppm.

In the 2-week study in rats, there were significant decreases in erythrocyte count, haemoglobin, and haematocrit in males exposed to anthracene at ≥ 2000 ppm and in females at ≥ 400 ppm. There was a significant increase in absolute and relative weights of spleen in males and females at ≥ 400 ppm.

In the 13-week study in male rats, there were significant decreases in erythrocyte count and haemoglobin and increases in mean corpuscular volume and platelet count at ≥ 400 ppm, and a decrease in haematocrit at ≥ 10 000 ppm. In females, there were significant decreases in erythrocyte count, haemoglobin, and mean corpuscular haemoglobin concentration, and increases in mean corpuscular volume and platelet count at ≥ 400 ppm, and a decrease in haematocrit at ≥ 2000 ppm. There was a significant decrease in absolute and relative weights of the thymus in males at 50 000 ppm, and a significant increase in absolute and relative weights of the spleen in males and females at ≥ 400 ppm. There was also a significant increase in the incidence of engorgement of erythrocytes in the spleen of males and females at ≥ 400 ppm, and a significant increase in the incidence of haematopoiesis in the bone

marrow of males at ≥ 2000 ppm and of females at ≥ 400 ppm (JBRC, 1994a, b, c, d). [The Working Group noted that changes in immune status parameters were observed only occasionally. These changes were inconsistent between species and sexes, with no dose–response relation, and were thus not supportive of immunosuppression.]

(ii) Non-human mammalian cells in vitro

Sonnenfeld et al. (1984) investigated the effects of several aromatic compounds, including anthracene, on polyriboinosinic:polyribocytidylic acid (poly I:C)-induced production of interferon alpha or beta (IFNα, IFNβ) (important in body defences against viral infection and with antitumour effects). Primary mouse fibroblasts were exposed in vitro for 24 hours to anthracene (10 or 100 μM) and then treated with poly I:C. At non-cytotoxic concentrations, anthracene did not affect IFNα and IFNβ production; non-statistically significant decreases of 17% and 27% were observed at 10 and 100 μM, respectively (Sonnenfeld et al., 1984). [The Working Group noted that fibroblasts are not immune cells and considered that the model was not relevant to address immunosuppression.]

[Overall, the Working Group noted that results from studies in experimental systems did not support a potential immunosuppressive effect for anthracene.]

4.2.7 Modulates receptor-mediated effects

(a) Humans

(i) Exposed humans

No data in exposed humans were available to the Working Group.

(ii) Human cell lines

See Table 4.10.

The effects of anthracene on the activation of estrogen receptor (ER), androgen receptor (AR), glucocorticoid receptor (GR), early growth response protein 1 (EGR-1), peroxisome proliferator-activated receptor alpha and beta/delta (PPARα, PPARβ/δ), constitutive androstane receptor (CAR), and the aryl hydrocarbon receptor (AhR) were investigated.

Two studies investigated the effect of anthracene on ER activation in a human breast carcinoma cell line (MCF-7) (Vondrácek et al., 2002; Gozgit et al., 2004). In the study by Vondrácek et al. (2002), several PAHs, including anthracene, were found to act as very weak inducers of ER-mediated activity in MCF-7 cells stably transfected with a luciferase reporter gene. The induction of luciferase was statistically significant at 6 hours with anthracene at ≥ 5 μM but not sufficient to calculate the induction equivalent factor (the ratio between the concentration of 17β-estradiol that was 25% effective and the concentration of PAH inducing the same level of luciferase activity). [The Working Group considered that the two studies above were informative and of good-quality design.]

Gozgit et al. (2004) investigated the estrogenicity of PAHs in the MCF-7 cell line, testing 14 PAHs for their ability to bind to either the ER or the AhR and to activate target gene expression. PAHs were tested at concentrations of 0.01–5 μM. PAHs that caused induction of estrogen-response element (ERE)-mediated luciferase expression were further studied using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to evaluate the expression of estrogen-responsive genes (HEM45, progesterone receptor, and pS2) and an aryl hydrocarbon-responsive gene (*CYP1A1*) in MCF-7 cells. Under conditions permissive of metabolism, anthracene was a weak inducer of ER-reporter luciferase activity (mean fold increase, 1.85) but did not induce mRNA expression of the three estrogen-responsive genes (HEM45, progesterone receptor, and pS2) or CYP1A1 mRNA expression, suggesting that the ER-reporter gene assay may detect concentrations of toxicants that are not physiologically active.

Table 4.10 End-points relevant to modulation of receptor-mediated effects with anthracene in human cells in vitro

End-point	Assay	Species, strain (sex), cell line	Tissue	Results	Dose or concentration	Route, duration, dosing regimen	Comments	Reference
CAR	Cell-based luciferase reporter assay	HepG2 cells	Liver	No differences	25 and 50 μM	24 h		
ER	[^{3}H]estradiol displacement	MCF-7 cells	Breast, adenocarcinoma	No differences	0–2 mM	10 min	Sigma; recrystallization and HPLC to ensure purity.	Chang & Liao (1987)
ERα	Luciferase reporter gene	MVLN cell line	Mammary gland, adenocarcinoma	No differences	Up to 400 μg/mL	72 h	Purity, 99%.	Villeneuve et al. (2002)
ERα	ERα CALUX	VM7Luc4E2 cell line	Mammary gland, adenocarcinoma	Weak activator, EC_{50} = 0.12 ± 0.02 mM	Up to 10 mM	19–22 h	Sigma, purity not reported.	Boonen et al. (2020)
PPARγ	PPARγ CALUX	U2OS cells	Osteosarcoma	Weak activator, EC_{50} = 0.13 ± 0.8 mM	Up to 10 mM	24 h	Sigma, purity not reported.	Boonen et al. (2020)

CALUX, chemical activated luciferase gene expression; CAR, constitutive androstane receptor; EC_{50}, half-maximal effective concentration; ER, estrogen receptor; h, hour(s); HPLC, high-performance liquid chromatography; min, minute(s); PPARγ, peroxisome proliferator-activated receptor gamma.

Concerning effects on other receptors, two studies were available: Kizu et al. (2003) and Kim et al. (2005). In the study by Kizu et al. (2003), the role of AhR on the anti-androgenic effects of PAHs was studied in human prostate carcinoma cells (LNCaP). The aims of the study were to determine whether AhR is involved in the anti-androgenicity of PAHs, and to obtain information on the molecular mechanisms of AhR-mediated anti-androgenic effects. Contrary to other PAHs, anthracene (1 μM) did not act as an AhR agonist, did not show anti-androgenic effects, and did not inhibit binding of the AR (in nuclear extracts) to oligonucleotide probes containing the AR-responsive element.

Kim et al. (2005) evaluated the ability of 15 PAHs to activate the EGR-1 gene and binding to PPARα and PPARβ/δ in cultures of human lung adenocarcinoma cells (A549) and human colorectal adenocarcinoma cells (HCT-116). The luciferase reporter gene was used to measure the activity of PPARs and transactivation of the EGR-1 promoter. Anthracene at 10 μM caused a significant increase in luciferase activity mediated by EGR-1, PPARα, and PPARβ/δ, which may be relevant in tumour progression and inflammation.

[The Working Group noted that, overall, data suggest that anthracene has weak estrogenic activity; however, this was not sufficient to calculate the induction equivalent factor or to induce mRNA expression of estrogen-responsive genes or CYP1A1 mRNA expression, suggesting that the ER-reporter gene assay may detect anthracene effects that do not result in biological activity. Concerning other receptors, anthracene caused a significant increase in EGR-1, PPARα, and PPARβ/δ luciferase activity, but no activation of AhR or activation/inhibition of AR was observed.]

(b) *Experimental systems*

See Table 4.11.

(i) *Non-human mammals in vivo*

Three studies were available to the Working Group (Chaloupka et al., 1994; Shimada et al., 2002; Yang et al., 2019). The study by Chaloupka et al. (1994) investigated the effect of several tricyclic hydrocarbons on hepatic microsomal methoxyresorufin *O*-demethylase (MROD) activity, Cyp1A2 and Cyp1A1 mRNA expression, and AhR binding in $B6C3F_1$ mice. Male $B6C3F_1$ mice were treated intraperitoneally with anthracene (0, 50, 100, 200, 300 mg/kg), and hepatic microsomal MROD activity was determined fluorimetrically 24 hours after treatment. Although it induced dose-dependent hepatic microsomal MROD activity and CyP1A2 expression without co-induction of CyP1A1, anthracene did not competitively displace radiolabelled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or B[a]P ([^{3}H]TCDD or [^{3}H]benzo[*a*]pyrene) from the mouse hepatic cytosolic AhR or the 4S carcinogen-binding protein. These data indicate that the induction of Cyp1A2 is independent from AhR activation. [The Working Group noted that this study was well conducted and informative.]

Shimada et al. (2002) investigated the effects of several PAHs and polychlorinated biphenyls on the induction of CYP1A1, 1A2, and 1B1 mRNA in the liver and lung of $AhR^{(+/+)}$ and $AhR^{(-/-)}$ mice of strain C57BL/6J. PAHs, including anthracene, and polychlorinated biphenyls were intraperitoneally injected at a dose of 100 mg/kg (olive oil was used as vehicle control). The mice were killed after 72 hours. Anthracene weakly induced expression of CYP1A1, 1A2 and 1B1 mRNA in the liver of $AhR^{(+/+)}$ mice. In $AhR^{(-/-)}$ mice, no induction was observed, indicating that the induction of CYP1A1, 1A2, and 1B1 occurred through an AhR-dependent mechanism. [The Working Group judged this study to be of low relevance because the number of animals investigated and statistical significance were not reported.]

Yang et al. (2019) investigated the effects of exposure to phenanthrene and anthracene on

Table 4.11 End-points relevant to modulation of receptor-mediated effects with anthracene in non-human mammalian systems in vivo and in vitro

End-point	Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose or concentration	Route, duration, dosing regimen	Comments	Reference
AhR	[^{3}H]TCDD or [^{3}H]-benzo[*a*]pyrene displacement	Mouse, B6C3F$_1$ (M)	Liver	No differences	50, 100, 200, 300 mg/kg	i.p., 24 h, single	Purity, 99%	Chaloupka et al. (1994)
MROD activity		Mouse, B6C3F$_1$ (M)	Liver	↑	50, 100, 200, 300 mg/kg	i.p., 24 h, single	Purity, 99%	
CYP1A2	Northern blot	Mouse, B6C3F$_1$ (M)	Liver	↑	50, 100, 200, 300 mg/kg	i.p., 24 h, single	Purity, 99%	
CYP1A1	Northern blot	Mouse, B6C3F$_1$ (M)	Liver	No differences	50, 100, 200, 300 mg/kg	i.p., 24 h, single	Purity, 99%	
CYP1A1, 1A2, 1B1	RT-PCR	Mouse, C57BL/6	Liver	(↑), weak induction only in AhR$^{(+/+)}$ mice	100 mg/kg	i.p., 72 h, single	Highest purity. Limitations: small sample size; no statistics reported.	Shimada et al. (2002)
CYP2B10	Real-time-PCR	Mouse, C57BL/6	Liver	No differences	350 mg/kg	Oral, 4 days, animals were killed 24 h after the last dose	From NTP repository. Limitations: lack of randomization, lack of blinding assessment for the animal studies, and the number of independent experiments performed for the in vitro experiments was not reported.	Yang et al. (2019)
AhR	Luciferase reporter gene	Rat, H4IIE-Luc hepatoma cell line	Hepatocarcinoma	No differences	Up to 400 µg/mL	72 h	Purity, 99%.	Villeneuve et al. (2002)
AhR	Luciferase reporter gene	Mouse, H1L1.1c2 hepatoma cell line	Hepatocarcinoma	No differences	Up to 10 µM	3 h	Sigma; purity, NR.	Ziccardi et al. (2002)
AhR	[^{3}H]TCDD displacement from AhR	Mouse, liver cytosol from C57BL/6N	Liver	+, 50 ± 8% displacement	1 µM	1 h	Sigma; purity, NR.	Bigelow & Nebert (1982)

Table 4.11 (continued)

End-point	Assay	Species, strain (sex), cell line	Tissue	Results[a]	Dose or concentration	Route, duration, dosing regimen	Comments	Reference
CYP1A1	Western blot	Rat, Fischer 344	Hepatocytes	No differences	10 µM	48 h	Purity, > 99%. Western blot not quantifiable.	Safa et al. (1997)
CYP2C11	Western blot	Rat, Fischer 344	Hepatocytes	+, ↓ 37%	10 µM	48 h		
AhR	[^{3}H]TCDD displacement	Rat, Fischer 344	Liver cytosol	Weak activator (IC_{50} binding affinity, > 100 µM AhR activation, 570 µM	Various concentrations tested	1 h		
AhR	AhR-CALUX	Mouse, H1L77.5c1	Hepatocarcinoma	Weak activator (no EC calculable, a fold induction of 1.60 ± 0.19 was calculated)	Up to 10 mM	48 h	Sigma; purity, NR.	Boonen et al. (2020)
AR	[^{3}H]R1881 displacement	Rat, Sprague-Dawley, tissue homogenate	Ventral prostate	No differences	0–2 mM	10 min	Sigma, recrystallization and HPLC to ensure purity.	Chang & Liao (1987)
GR	[^{3}H]dexamethasone displacement	Rat, Sprague-Dawley, tissue homogenate	Liver	No differences	0–2 mM	10 min	Sigma, recrystallization and HPLC to ensure purity.	
AhR	Yeast-based bioassay	*Saccharomyces cerevisiae* strain, YCM3	Yeast	Weak activator (< 25% of the maximal β-NF activity)	10 µM	18 h	Purity, > 99%.	Alnafisi et al. (2007)
ER	Yeast two-hybrid system	Y190	Yeast	No differences	Up to 1 mg/mL	NR	Purity, 99%.	Kurihara et al. (2005)

AhR, aryl hydrocarbon receptor; AR, androgen receptor; CALUX, chemical activated luciferase gene expression; CYP, cytochrome P450; EC, effective concentration; ER, estrogen receptor; GR, glucocorticoid receptor; h, hour(s); HPLC, high-performance liquid chromatography; IC_{50}, half-maximal inhibitory concentration; i.p., intraperitoneal; min, minute(s); MROD, methoxyresorufin *O*-demethylase; β-NF, beta-naphthoflavone; NR, not reported; NTP, National Toxicology Program; RT-PCR, reverse transcription-polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[a] +, positive; ↑, increase; ↓, decrease; (↑), decrease, not quantitated.

the liver, and the underlying mechanisms. In the study, C57BL/6 mice and human hepatocytes (HepG2 cells and primary human hepatocytes) were used. Phenanthrene or anthracene (350 mg/kg per day) was orally administered daily to groups of 5–6 C57BL/6 male mice (age, 8 weeks) for four consecutive days. Corn oil was used as the vehicle. This study demonstrated that phenanthrene, but not anthracene, effectively activates both human and mouse nuclear receptor CAR and induces promoter activity and gene expression of human CYP2B6 and mouse CYP2B10, and that CAR is essential for mediating phenanthrene-induced hepatotoxicity. [The Working Group identified some limitations, including the lack of randomization, lack of blinding assessment for the animal studies, and the fact that the number of independent experiments performed for the in vitro experiments was not reported.]

[The Working Group noted that the role of AhR in anthracene-induced hepatic enzymes in vivo remains unclear since contradictory results were reported. Both studies, however, demonstrated the induction of CYP1A2. No CAR activity was observed.]

(ii) Non-human mammalian cells in vitro

Six studies investigated the effect of anthracene on AhR activation. Two studies showed no activation (Villeneuve et al., 2002; Ziccardi et al., 2002), whereas the other studies reported weak or very poor activation (Bigelow & Nebert, 1982; Safa et al., 1997; Alnafisi et al., 2007; Boonen et al., 2020). In the study by Alnafisi et al. (2007), anthracene caused very weak AhR signalling (less than 25% of the maximal activity of β-naphthoflavone) at a high concentration (10 μM). In the study by Boonen et al. (2020), anthracene showed weak AhR agonist activity (effective concentration, EC_{50}, not determined; induction, 1.60 ± 0.19-fold) in the AhR-CALUX bioassay. [The Working Group noted that, overall, in vitro data indicate that anthracene has no effect on AhR activation or very modest effects that are observed only at high concentrations, compared with other polycyclic hydrocarbons.]

Four studies investigated the effect of anthracene on ER activation. Three studies showed no activation (Chang & Liao, 1987; Villeneuve et al., 2002; Kurihara et al., 2005), whereas Boonen et al. (2020) reported weak ER activation. In the study by Boonen et al. (2020), anthracene had weak ERα agonist activities (EC_{50} = 1.21 ± 0.62 × 10^{-5} M; induction, 1.62 ± 0.10-fold) in the ERα-CALUX bioassay. In the PPARγ-CALUX bioassay, anthracene showed weak agonistic activity (EC_{50} = 1.27 ± 1.8 × 10^{-4} M; induction, 1.36 ± 0.22-fold).

Jung et al. (2001) investigated the effects of several nitrated PAHs and azoarenes in a fish hepatoma cell line (PLHC-1). Anthracene was tested only in one experiment, and no induction in ethoxyresorufin-*O*-deethylase (EROD) activity was observed. [The Working Group noted that this study, although relevant for ecotoxicological evaluation, was not considered to be relevant since the transferability of results to humans is questionable.]

[The Working Group noted that, overall, there was no strong consensus in the literature on the effects of anthracene on AhR, ER, and other nuclear receptors. Some results suggested that anthracene may have multiple modes of action and may activate or inhibit multiple receptor-signalling pathways known to play critical roles in mediating endocrine disruption. However, where observed, these effects were shown at high concentrations, several orders of magnitude higher than for other PAHs.]

4.2.8 Causes immortalization

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

See Table 4.12.

(i) Non-human mammals in vivo

Three studies evaluated the effects of anthracene on the morphological transformation of embryo cells derived from pregnant animals. In the study by DiPaolo et al. (1972), cloned Balb/3T3 embryonic fibroblast cell lines were exposed to 0.1% anthracene in vitro for 48 hours; transformed colonies were counted and reseeded. To assess for tumourigenicity, transformed cells were injected subcutaneously into X-irradiated and non-irradiated weanling Balb/c mice and assessed after 13–35 days. The same group treated pregnant Syrian Golden hamsters with 0.5 mL of anthracene (1.0–3.0 mg/100 g maternal weight) by intraperitoneal injection on days 10–11 of gestation. Embryos were excised 48–72 hours after the injection and cells from the whole embryo were cultured in vitro. Derived transforming colonies were finally injected into X-irradiated weanling male hamsters and tumourigenicity was assessed (DiPaolo et al., 1973). In a third study (Evans & DiPaolo, 1975), growth in soft agar was assessed for primary embryonic fibroblast-like cells derived from pregnant inbred syngeneic strain 2 Sewall Wright guinea-pigs on day 32 of gestation and exposed to anthracene at a dose of 0.5 µg/mL in medium. Finally, transformed colonies were injected into X-irradiated guinea-pigs and assessed for tumourigenicity. All three studies gave negative results.

(ii) Non-human mammalian cells in vitro

The Working Group identified 21 relevant original in vitro studies related to morphological transformation and immortalization. Of these, four studies investigated tumorigenicity in addition to morphological transformation (DiPaolo et al., 1972; Evans & DiPaolo, 1975; Pienta et al., 1977; Laaksonen et al., 1986). Three studies used fetal cells (DiPaolo et al., 1972; Evans & DiPaolo, 1975; Pienta et al., 1977), and one study used newborn mouse skin fibroblasts, treated in vitro (Laaksonen et al., 1986). [The Working Group noted that in all four studies, anthracene gave negative results (no morphological transformation, no tumours), whereas several other chemicals, such as B[a]P, 3-MC, or DMBA, gave positive results.]

In a series of studies, Laaksonen et al. (1983, 1984, 1986) found anthracene to give negative results in cell transformation assays. Foci formation in nude mouse fibroblasts was increased by B[a]P, 3-MC, and benz[*a*]anthracene (Laaksonen et al., 1983), and 3-MC increased SV40-induced cell transformation (Laaksonen et al., 1984, 1986), whereas anthracene gave negative results in all these studies. Similarly, in a study by Lubet et al. (1983), anthracene gave negative results whereas B[a]P, DMBA, and 3-MC gave positive results in the C3H/10T1/2 clone 8 cell transformation assay.

The Bhas 42 cell transformation assay (carried out in BALB/c 3T3 murine cells transfected with v-Ha-*ras*) was used by Asada and co-workers (Asada et al., 2005) to compare the initiation and promotion capacity of several PAHs. Anthracene gave negative results. The Bhas 42 cell transformation assay was also used in a validation study conducted by three laboratories (Sakai et al., 2011). Anthracene gave negative results in the initiation assay in three laboratories, but positive results in the promotion assay in one laboratory. [The Working Group noted that in the laboratory in which the positive result was obtained, the solvent control (DMSO, 0.5%) gave 1.8 ± 1.3 foci/well, and there was a dose-dependent and statistically significant increase in the number of foci in wells treated with anthracene (1.25 µg/mL, 5.5 ± 2.3 foci/well; 2.5 µg/mL, 6.7 ± 2.3 foci/well; 5 µg/mL, 6.7 ± 4.4 foci/well; 10 µg/mL, 9.8 ± 4.4 foci/well; and 20 µg/mL, 10.2 ± 3.5 foci/well). The solvent control gave somewhat higher results in the other two laboratories, where it was 2.3 ± 1.0 and 2.3 ± 1.2 foci/well. The positive controls, 3-MC for the initiation assay and TPA for the promotion assay, gave clearly positive results (> 14 foci/well). However, the purity of anthracene (from Aldrich) was not reported. The

Table 4.12 End-points relevant to immortalization with anthracene in non-human mammals in vivo and in vitro

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation	Visual assessment	Mouse, Balb/3T3, clones of embryonic fibroblasts Mouse, Balb/3T3 (F), irradiated weanling mice	Cells treated for 48 h and cultured for 8 days (n = 5) Tumourigenicity, subcutaneous inoculation of 10^6 transformed (30 colonies) or non-transformed (20 colonies) cells Mice (10/point), maintained for 180 days	10 µg/mL	–	Source and purity of chemicals, NR; solvent (acetone, ≤ 0.1% in medium); anthracene used as negative control.	DiPaolo et al. (1972)
Tumourigenicity of inoculated cells	Palpation, histology						
Morphological transformation Tumourigenicity	Visual assessment Palpation, histology	Hamster, Syrian Golden (F), pregnant Hamster, Syrian Golden (M), weanling	Pregnant animals (n = 4) at days 10–11 of gestation were injected intraperitoneally Cells from embryos prepared for culture on day 13 10^7 cells from colonies injected subcutaneously into irradiated male hamsters Hamsters were observed for tumour development for 1 yr	1–3 mg/100 g	–	Purity, NR; treatment of pregnant hamsters varied from 48 to 72 h; solvent (70% ethanol, DMSO, or trioctanoin) control and several positive-control chemicals included (e.g. B[a]P, DMBA).	DiPaolo et al. (1973)

Table 4.12 (continued)

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation Immortalization Tumourigenicity	Visual assessment; microscopy Growth of colonies in soft agar NR	Guinea-pig (F), pregnant; and guinea-pig fetal cells; days 32–49 of gestation Guinea-pig, irradiated syngeneic newborns	Anthracene was administered in utero (32-day fetus), or during in vitro culture of fetal cells, passage 68; 4–24 mo of continuous culture; $n = 6$ Inoculation of 10^8 cells; guinea-pigs were observed for 1 year for tumour development; $n = 6$	0.5 µg/mL medium	–	Synthetic-grade anthracene, purity, NR; under similar conditions, DMBA gave positive results; negative control, acetone (solvent).	Evans & DiPaolo (1975)
Morphological transformation Tumourigenicity	Stereomicroscopy after Giemsa staining Palpation, histology	Hamster, Golden Syrian, embryo cells, cryopreserved primary cultures Non-immuno-suppressed suckling hamsters	Cells were previously tested with 3-MC; anthracene treatment, 8 days; $n = 6$ 10^6 cells inoculated; animals were followed for ≥ 6 mo	1, 5, 10, 25, 50 µg/mL medium	–	Purity, NR; solvent control, 0.2% DMSO; other tested chemicals induced transformation.	Pienta et al. (1977)
Morphological transformation	Visual assessment after Giemsa staining	Mouse, NMRI nu/nu (nude) newborn, skin fibroblasts	Incubation for 25 days after treatment	14, 28, 56, 112 µM; 16 dishes/dose	–	Purity, NR; positive controls, PAHs; negative control, DMSO (solvent).	Laaksonen et al. (1983)
Morphological transformation	Scoring after Giemsa staining	Mouse, NMRI nu/nu (nude) newborn, skin fibroblasts	Anthracene for 72 h, SV40 for 2 h, follow-up for 25 days	14 and 28 µM +/–SV40	–	Purity, NR; 3-MC positive; negative controls, DMSO + SV40 and medium + SV40.	Laaksonen et al. (1984)

Table 4.12 (continued)

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation Tumourigenicity	Counting foci after Giemsa staining Inspection	Mouse, NMRI nu/nu (nude) newborn, skin fibroblasts Adult nude mice (F) (10/group)	SV40 for 2 h followed after 24 h by treatment with PAH for 3 days 10×10^6 cells from transformed foci were inoculated; mice were followed for their lifetime	Concentration, NR	–	Purity, NR; very poor data about anthracene experiments (concentration and time, NR); 3-MC gave a positive result.	Laaksonen et al. (1986)
Morphological transformation	Scoring after Giemsa staining	Mouse, C3H 101/2 clone 8	24 h treatment, 4–6 wk culture for foci	3, 10, 30 µg/mL medium	–	Purity, NR; solvent controls gave negative results; some tested chemicals induced transformation.	Lubet et al. (1983)
Morphological transformation	Visual assessment after Giemsa staining	Mouse, v-Ha-*ras*-transfected BALB/c 3T3 (Bhas 42) cells	Initiation assay (2-day treatment): cells were treated with anthracene until day 3 and fixed on day 24 Promotion assay (12-day treatment): fresh medium containing anthracene was applied on days 3, 7, and 10; fresh medium without chemical was applied on day 14; cells were fixed on day 21	0–10 µg/mL	–	Purity, NR; negative control, solvent; B[a]P was the positive control in the initiation assay, TPA was the positive control in the promotion assay.	Asada et al. (2005)

Table 4.12 (continued)

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation	Visual assessment after Giemsa staining (criteria for positivity defined)	Mouse, v-Ha-*ras*-transfected BALB/c 3T3 cell line (Bhas 42)	Validation study in 6 (anthracene in 3) laboratories from 3 countries Initiation assay: treatment for 72 h, cells were fixed on day 21 Promotion assay: treatment on days 4, 7, and 11, fresh medium on day 14	0–50 µg/mL	–, In 3 of 3 laboratories in initiation assay +, In 1 of 3 laboratories in promotion assay (dose-dependent, statistically significant increase)	Purity, NR; negative control, solvent; positive controls, 3-MC for the initiation assay and TPA for the promotion assay.	Sakai et al. (2011)
Morphological transformation Immortalization	Stereomicroscopy after staining Growth of transformed cells in soft agar	Hamster, Syrian, kidney cells (BHK 21/Cl 13)	Anthracene treatment for 18 h, S9 mix added; centrifuged cells cultured for 6 days	0.025, 0.25, 2.5, 10, 25, 250 µg/mL	+, Increase in transformation rate at LC_{50} (25 µg/mL), 1.4, but no dose–response relation; results of growth in soft agar, NR	Purity, NR; negative control, DMSO; B[a]P, chrysene, and 3-MC gave the highest positive results.	Greb et al. (1980)
Morphological transformation	Infrared spectroscopy scoring	Hamster, Syrian, embryo cells (SHE)			+, Transformation rates: anthracene, 5.58%; D-mannitol, 3.36%; and B[a]P, 17.2%	B[a]P and 3-MC as positive controls; D-mannitol as negative control.	Ahmadzai et al. (2012)
Morphological transformation	Microscopy; transformation score based on size of colonies	Rat, Wistar, kidney cells (BRK) from baby rats aged 9 days	Co-transforming ability of PAHs; cells transfected with HPV16E7-t and plasmid pEJ6.6, which carries the H-*ras* oncogene and treated with anthracene; cells fixed 18 days after transfection	1 µM	+, Colony-forming index was increased (but not statistically significantly; high variation; $n = 3$) for anthracene, and was higher than that for fluoranthene or benzo[*ghi*]perylene (statistically significant increase)	Purity, NR; total duration of PAH treatment was unclear; negative control, NR; many PAHs included that gave positive results.	Zhang et al. (2019)

Table 4.12 (continued)

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation	Stereomicroscopy after Giemsa staining	Hamster, Syrian Golden, embryo cells (SHE)	Incubation for 7 days	2.5–100 µg/mL	–	Chemicals of the highest purity available, generally > 97%; solvent controls gave negative results; B[a]P and DMBA gave clearly positive results; validation study; interlaboratory comparison.	Tu et al. (1986)
Morphological transformation	Microscopy after Giemsa staining	Hamster, Syrian, embryo cells (SHE)	After chemical treatment (approximately 20 h), the cells were subcultured and assayed for viability and enhancement of virus transformation (simian adenovirus SA7 transformation enhancement assay)	0–1100 µM	–	Purity, NR; negative control undefined, (solvent, acetone); B[a]P and DMBA gave positive results; doses up to the limit of solubility; validation study; two laboratories.	Schechtman et al. (1986)
Morphological transformation	Scoring for foci after Giemsa staining	Mouse, C3H/10T1/2 clone 8, embryo cells	Cells treated for 24 h, and cultured for 4–6 wk with or without subculture; method development to amplify expression of phenotypical transformation; amplification by replating (and rat S9 mix)	3, 10, and 30 µg/mL	–	Purity, NR; negative control, solvent (acetone); several PAHs induced transformation.	Schechtman et al. (1987)

Table 4.12 (continued)

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation	Counting only type II and III foci (degree of morphological aberration); criteria for transformation established	Mouse, C3H/10T1/2, embryo cells	Comparison between two laboratories	0.8–100 µg/mL; 12–25 plates/dose	–, in both laboratories (in one laboratory, anthracene was not tested at a dose level giving 25% cytotoxicity as the highest dose level)	Chemicals from NCI Chemical Repository, purity, NR; anthracene coded before assay, not when delivered; negative control, solvent (acetone or DMSO); positive control, 3-MC.	Dunkel et al. (1988)
Morphological transformation	Visual assessment of transformed (according to set criteria) colonies	Hamster, Syrian, embryo cells (SHE)	Enhanced transformation assay (pH 7.35 or pH 6.7, culture for 7 days); comparison between two laboratories	0.63, 1.25, 2.5, 5, 10 µg/mL for each pH	–	Purity, NR; negative controls included separately for each chemical (different solvents); B[a]P gave a positive result.	LeBoeuf et al. (1989)
Morphological transformation	Stereomicroscopy after staining	Hamster, Syrian, embryo cells (SHE)	Syrian hamster embryo cell transformation assay, pH 6.7	Concentration, NR, only that concentrations were based on cytotoxicity assay	–	Purity, > 99%; solvent as negative control; B[a]P as positive control.	LeBoeuf et al. (1996)
Morphological transformation	Stereomicroscopy after Giemsa staining; morphological transformation defined	Hamster, Syrian, embryo cells (SHE)	Prevalidation study; 4 laboratories; 6 chemicals including anthracene, B[a]P, and 3-MC	0.001–100 µg/mL	–	Purity, NR; negative control, DMSO; positive control, B[a]P (positive in all laboratories).	Maire et al. (2012)
Morphological transformation	Stereomicroscopy after Giemsa staining	Hamster, Syrian, embryo cells (SHE)	Prevalidation study; 3 laboratories; 6 chemicals including anthracene, B[a]P, 3-MC; treatment for 7 days after which cells were fixed	0, 2.5, 5, 10, 25, 50, 100 µg/mL	–	Purity, NR; negative control, DMSO; positive control, B[a]P (positive in all laboratories).	Pant et al. (2012)

Table 4.12 (continued)

End-point	Assay or method	Species, strain (sex), cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Morphological transformation	Visual assessment; only type III foci recorded (morphological criteria given)	Mouse, BALB/c 3T3, two different lineages of the A31-1-1 clone were used (ECVAM and HRI)	Prevalidation study; 3 laboratories; 6 chemicals including anthracene, B[a]P, and 3-MC; treatment for 72 h, cells fixed on day 24 or 25	0, 1, 10, 100, 1000 µg/mL	–	Purity, NR; negative control, solvent; positive control, 3-MC (positive in all laboratories).	Tanaka et al. (2012)

B[a]P, benzo[*a*]pyrene; DMBA, dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; F, female; h, hour(s); HPV, human papilloma virus; 3-MC, 3-methylcholanthrene; LC_{50}, median lethal dose; M, male; mo, month(s); NCI, National Cancer Institute; NR, not reported; PAH, polycyclic aromatic hydrocarbon; SHE, Syrian hamster embryo; SV40, simian virus 40; S9, 9000 × *g* supernatant; TPA, 12-*O*-tetra-decanoylphorbol-13-acetate.

[a] –, negative; +, positive.

chemicals to be tested were coded before being distributed to the test laboratories.]

Three in vitro studies showed that anthracene did not induce cell transformation (Greb et al., 1980; Ahmadzai et al., 2012; Zhang et al., 2019); however, some inconsistencies in the results were observed. In the study by Greb et al. (1980), anthracene (with metabolic activation by S9 mix from Aroclor-treated rats) induced an increase of only 1.4-fold in the transformation rate at the LC_{50} (concentration that is lethal to 50% of cells) in Syrian Golden hamster kidney fibroblasts (BHK 21/CL 13) cells. The increase was higher than that induced by B[a]P without metabolic activation (0.9-fold) or phenanthrene with metabolic activation (0.9-fold); however, it was less than 2-fold and with no dose–response relation, and thus did not fulfil either of the set criteria for positivity.

Ahmadzai et al. (2012) determined the cell transformation rate for anthracene, B[a]P, and 3-MC, and other chemicals using a new type of scoring by infrared spectroscopy in Syrian hamster embryo cells. The rate of transformation for anthracene (5.58%) was higher than that induced by the negative control D-mannitol (3.36%), and lower than that for B[a]P (17.2%). Zhang et al. (2019) found that in kidney cells from Wistar rats (age, 9 days), anthracene exhibited a higher mean colony-forming unit index (not statistically significant compared with controls) than did either fluoranthene or benzo[*ghi*]-perylene (both statistically significant).

Negative results for morphological cell transformation were reported in six studies in Syrian hamster embryo cells (Schechtman et al., 1986; Tu et al., 1986; LeBoeuf et al., 1989, 1996; Maire et al., 2012; Pant et al., 2012), in two studies in C3H/10T1/2 mouse embryo cells (Schechtman et al., 1987; Dunkel et al., 1988), in one study in v-Ha-*ras*-transfected murine BALB/c 3T3 cells (Bhas 42) (Asada et al., 2005), and in one study in two clones of BALB/c 3T3 cells (ECVAM and HRI) (Tanaka et al., 2012). [The Working Group noted that, despite these negative results, the potential tumour-promoting activity of anthracene should not be dismissed. Few studies examined the promotion potential of anthracene; different transformation results might be obtained if, for example, an initiator was used first, followed by anthracene. In addition, the Working Group noted that the effects of UV light in combination with anthracene were not evaluated in these cell transformation assays.]

4.2.9 Alters cell proliferation, cell death, or nutrient supply

See Table 4.13.

No data for the key characteristic "alters cell proliferation, cell death, or nutrient supply" in exposed humans were available to the Working Group. Most of the available literature evaluated specific end-points, i.e. cell junctions, cell proliferation, and calcium signalling in vitro in various cell types, and the evidence was organized accordingly. Of note, dysregulated gap junction intercellular communication (GJIC) and related connexin proteins can lead to alterations in cell survival, proliferation, and calcium and other cell signalling pathways (Goodson et al., 2015; Sinyuk et al., 2018; Siegrist et al., 2019). The integrity of cell junctions (tight junctions, adherens junctions, gap junctions) and their complex crosstalk is vital for maintaining tissue homeostasis (Naser et al., 2022).

(a) Cell junctions

(i) Human primary cells and cell lines

Wu et al. (2022) showed that anthracene of high purity (99%), at concentrations found in human blood, disrupted endothelial barrier function via disruption of cell junctions in human umbilical vein endothelial cells (HUVEC). There was an increase of 1.15–1.42-fold in fluorescein leakage and a dose-dependent and statistically significant decrease in trans-endothelial electrical resistance. Paracellular gap formation was

Table 4.13 End-points relevant to cell proliferation, cell death, or nutrient supply with anthracene in human cells in vitro and non-human mammalian experimental systems in vitro

End-point	Assay or method	Species, strain, cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Cell junctions							
Endothelial barrier function	Permeability (FITC-dextran fluorescence in Transwell monolayer, TER); barrier protein (immunoblotting) and mRNA (qPCR) expression (VE-cadherin, ZO-1, occludin) Morphology (TEM); VE-cadherin internalization (immunofluorescence)	Human umbilical vein endothelial cells (HUVEC)	Treatment for 24 h (VE-cadherin mRNA, 12 h);	0.01–1 µM [1.78 ng/mL–178 ng/mL] (anthracene in human whole blood, 79 ng/mL)	↑ FITC-dextran fluorescence ↓ TER (dose-dependent) ↓ mRNA expression of VE-cadherin and occluding ↓ Protein expression ZO-1 and occludin ↑ Intracellular gaps formation	Purity, 99%; EDTA, 2.5 mM, as positive control; DMSO (solvent), as negative control; $n = 3$ (except for immunoblotting, $n = 1$).	Wu et al. (2022)
GJIC inhibition	SL/DT assay	Human bronchial epithelial (HBE1) cell line	Exposure, 1 h, 24 h	0–100 µM	No differences	Purity, NR; for 1- and 9-methylanthracene, there was a dose-dependent, statistically significant decrease.	Brózman et al. (2020)
MAPK activation	Immunoblotting	Human bronchial epithelial (HBE1) cell line	Exposure, 1 h, 24 h	100 µM	Increased p38 phosphorylation at 1 h		
Connexin 43 protein	Immunoblotting	Human bronchial epithelial (HBE1) cell line	Exposure, 1 h, 24 h	100 µM	No differences		
GJIC inhibition	SL/DT assay	Rat liver WB-F344 epithelial cells	Treatment, 90 min; $n = 2$	5–20 mg/L	↓, 40% decrease compared with controls	Purity, NR; solvent control (acetonitrile); 9-methylanthracene and fluoranthene gave positive results. No statistical analysis provided.	Upham et al. (1994)

Table 4.13 (continued)

End-point	Assay or method	Species, strain, cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
GJIC inhibition	SL/DT assay	Rat liver WB-F344 epithelial cells	Treatment, 30 min; *n* = 3	0–350 µM	No differences	Purity, NR; solvent control (acetonitrile); 1- and 9-methylanthracene gave positive results.	Upham et al. (1996)
GJIC inhibition	SL/DT assay	Rat liver WB-F344 epithelial cells	Treatment, 10 min; *n* = 3	0–350 µM	No differences	Purity, NR; solvent control (acetonitrile); several positive chemicals. high variation at the highest concentrations.	Weis et al. (1998)
GJIC inhibition	SL/DT assay	Rat liver WB-F344 epithelial cells	Treatment, 15 min; *n* = 3	100–350 µM	No differences	Purity, NR; solvent control (acetonitrile); concentrations used based on Rummel et al. (1999); high variation at the highest concentrations.	Rummel et al. (1999)
GJIC inhibition	SL/DT assay	Rat liver WB-F344 epithelial cells	Treatment, 30 min; *n* = 3	0–100 µM	No differences	Purity, NR; DMSO as solvent control; 1- and 9-methylanthracene gave clearly positive results.	Vondrácek et al. (2007)
Connexin 43 phosphorylation	Immunoblotting (western blotting), densitometry	Rat liver WB-F344 epithelial cells	Treatment, up to 30 min.	60 µM	No differences	Purity, NR; TPA clearly inhibited.	Upham et al. (2008)
GJIC inhibition	SL-DT assay	Mouse testicular Leydig TM3 cells and Sertoli TM4 cells	Treatment, 0.5 h; *n* = 3	0–200 µM	Dose-dependent trend decrease from 50 µM but not statistically significant.	Purity, > 98.5%; vehicle controls, DMSO (maximum, 1% v/v in medium); TPA as positive control.	Kubincová et al. (2019)

Table 4.13 End-points (continued)

End-point	Assay or method	Species, strain, cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Cell proliferation and cell death							
Cell proliferation	Alamar Blue	Human placental choriocarcinoma BeWo and JEG-3 cell lines	Anthracene dissolved in methanol (< 0.1% in medium); doses determined by in vivo levels; treatment, 24 h and 72 h; $n = 3$	10 and 80 ng/mL	No differences	Purity, NR; negative controls not defined; no positive control.	Drwal et al. (2017)
Cyclin D1 Cyclin A2; Cdk2, Cdk4; Bax; Bcl-xl; caspase-3	Immunoblotting	Human placental choriocarcinoma BeWo and JEG-3 cell lines	Anthracene dissolved in methanol (< 0.1% in medium); doses determined by in vivo levels; treatment, 24 h and 72 h; $n = 3$	10 and 80 ng/mL	JEG-3: ↑ Bax; ↑ cyclinA2; ↓ cyclin D1; ↑ cdk2 BeWo: ↑Bax; ↑ caspase	Purity, NR; negative controls not defined; no positive control.	
Cell viability	XTT assay	Human placental choriocarcinoma BeWo and JEG-3 cell lines	anthracene dissolved in methanol (< 0.1% in medium); doses determined by in vivo levels; treatment 24 h and 72 h; $n = 3$	10 and 80 ng/mL	↑ after 72 h JEG-3: 80 ng/mL BeWo: 10 ng/mL	Purity, NR; negative controls not defined; no positive control.	Drwal et al. (2017)
Cell death	Annexin-V-FITC apoptosis detection kit, flow cytometry; DNA ladder kit, agarose gel separation	Human monocytic (THP-1) cell line	Treatment for 24 h	50 µM	No differences	Purity, NR; vehicle control, 0.1% DMSO; positive control, staurosporin	Wan et al. (2006)

Table 4.13 End-points (continued)

End-point	Assay or method	Species, strain, cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Epidermal proliferation	Thymidine incorporation, ODC activity	Mouse, albino hairless (F), (age 4–6 mo); *n* = 4–5	Dorsal skin painted; UV-A exposure 2 h later; skin studied at 4, 24, 48, 72, and 96 h (thymidine) or 4, 24, 48 h (ODC) after treatment	1% in petrolatum (Vaseline)	↑ Thymidine incorporation ↑ ODC	Dose unclear; purity, NR; ventral skin as negative control.	Gange (1981)
No. of dopa-positive melanocytes in epidermis	10% formalin-fixed skin incubated in dopa solution; light microscopy	Mouse, C57BL/6 (age 6–8 wk)	Area of 4 cm^2 shaved 1–2 days before skin painting; incubation of treated skin with dopa 6 days after last application	200 µg × 2/mouse, 2 consecutive days	No differences	Purity, NR; skin area for analysis was unclear; solvent (acetone) control; chemicals that gave positive results were included.	Iwata et al. (1981)
Hyperplasia	Histology	Rat, Fischer 344 (M); 2 tracheas per rat transplanted subcutaneously into post-scapular region, exposed 4 wk later	Pellets containing anthracene were implanted into tracheal explants (6 tracheas/group) for 3 days or 1, 2, 4 or 8 wk	Beeswax pellets containing 1 mg PAH	Mild changes (hyperplasia; similar to the beeswax control, but lasting longer) in 25–50% of explants during 8 wk and moderate changes (transitional epithelium) in 10–20% during 4 wk	PAHs recrystallized; anthracene release from pellets: in vitro/in vivo, 1.2; DMBA and B[a]P: moderate to severe changes in 50–100%.	Topping et al. (1978)

Table 4.13 End-points (continued)

End-point	Assay or method	Species, strain, cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Liver regeneration	Liver (dry) weight compared with controls	Rats (male), strain not reported	Partially hepatectomized male rats (n = 12) fed anthracene for 10 days	1% in food	No differences	Purity, ≥ 98%; negative control and positive compounds included.	Gershbein (1975)
Intracellular calcium signalling							
Intracellular calcium	Flow cytometry	Primary human mammary epithelial cells (mammoplasty; n = 7)	Incubations, 2 h and 18 h	0.03, 0.3, 3 µM	No differences	Purity, > 95%; PAHs did not interfere with fluorescence; B[a]P and DMBA gave positive results.	Tannheimer et al. (1997)
Intracellular calcium	Flow cytometry	HBP-ALL human T-cell line	Rapid (3 min.); Sustained (4 h);	10 µM	↑, at 3 min No differences at 4 h	All chemicals and reagents were ACS or molecular-biology grade.	Krieger et al. (1994)
Calcium uptake	Filtration method for $^{45}Ca^{2+}$ uptake	Human HBP-ALL T-cell line; 15 000 × g supernatant from cell lysate	In vitro incubation of microsomes with $^{45}Ca^{2+}$, 5 mM ATP and PAH for 5 min; n = 1	0.1, 1, 10 µM	No differences	Purity, NR; DMBA and B[a]P gave dose-dependent statistically significant inhibition.	Krieger et al. (1995)
Ca-ATPase activity	(γ-^{32}P)ATP hydrolysis	Human HBP-ALL T-cell line, 15 000 × g supernatant from cell lysate	Incubation, 30 min at 37 °C; 5 mM ATP; n = 3	10 µM	No differences	Purity, NR; solvent control; B[a]P gave a statistically significant decrease.	
Ca-ATPase activity (SERCA)	(γ-^{32}P)ATP hydrolysis	HPBMC (n = 4); 15 000 × g supernatant from cell lysate		10 µM	No differences	Purity, NR; B[a]P and DMBA gave a dose-dependent positive result, with 99% inhibition at 10 µM.	

Table 4.13 End-points (continued)

End-point	Assay or method	Species, strain, cells	Study design, culture time	Dose or concentration	Results[a]	Comments	Reference
Intracellular calcium	Flow cytometry	HPBMC (n = 10); CD3+ T cells, CD19+ B cells and CD14+ monocytes treated separately	Treatment at 20, 42, or 66 h	10 µM	↑	Purity, > 95%; DMSO at < 0.1% did not differ from non-DMSO control; B[a]P and DMBA gave strong inhibition.	Mounho et al. (1997)
PTK activity	Modification of PTK assay (Pierce Bioproducts, Rockford, Illinois, USA); also, Fyn and ZAP-70 removed by immunoprecipitation	Human HBP-ALL T-cell line (calcium increase dependent on PTK)	Total PTK activity; specific (Fyn and ZAP-70) activity; 5 min exposure; n = 3	10 µM	↑ Transient only for PTK, but not other kinases	Purity, > 95%; DMSO solvent control (DMSO, < 0.1%); DMBA and 3-MC, but not B[a]P, caused statistically significant increases.	Davila et al. (1999)

ACS, American Chemical Society; ATP, adenosine triphosphate; B[a]P, benzo[*a*]pyrene; Ca^{2+}, calcium; dopa, dihydroxyphenylalanine; DMBA, dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; F, female; FITC, fluorescein isothiocyanate; GJIC, gap junctional intercellular communication; h, hour(s); HPBMC, human peripheral blood mononuclear cell; HUVEC, human umbilical vein endothelial cells; M, male; MAPK, mitogen-activated protein kinases; 3-MC, 3-methylcholanthrene; min, minute; mo, month(s); NR, not reported; ODC, ornithine decarboxylase; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; PTK, protein tyrosine kinase; qPCR, quantitative polymerase chain reaction; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPases; SL/DT, scrape-loading/dye transfer; TEM, transmission electron microscopy; TER, transepithelial resistance; TPA, 12-*O*-tetra-decanoylphorbol-13-acetate; UV, ultraviolet; VE-cadherin, vascular endothelial cadherin; v/v, volume/volume; wk, week(s); XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide, disodium salt; ZO-1, zonula occludens-1.

[a] ↑, increase; ↓, decrease.

shown by transmission electron microscopy. After a 24-hour exposure to anthracene, mRNA expression of vascular endothelial cadherin (VE-cadherin), zona occludens-1 (ZO-1), and occludin was downregulated by 33.2–71.4%, 19.1–21.0%, and 31.9%, respectively. Protein expression of ZO-1 and occludin was downregulated, and VE-cadherin was internalized.

The effects of anthracene were also investigated in rapid (< 1 hour) and sustained (up to 24 hours) inhibition of GJIC in the human bronchial epithelial cell line HBE1 (immortalized by human papillomavirus type 18, HPV18, E6/E7 proteins). Connexin 43 was not inhibited by anthracene (Brózman et al., 2020), whereas p38 MAPK, which is probably involved in GJIC regulation (see Siegrist et al., 2019), was activated (a statistically significant increase in phosphorylation at 1 hour) by anthracene at a concentration of 100 μM.

(ii) Non-human mammalian cells in vitro

The effects of anthracene exposure on GJIC inhibition were evaluated in rat liver epithelial cells (WB F344) (Upham et al., 1994, 1996; Weis et al., 1998; Rummel et al., 1999; Vondrácek et al., 2007), and in murine testicular cells (Kubincová et al., 2019). All the studies used a scrape-loading/dye-transfer assay for GJIC, and none showed statistically significant inhibition of GJIC. [The Working Group noted some inconsistencies in the results among the studies (Upham et al., 1994; Vondrácek et al., 2007; Kubincová et al., 2019). Upham et al. (1994) observed a 60% inhibition of GJIC, when compared with the controls, at all tested doses in rat liver cells treated with anthracene for 90 minutes. In a follow-up study (Upham et al., 1996), however, a shorter treatment time (30 minutes) did not induce GJIC inhibition (no statistics were reported, because the experiment was only carried out twice). In the studies by Weis et al. (1998), Rummel et al. (1999), Vondrácek et al. (2007), and Upham et al. (2008), carried out in rat liver epithelial WB-F344 cells, anthracene did not inhibit GJIC at concentrations up to 350 μM. In the study by Kubincová et al. (2019), anthracene did not inhibit GJIC at concentrations up to 200 μM in mouse testicular Leydig TM3 cells or Sertoli TM4 cells.]

(b) Alters cell proliferation or cell death

(i) Human cell lines

Drwal et al. (2017) studied the effect of anthracene on cell proliferation in human placental choriocarcinoma cell lines BeWo and JEG-3. The doses used were those identified in studies of exposure of the general population: 10 ng/mL (placenta) and 80 ng/mL (maternal blood). Treatment with anthracene for 24 hours or 72 hours did not alter cell proliferation, as measured by the Alamar Blue assay, but significantly increased cell viability in both cell lines, JEG-3 (80 ng/mL) and BeWo (10 ng/mL), at 72 hours. In the same study, Drwal et al. (2017) showed that exposure of JEG-3 cells to anthracene for 72 hours increased cyclin D1 and CDK2 but decreased cyclin A2 at 10 ng/mL and decreased BAX at 10 and 80 ng/mL. On the other hand, identical exposure of BeWo cells resulted in increased BAX at 10 and 80 ng/mL, and also in increased caspase-3 at 10 mg/mL. The study showed different cell type-dependent actions on apoptosis; a pro-apoptotic effect (increased BAX and caspase-3) in BeWo cells and an anti-apoptotic effect (decreased BAX and increased CDK2 and cyclin D1) in JEG-3 cells. In another study, Wan et al. (2006) compared induction of apoptosis in human monocytic THP-1 cells by flow cytometry. Anthracene did not alter the percentage of cells in apoptosis and necrosis, compared with controls.

(ii) Non-human mammals in vivo

Gange (1981) studied epidermal cell proliferation, using ornithine decarboxylase (ODC) and thymidine incorporation, in groups of four or five female albino hairless mice (age, 4–6 months). The dorsal skin was painted with 1% anthracene

in petrolatum and irradiated with UV-A light 2 hours later; the ventral skin of the same animal served as the matched control. Anthracene plus UV-A irradiation caused statistically significant increases in thymidine incorporation (at 48 hours and 96 hours after treatment) and in ODC activity (at 4 hours and 24 hours). Anthracene without irradiation caused a small but statistically significant increase in ODC (at 4 hours).

Iwata et al. (1981) studied the activation of melanocytes (as number of dopa-positive melanocytes in epidermis) by several PAHs in C57BL/6 mice. Anthracene (two consecutive days) and DMBA, the control (1 day only), were painted onto the skin and three or four (width, 2 mm) sections of fixed skin were incubated in dihydroxyphenylalanine solution (0.1%; 24 hours), for 6 days after the last application. Although the area for analysis was unclear, anthracene induced an average of 2.0 active melanocytes, compared with > 100 active melanocytes induced by the positive control (DMBA).

Topping et al. (1978) subcutaneously transplanted tracheas from male Fischer 344 rats into isogenic animals and, 4 weeks later, implanted beeswax pellets containing 1 mg of anthracene (recrystallized before use) into the tracheas. Beeswax pellets without PAH were used as a negative control. Beeswax pellets caused mild changes, but beeswax pellets releasing anthracene induced mild to moderate epithelial changes of 10–20%, as hyperplastic responses, within 4 weeks (Topping et al., 1978). [The Working Group noted that DMBA and B[a]P induced moderate to severe changes, including squamous metaplasia, in 50–100%.]

In another study, Gershbein reported that exposure of partially hepatectomized Holtzman (HLZ) or Charles River male rats to anthracene (at 1% in the feed; daily for 7 days) had no effect on the extent of liver regeneration over a period of 10 days post-operation (Gershbein, 1975).

(iii) Non-human mammalian cells in vitro

Shabad et al. (1972) described epithelial changes in tissue cultures of embryonic kidney (embryo age 19–21 days) from mice (BALBc, C3H/A, or C57BL/CBA F1 hybrids) treated daily with 8 mg of anthracene (purity not reported) during the last week of pregnancy. Anthracene induced diffuse hyperplasia and solid epithelial areas that were not seen in the controls but did not induce nodular proliferation or papillary growth as was observed for the positive control (DMBA).

Nuclear size, as a measure of cell proliferation, was assessed in rat trachea epithelial cells exposed for 3 hours to anthracene, other PAHs, various activation-dependent carcinogens, and direct-acting carcinogens. Anthracene did not induce an increase in nuclear size after cells were further cultured for 24, 72, or 120 hours, compared with other compounds (Fowlie et al., 1991).

Anthracene did not alter cell proliferation, as measured by cell number and percentage S-phase cell count, in hepatic epithelial stem-like rat cell (WB-F344) (Chramostová et al., 2004).

(c) Intracellular calcium

In human primary mammary epithelial cells from mammoplasty (n = 7) cultured for up to 18 hours with various PAH-compounds, anthracene (purity, > 95%), compared with the positive control (B[a]P), did not significantly alter intracellular Ca^{2+} levels at any time point or concentration, or in any cell preparation from different individuals (Tannheimer et al., 1997). In a series of studies, anthracene was reported to induce a small and transient Ca^{2+} mobilization response (Krieger et al., 1994, 1995; Mounho et al., 1997) in a human T-cell line (HPB-ALL) and human peripheral blood mononuclear cells. However, in human peripheral blood mononuclear cells, anthracene did not inhibit the activities of transmembrane sarcoendoplasmic reticulum calcium

ATPases SERCA2b or SERCA3, or plasma membrane Ca^{2+}-ATPase in human erythrocyte ghosts (Krieger et al., 1995). Anthracene at 10 µM significantly increased protein tyrosine kinase (PTK) activity in HPB-ALL human T cells, but did not alter other kinases (i.e. Fyn and ZAP-70) known to play important roles in T-cell activation and that have been observed to be activated by a 10-minute exposure to PAHs (Davila et al., 1999). [The Working Group noted that the effects of anthracene on Ca^{2+} signalling are smaller and often transient when compared with the effects of other PAHs.]

4.3 Evaluation of high-throughput in vitro toxicity screening data

Anthracene was tested in high-throughput toxicity screening assays under the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2019). Chemical samples of high purity were procured, prepared in DMSO stock solutions at a concentration of approximately 20 mM, and tested over a period of several years in biochemical and cellular bioassays measuring a wide variety of biological end-points. In addition, chemical analysis of the samples was done in high-throughput fashion at an early and a late stage of the sample testing lifetime, as described in Tice et al. (2013). Testing results data from the concentration–response testing design for all end-points were analysed for significant activity, and an active/inactive "hit call" was made for each response, together with a potency value (Filer et al., 2017). For all active calls, individual concentration–response curves were examined to ensure that biologically meaningful activity was detected. Bioassay end-points were mapped, where possible, to the key characteristics of carcinogens using the "kc-hits" software (the key characteristics of carcinogens – high-throughput screening discovery tool, available from: https://gitlab.com/i1650/kc-hits; Reisfeld et al., 2022) to aid in providing mechanistic insights (Chiu et al., 2018). The detailed results are available in the supplementary material for this volume (Annex 2, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/631) and are briefly summarized below.

The results for anthracene high-throughput toxicity testing in the CompTox Chemicals Dashboard encompassed 979 assay end-points, of which 280 were mapped to the key characteristics of carcinogens. The cytotoxicity limit for anthracene, on the basis of a panel of cellular cytotoxicity and viability assays, was estimated to be > 1 mM (US EPA, 2022). There were 17 positive hit calls for concentration–response curves mapped to end-points relevant to the key characteristics, but 12 of these had quality control flags indicating low confidence results. The five without flags mapped to the key characteristics "modulates receptor-mediated effects" (four end-points) and "alters cell proliferation, cell death, or nutrient supply" (one end-point). Three of the positive results for "modulates receptor-mediated effects" concerned upregulation of cytochrome P450 gene expression (CYP1A1, CYP1A2, and CYP2B6) in the human liver cell line HepaRG, for which the half-maximal activity concentrations (AC_{50}s) were 10.1, 18.0, and 14.7 µM, respectively. [The Working Group noted that HepaRG cells have xenobiotic metabolic activity.] The fourth positive result was for AR antagonist activity in the 22Rv1 human prostate carcinoma epithelial cell line, for which the AC_{50} was 22.3 µM. An additional AR antagonist assay had a positive hit call but was flagged for "less than 50% efficacy"; anthracene was inactive in two other AR antagonist assays. [The Working Group considered this to be weak evidence of AR modulation activity.] A single positive hit call (without flags) for the key characteristic "alters cell proliferation, cell death, or nutrient supply",

for cell cycle arrest in the human liver HepG2 cell line, had an AC_{50} of 115.8 μM.

The chemical analysis of anthracene included two different stock solutions. For one (Tox21_202226), the expected structure and purity were confirmed on initial testing but "low concentration 5–30% of expected value" was found on later analysis. The second sample (Tox21_300014) gave inconclusive results on initial testing, and an incorrect molecular weight was found on the second analysis (NIH, 2022). Mapping of specific samples to bioactivity testing results was not available in the public data. [The Working Group considered the testing results for anthracene to be of low confidence since it was not possible to link specific samples to bioactivity testing.]

5. Summary of Data Reported

5.1 Exposure characterization

Anthracene is a three-ring polycyclic aromatic hydrocarbon (PAH) mainly produced from coal tar. It is a High Production Volume chemical with a world production of about 20 000 tonnes per year. Anthracene is mainly used as an intermediate in the manufacture of dyes and pigments, pyrotechnics, coatings, wood preservatives, pesticides, and organic chemicals.

Anthracene release or disposal into the environment takes place because of industrial use or unintended formation during industrial processes. Additionally, anthracene is formed together with other PAHs during the incomplete combustion or pyrolysis of organic matter from both natural and anthropogenic sources, with predominance of the latter. Therefore, anthracene is ubiquitous in the environment. Anthracene has been detected in foodstuffs as a result of environmental contamination (via water, soil, and/or air) and/or unintended formation during food processing.

Occupational exposure to anthracene occurs in a variety of industries and activities, including carbon black manufacture, coking, tear-off of old coal-tar roofs, asphalt paving, firefighting, manufacture of creosote or creosote-containing products, production of carbon anodes for aluminium electrolysis, and production of fireproof material. In these diverse settings, exposure to anthracene is mainly by inhalation and dermal contact.

Anthracene exposure of the general population occurs from multiple routes, i.e. via ingestion of food and beverages/water, inhalation of polluted air, and through contact with contaminated soils or consumer products. Contaminated food is the major route of anthracene intake by the non-smoking and non-occupationally exposed population. The most significant sources of exposure of the general population via inhalation are tobacco smoke, biomass burning (indoors or outdoors), and traffic and industry emissions.

Biological monitoring of anthracene exposure in workers and the general population has seldom been performed.

Overall, occupation, smoking habits, living or working in industrial or urban polluted areas, and cooking using biomass as fuel are the main determinants of anthracene exposure.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with anthracene caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species (mouse and rat).

Anthracene was administered orally (in feed) in one study that complied with Good Laboratory

Practice (GLP), in male and female Crj:BDF_1 mice. In females, anthracene caused an increase in the incidence of hepatocellular neoplasms, including carcinoma and adenoma or carcinoma (combined) of the liver, and histiocytic sarcoma at multiple tissue sites.

Anthracene was administered orally (in feed) in one study that complied with GLP in male and female F344/DuCrj rats. In males, anthracene caused an increase in the incidence of hepatocellular neoplasms including carcinoma and adenoma or carcinoma (combined) of the liver, and transitional cell papilloma or carcinoma (combined) of the urinary bladder. In females, anthracene caused an increase in the incidence of renal cell adenoma or carcinoma (combined) of the kidney and endometrial stromal sarcoma of the uterus.

Anthracene was tested by oral administration (gavage) in male and female transgenic Hras128 and non-transgenic Sprague-Dawley rats. In male transgenic rats, anthracene caused a significant increase in the incidence of mammary adenoma or adenocarcinoma (combined).

5.4 Mechanistic evidence

The absorption, distribution, and excretion of anthracene in humans is documented by its presence in the urine after experimental cutaneous exposure and as a result of occupational exposures. Anthracene has also been detected in the urine, blood, and several tissues of the general population. A single study reported on 1-hydroxyanthracene in human hair. Anthracene is absorbed through rat skin and from the stomach in vivo, and through natural or excised skin of several mammalian species in vitro. Anthracene is extensively metabolized in rats or rabbits via its 1,2-oxide to 1,2-dihydrodiol (major product) and 1,2-dihydro-2-hydroxy-1-anthryl-mercapturic acid, whereas oxidation at positions 9 and 10 results in the formation of 9,10-dihydrodiol and related more oxidized products including 9,10-anthraquinone. Anthracene metabolism in rats or rat liver microsomes also produces 9-methyl or 9,10-dimethyl derivatives that undergo further oxidation at methyl groups and/or aromatic rings.

Data were available for anthracene for the following key characteristics of carcinogens: "is electrophilic or can be metabolically activated to an electrophile", "is genotoxic", "induces epigenetic alterations", "induces oxidative stress", "induces chronic inflammation", is immunosuppressive", "modulates receptor-mediated effects", "causes immortalization", and "alters cell proliferation, cell death, or nutrient supply".

Overall, the mechanistic evidence for anthracene with regard to the key characteristics of carcinogens "is genotoxic", "induces oxidative stress", and "modulates receptor-mediated effects" is suggestive in experimental systems.

There is suggestive evidence that anthracene is genotoxic in experimental systems. No data were available in humans exposed to anthracene or in human primary cells. DNA damage was shown in several studies performed in human cell lines, mammalian cells in vitro, and in several non-mammalian experimental systems. This was especially true when anthracene photoactivation and/or modification by interaction with nitrogen dioxide (NO_2) occurred. However, DNA damage was not shown in all the studies, leading to unexplained incoherence across studies of different end-points and different systems. Thus, anthracene could be considered a pro-genotoxic compound. High mutagenicity in urine from ICR mice exposed to anthracene in combination with NO_2 was shown by the Ames test. In non-human mammalian in vitro models, three out of eight studies showed positive results for several genotoxicity end-points. In addition, in non-mammalian experimental systems, positive findings for genotoxicity were noted in 10 out of 29 studies without metabolic activation, whereas 14 out of 29 studies gave positive results with metabolic activation.

There is suggestive evidence that anthracene induces oxidative stress. In exposed humans and human primary cells, there was inconsistent data, including oxidative damage to DNA. Only one study, in human peripheral blood lymphocytes, with the limitations on understanding the source of cells, reported a significant association between superoxide anion radicals and anthracene exposure. There is also suggestive evidence that anthracene induces oxidative stress in experimental systems. In the human cell line HaCaT, anthracene combined with ultraviolet (UV) induced oxidative stress, as measured by the production of reactive oxygen species. In other experimental systems including two studies in non-human mammalian cells in vitro and several in non-mammalian models, anthracene increased oxidative stress measured by a range of oxidative stress markers, such as malondialdehyde, reactive oxygen species, protein carbonyls, and altered antioxidant enzyme and glutathione-*S*-transferase activities.

There is suggestive evidence that anthracene modulates receptor-mediated effects. No data were available in humans exposed to anthracene or human primary cells. Evidence from experimental systems suggests that anthracene may activate multiple receptor-signalling pathways known to play critical roles in mediating endocrine disruption, including the aryl hydrocarbon receptor (AhR), estrogen receptor (ER), early growth response protein 1 (EGR-1), and peroxisome proliferator-activated receptor alpha and beta/delta (PPARα and PPARβ/δ). There are four studies in human cancer cells in vitro (MCF7, A549, and HCT-116) showing weak estrogenic activity and a significant increase in EGR-1, PPARα, and PPARβ/δ luciferase activity.

There was a paucity of data or inconsistent evidence for the following key characteristics, "is electrophilic or can be metabolically activated to an electrophile", "induces epigenetic alterations", and "induces chronic inflammation".

No data were available for the other key characteristics.

Data for anthracene from the assay battery of the Toxicity Forecaster (ToxCast) research programme in the USA were considered supportive for the key characteristic "modulates receptor-mediated effects" on the basis of induction of target genes for AhR (CYP1A1 and CYP1A2) and constitutive androstane receptor (CYP2B6) in the human liver cell line HepaRG. Anthracene was active in two androgen receptor (AR) assays, but there is low confidence in the results because additional AR assays were without activity.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of anthracene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of anthracene.

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

Anthracene is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The Group 2B evaluation for anthracene is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* for cancer in experimental animals is based on an increase

in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species (mouse and rat) in two studies that complied with GLP. The mechanistic evidence was *limited*. There is suggestive evidence that anthracene is genotoxic, induces oxidative stress and modulates receptor-mediated effects in experimental systems. The evidence regarding cancer in humans was *inadequate*, as no studies were available.

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2-BROMOPROPANE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 75-26-3 (NCBI, 2022)

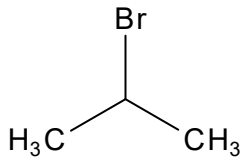
EC/List No.: 200-855-1 (NCBI, 2022)

IUPAC systematic name: 2-bromopropane (NCBI, 2022)

Synonyms: isopropyl bromide; propane, 2-bromo-; 1-bromo-1-methylethane; 2-BP (NCBI, 2022).

1.1.2 Structural and molecular information

Chemical structure:



Molecular formula: C_3H_7Br (NCBI, 2022)

Relative molecular mass: 122.99 (NCBI, 2022).

1.1.3 Chemical and physical properties

Description: clear colourless to slightly yellow liquid (NCBI, 2022)

Boiling-point: 59–60 °C (Royal Society of Chemistry, 2022; NCBI, 2022)

Melting-point: –89 to –92 °C (Royal Society of Chemistry, 2022; ECHA, 2022b; NCBI, 2022)

Flash-point: 19–20 °C at 101.3 kPa (Royal Society of Chemistry, 2022; ECHA, 2022b; NCBI, 2022)

Density: 1.31 g/mL at 20 °C (Royal Society of Chemistry, 2022; NCBI, 2022)

Vapour pressure: 26 kPa at 20 °C (ECHA, 2022b)

Solubility: 3.18 g/L at 20 °C in water; miscible with alcohol, benzene, chloroform, and ether (Royal Society of Chemistry, 2022; NCBI, 2022)

Octanol/water partition coefficient (P): log K_{ow} = 2.14 (Royal Society of Chemistry, 2022; NCBI, 2022)

Stability: 2-Bromopropane is not expected to undergo direct photolysis in the environment because it lacks functional groups that absorb light at wavelengths greater than 290 nm (NCBI, 2022).

[The Working Group used a conversion factor of 1 ppm ≈ 5.03 mg/m^3 at 25 °C (NTP, 2003).]

1.1.4 Impurities

2-Bromopropane of high purity (≥ 99%) is available commercially (NCBI, 2022).

Two studies in Asia reported on impurities associated with 2-bromopropane. In a plant in the Republic of Korea, the purity of 2-bromopropane used was 97.4% and impurities included *n*-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%) (Park et al., 1997). In a plant in China, the reported purity of 2-bromopropane was 98.08% and impurities consisted of 2-propanol (isopropyl alcohol) (1.76%), dibromopropane (0.085%), benzene (0.055%), and trichloroethylene (0.010%) (Ichihara et al., 1999).

In the above-mentioned plant in the Republic of Korea, a mixture of two 2-bromopropane-containing solutions was used. SPG-6AR contained 60.7% of 2-bromopropane, 33% of *n*-heptane, and 1.55% of 1.1.1-trichloroethane; other chemicals, such as 1,2-dibromopropane and nitromethane, were detected at a level below 1%. Solvent 5200, used for cleaning, contained 99.1% of 2-bromopropane, 0.2% of 1,2-dibromopropane, and impurities at a level below 1% (Kim et al., 1996a; Park et al., 1997).

1.2 Production and use

1.2.1 Production process

2-Bromopropane is synthesized by heating 2-propanol together with hydrogen bromide (Ichihara et al., 1999; NCBI, 2022) and also occurs as an impurity of commercial-grade 1-bromopropane, historically at concentrations of 0.1–0.2% (IARC, 2018). In the American Society for Testing and Materials standards for 1-bromopropane used in vapour degreasing, 2-bromopropane is listed as an impurity at a maximum of 0.1% by weight (ASTM, 2000), but the standard was updated in 2018 to a maximum of 0.05% by weight (ASTM, 2018). [The Working Group noted that for other uses of 1-bromopropane, such as adhesives, no maximum level of 2-bromopropane impurity in 1-bromopropane has been set.]

1.2.2 Production volume

Production volumes for 2-bromopropane were reported to be < 1 000 000 pounds/year [< 450 tonnes/year] from 2016 to 2019 in the USA (US EPA, 2022). Manufactured and/or imported quantities of 2-bromopropane were 1 to < 1000 tonnes/year in Japan between 2012 and 2020, except for 1000–2000 tonnes in 2014 (NITE, 2023). [The Working Group could not identify any national production volume data outside of Japan and the USA. However, at least 13 manufacturers in China are known (ChemicalBook, 2023a), and one manufacturer in China reported a production volume of 2000 tonnes in 2015 (Jiangsu JiuLi Environmental Technology Co., Ltd, 2015).]

1.2.3 Uses

2-Bromopropane has been reported to be used in the synthesis of pharmaceuticals as an alkylating agent, and as an intermediate for dyes, pesticides, and other chemicals (Lewis, 2001; ChemicalBook, 2023b; ECHA 2023). [The Working Group was not able to identify for which specific pharmaceuticals 2-bromopropane is used in the synthesis.] Also, 2-bromopropane was used as a solvent to replace Freon 113 in an electronic component manufacturing factory in the Republic of Korea (Park et al., 1997). The average monthly use as a solvent was reported as 1301 kg (range, 750–2500 kg) from February 1994 to July 1995 (KOSHA, 1995). [The Working Group noted that no information was available on the locations of continuing use of 2-bromopropane.]

1.3 Detection and quantification

1.3.1 Air

2-Bromopropane can be determined in air samples using National Institute for Occupational Safety and Health (NIOSH) method 1025. The method uses a solid sorbent tube for sampling 2-bromopropane at 0.01–0.2 L/min, followed by quantitative analysis with gas chromatography-flame ionization detection (GC-FID). This method is linear over a wide range of concentrations (4.5–393 µg/sample), with a limit of detection (LOD) of 1 µg/sample (NIOSH, 2003a). The United States Occupational Safety and Health Administration (OSHA) published a validated method for analysis of 2-bromopropane in air samples using charcoal tubes and personal sampling pumps to collect 12 L air samples, followed by analysis with GC-electron capture detection (GC-ECD). The method's limit of quantification (LOQ) for 2-bromopropane is 1.8 ppb [9.2 µg/m^3] (OSHA, 2013).

1.3.2 Water

No data on 2-bromopropane analysis in water samples were available to the Working Group.

1.3.3 Consumer products

Because of its wide use in synthesis of pharmaceuticals, sensitive methods have been developed for analysis of 2-bromopropane impurities in bulk drugs. A GC-mass spectrometry (GC-MS) method was reported for determination of 2-bromopropane impurities in divalproex sodium (an anticonvulsant medication), with an LOQ of 5 ng/mL (Reddy et al., 2019). More recently, GC-MS/MS was applied to measure 2-bromopropane impurities in abiraterone acetate, which is used to treat prostate cancer, with an LOQ of 60 ng/mL (Zhong et al., 2022).

1.3.4 Human biomarkers

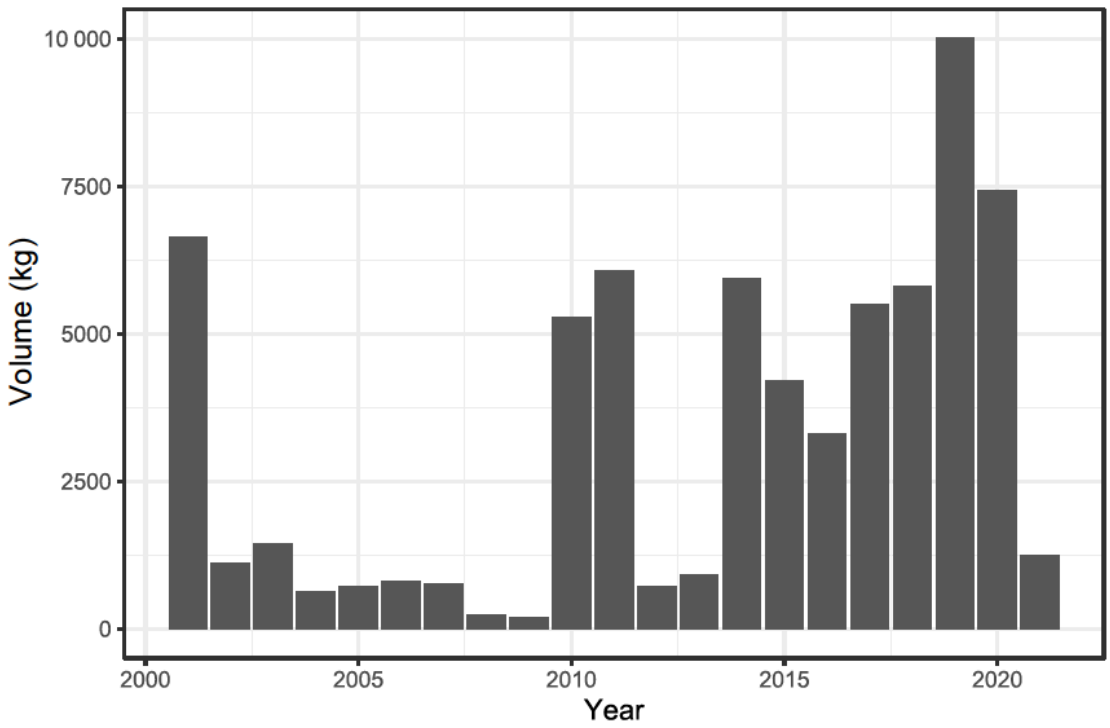
2-Bromopropane and its potential metabolites acetone and 2-propanol were determined in human and rat urine using a GC-FID method with LODs of 0.01, 0.05, and 0.10 µg/mL for 2-bromopropane, acetone, and 2-propanol, respectively (Kawai et al., 1997). A more sensitive method was reported for analysis of 2-bromopropane in human urine with headspace GC-ECD. The method was linear over a wide range of concentrations (0.03–12.5 µg/mL), with an LOD of 7 ng/mL (B'Hymer & Cheever, 2005). Column-switching liquid chromatography-tandem mass spectrometry (CSLC-MS/MS) was successfully applied for determination of isopropyl mercapturic acid, as a biomarker of 2-bromopropane exposure, in human urine samples. This rapid method was linear over a wide range of concentrations up to 2500 µg/L, with an LOD of 2.5 ng/mL (Eckert & Göen, 2014).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

In Japan, the total release volume of 2-bromopropane into the environment was estimated to be 189–10 007 kg/year in 2001–2021 (Fig. 1.1). Almost all were air emissions (Japan Ministry of the Environment, 2023). In Japan, 2-bromopropane measurements in 13 and 19 air samples were below the LODs (0.17 and 0.20 µg/m^3, respectively) in 1998–1999 and 1997–1998, respectively. Measurements in six samples each from rivers and the sea were all below the LOD (0.01 µg/L) in 1997 (Japan Ministry of the Environment, 2005). No quantitative information on occurrence of 2-bromopropane in drinking-water, food, or consumer products was available to the Working Group.

Fig. 1.1 Release of 2-bromopropane into the environment in Japan between 2001 and 2021



Source: Japan Ministry of the Environment (2023).

1.4.2 Occupational exposure

The NIOSH National Occupational Exposure Survey (NOES) of 1981–1983 estimated that about 1582 chemists were potentially exposed to 2-bromopropane in the USA (NIOSH, 1983). [The Working Group estimated a confidence interval of 1029–2135 exposed workers. This estimate did not include workers potentially exposed to 2-bromopropane because of its presence as an impurity of 1-bromopropane.]

Occupational exposure to 2-bromopropane can occur via inhalation and/or dermal uptake (NCBI, 2022). Several studies have quantified 2-bromopropane in the air of workplace settings (Table 1.1). The geometric mean ambient concentration of 2-bromopropane in a workshop staffed with five workers in an integrated circuits parts factory in Japan was 3 mg/m^3. The workers were engaged in soldering of integrated circuits parts on the boards in which 2-bromopropane was applied by an automated process. There was no skin contact with liquid 2-bromopropane. One worker who was responsible for the operation of the machine for cleaning with 2-bromopropane, and checked the machine operation frequently, had the highest exposure. Personal air sampling was not carried out (Kawai et al., 1997).

After the reports of haematopoietic and reproductive disorders among workers using 2-bromopropane as a solvent in an electronics factory in the Republic of Korea (as detailed in Section 4.2), the use of 2-bromopropane was stopped. The mean duration of exposure was 10.1 months (range, 4–16 months) for the 15 women with primary ovarian failure. To characterize the workers' exposure, sampling was performed under simulated conditions. Stationary 3-hour

concentrations of 2-bromopropane ranged from 9.2 to 19.6 ppm [46.3–98.6 mg/m³]. Three short-term (23-minute) air monitoring measurements in the enclosure around the cleaning baths were performed at different heights above the cleaning solution and resulted in concentrations of 4359.5, 105.9, and 4140.9 ppm [21 928, 533, and 20 829 mg/m³] (details of the workers' tasks and exposures are described in Section 1.6) (KOSHA, 1995; Park et al., 1997). [The Working Group noted that the assessment under simulated conditions may have resulted in underestimated values of exposure.] Because of a lack of personal protective equipment and hand-dipping practices, dermal exposure to 2-bromopropane was likely but was not quantified (Kim et al., 1996b).

A study of 2-bromopropane exposure was conducted at a factory manufacturing the chemical in Yixing City, Jiangsu Province, China, in 1996 (Ichihara et al., 1999). The factory had been producing 2-bromopropane at a rate of 5 tonnes/year since 1991. Production did not take place in the month of August every year, because of solvent evaporation at high summer temperatures. The study included 25 workers (11 male and 14 female) at the plant (employed for an average of ~2.5 years) who had time-weighted average (TWA) concentrations of 2-bromopropane exposure measured using personal passive samplers. Measurements were carried out in December; the room temperature was 10.5 °C, and the windows were half-open. [The Working Group noted that the exposures were probably lower at the time of the measurement compared with other times of the year.] Personal passive samplers were attached to each worker for one 8-hour daytime shift. Ambient concentrations of 2-bromopropane were also measured in the breathing zone of workers with detection tubes in four process-specific areas of the factory. Interviews with workers were also conducted to ascertain information on job tasks and exposure opportunities during work shifts. The TWA 2-bromopropane exposure concentration ranged from below the LOD (0.2 ppm [1.0 mg/m³]) to 5.84 ppm [29.4 mg/m³] among male workers and from below the LOD to 16.18 ppm [81.4 mg/m³] among female workers. The 10 female workers and one male worker with the job tasks of operator (n = 9) and mixer (n = 2) had the highest median 2-bromopropane concentrations [6.77 ppm; 34.1 mg/m³] and [6.30 ppm; 31.7 mg/m³], respectively and were reported to spend almost their entire shift inside the factory. All female workers at this factory were non-smokers. Workers in non-production jobs [71% male], such as boilers, accountants, salespeople, and managers, had non-detectable levels of exposure (except one accountant). The median instantaneous ambient concentrations of 2-bromopropane measured in the breathing zones of the process areas were 4.0 ppm [20.1 mg/m³] (range, 2.5–17.2 ppm [12.6–86.5 mg/m³]) for observing the temperature of reaction pots, 27.6 ppm [139 mg/m³] (range, 8.2–90.9 ppm [41.2–457 mg/m³]) for pouring distilled product into plastic containers, 38.8 ppm [195 mg/m³] (range, 17.6–57.6 ppm [88.5–290 mg/m³]) for mixing product with sodium hydrogen carbonate, and 88.6 ppm [446 mg/m³] (range, 19.8–110.8 ppm [99.6–557 mg/m³]) for pouring processed product into drums. The analysis of the 2-bromopropane produced showed 98.08% volume per volume (v/v) purity.

NIOSH performed 10 studies measuring exposure to 1-bromopropane, which also measured exposure to 2-bromopropane alongside 1-bromopropane, because of the presence of 2-bromopropane as an impurity in the material being used (Table 1.1). Exposures were evaluated among workers at furniture and adhesive manufacturing companies, where exposures originated from the adhesives being used or manufactured. Exposures were also evaluated at companies where 2-bromopropane exposure was being measured as a result of impurities in solvents used during degreasing operations. In 1998, at an aircraft cushion manufacturing plant

Table 1.1 Occupational exposure to 2-bromopropane measured in air samples

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
Machine operation/ machine cleaning with 2-BP, integrated circuits parts factory Japan, 1995	Ambient workplace air	5 (different sites in the workshop)	GC-FID (0.01 µg/mL)	3 mg/m³ (GM), 1.47 mg/m³ (GSD) (NR)	NR		Kawai et al. (1997)
Tactile switch assembly operation section, electronics factory Republic of Korea, 1995	Ambient workplace air (simulated setting)	14 (stationary samples near each cleaning bath, for 3 h) 3 short-term (15-min) samples inside the hood of each cleaning bath	GC-MS (NR)	Stationary samples: 12.4 ppm (9.2–12.6 ppm) [62.4 mg/m³ (46.3–98.6 mg/m³)] Short-term samples: 4140.7 ppm [20 828 mg/m³] (NR)	NR NR		KOSHA (1995); Park et al. (1997)
2-BP production factory China, 1996	Ambient workplace air, personal monitor	24	GC-EID (0.2 ppm [1.0 mg/m³] TWA)	Breathing zone samples (short-term): Observing the temperature of reaction pots: (2.5–17.2 ppm) [(12.6–86.5 mg/m³)] Pouring distilled product into plastic containers: (8.2–90.9 ppm) [(41.2–457 mg/m³)] Mixing product with sodium hydrogencarbonate: (17.6–57.6 ppm) [(88.5–290 mg/m³)]	4.0 ppm [20.1 mg/m³] 27.6 ppm [139 mg/m³] 38.8 ppm [195 mg/m³]	Duration of exposure: 5–69 months. Breathing zone sampling in ambient air was performed with detector tubes. Personal sampling was performed with passive samplers.	Ichihara et al. (1999)

Table 1.1 (continued)

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
2-BP production factory China, 1996 (cont.)				Pouring processed product into drums: (19.8–110.8 ppm) [(99.6–557 mg/m³)]	88.6 ppm [446 mg/m³]		Ichihara et al. (1999) (cont.)
				Personal samples (8-h TWA):			
				Operator (n = 9): (4.09–16.18 ppm) [(20.6–81.4 mg/m³)]	[6.77 ppm] [34.1 mg/m³]		
				Mixer (n = 2): (5.84–6.76 ppm) [(29.4–34.0 mg/m³)]	[6.30 ppm] [31.7 mg/m³]		
				Laboratory (n = 1): (NA)	[2.87 ppm] [14.4 mg/m³]		
				Repair (n = 2): (0.95–1.20 ppm) [(4.78–6.04 mg/m³)]	[1.08 ppm] [5.41 mg/m³]		
				Boiler (n = 2) : (< 0.2–0.80 ppm) [(< 1.0–4.02 mg/m³)]	[< 0.42 ppm] [< 2.1 mg/m³]		
				Other (white collar) (n = 7): (< 0.2–0.88 ppm) [(< 1.0–4.43 mg/m³)]	[< 0.2 ppm] [< 1.0 mg/m³]		

Table 1.1 (continued)

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
Adhesive use in aircraft cushion manufacturing plant North Carolina, USA, 1998	Personal monitoring (full-shift, short-term)	6930 full-shift TWA 11 area samples	GC-FID (1.0 µg/sample)	Overall: 0.14 ppm (< 0.01–0.55 ppm) [0.70 mg/m³ (< 0.05–2.77 mg/m³)]	NR	Adhesive containing low concentrations of 2-BP. Assembly sprayers and assemblers and covers workers worked directly with the adhesive formulations.	NIOSH (2002a)
				Assembly department: 0.30 ppm (0.10–0.55 ppm) [1.51 mg/m³ (0.50–2.77 mg/m³)]	NR		
				Covers department: 0.06 ppm (0.02–0.11 ppm) [0.30 mg/m³ (0.10–0.55 mg/m³)]	NR		
		12 short-term (15-min) samples among sprayers		Assembly department: [0.27 ppm] (0.2–0.4 ppm) [1.36 mg/m³ (1.0–2.0 mg/m³)] Covers department: NR (ND–0.1 ppm) [(ND–0.5 mg/m³)]	[1.26 mg/m³]		
Radio frequency and microwave communications component manufacturing plant, parts degreasing Indiana, USA, 2000	Personal air monitoring	20 full-shift TWA from 6 departments 2 short-term samples of several 1–5-min tasks for multiple workers	GC-FID (0.004 ppm [0.020 mg/m³] MDC for full-shift samples; 0.06 ppm [0.30 mg/m³] MDC for task-based samples)	Full-shift: all ND Task: all ND		Monitoring conducted after ventilation installation. Degreaser with possible 2-BP impurity. 20 workers sampled for 1 full shift each. Task samples combined 5 or 6 workers doing 1–5-minute tasks at degreaser.	NIOSH (2001)
	Stationary air monitoring	7	GC-FID (0.004 ppm [0.020 mg/m³] MDC)	1 sample 0.02 ppm [0.10 mg/m³], 6 samples ND			

Table 1.1 (continued)

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
Adhesive use in furniture cushion manufacturing company North Carolina, USA, 2000	Personal monitoring (full-shift/short-term (15-min)/ ceiling (5-min))	Personal monitoring (TWA) 12 (sprayers) 2 (floaters)	GC-FID (1 µg/sample)	Sprayers: 0.66 ppm (0.2–1.35 ppm) [3.32 mg/m³ (1.01–6.79 mg/m³)] Floaters: [0.15 ppm] (0.1–0.2 ppm) [0.75 mg/m³ (0.50–1.01 mg/m³)]	[2.77 mg/m³] [0.75 mg/m³]	Adhesive containing low concentrations of 2-BP.	NIOSH (2002b)
		Short-term (15-min) samples 9 (sprayers)	(0.06 ppm [0.30 mg/m³] MDC)	0.779 ppm (0.30–1.56 ppm) [3.92 mg/m³ (1.51–7.85 mg/m³)]	[3.12 mg/m³]		
		Ceiling measurements 11 (sprayers)	(0.12 ppm [0.60 mg/m³] MDC)	0.753 ppm (0.37–1.13 ppm) [3.79 mg/m³ (1.86–5.68 mg/m³)]	[3.47 mg/m³]		
Foam cushion manufacturing plant, adhesive spraying North Carolina, USA, 1999	Personal air monitoring (full-shift TWA)	Glue line: 7 (sprayers) 1 (cushion bundler) 1 (supervisor, setup) Springs line: 5 (sprayers) 1 (cushion bundler) 1 (setup)	GC-FID (2 µg/sample) (0.02 ppm) [0.10 mg/m³] MDC)	All positions: 0.24 ppm (GM) (0.08–0.68 ppm) [1.21 mg/m³ (0.40–3.42 mg/m³)] Glue line sprayers: 0.26 ppm (GM) (0.19–0.35 ppm) [1.31 mg/m³ (0.96–1.76 mg/m³)] Springs line sprayers: 0.38 ppm (GM) (0.24–0.68 ppm) [1.91 mg/m³ (1.21–3.42 mg/m³)]	NR [1.26 mg/m³] [1.76 mg/m³]	2-BP was measured in 2 bulk adhesive formulations at 0.135% and 0.0265%. Unclear whether measured in 1999 or 2001. Relationship between 2-BP exposure and semen quality and nerve conduction tests not assessed. End-of-week bromide ion (Br) in urine included in statistical analysis. Each worker sampled for 1 full shift.	NIOSH (2003b)

Table 1.1 (continued)

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
Foam cushion manufacturing plant, adhesive spraying North Carolina, USA, 1999 (cont.)				Single measures: Glue line doffer (cushion bundler): 0.16 ppm [0.80 mg/m³] Supervisor/ setup: 0.08 ppm [0.40 mg/m³] Springs line doffer (cushion bundler): 0.14 ppm [0.70 mg/m³] Foam setup: 0.11 ppm [0.53 mg/m³]			NIOSH (2003b) (cont.)
	Stationary monitoring			0.06 ppm[a] [0.30 mg/m³]			
Foam cushion manufacturing plant, adhesive spraying North Carolina, USA, 2001	Personal air monitoring (full-shift TWA)	Total: 40 Unexposed: 27 Exposed: 13 (including 8 sprayers and 5 non-sprayers)	GC-FID (0.8 µg/sample) (0.003 ppm [0.015 mg/m³] MDC)	Unexposed: NR (ND–< 0.01 ppm) [(ND–< 0.05 mg/m³)]	NR	Exposure designation based on job task review. Each worker sampled for 1 full shift.	NIOSH (2003b)
				Exposed: 0.066 ppm (GM) (ND–0.52 ppm) [0.33 mg/m³ (ND–2.62 mg/m³)]	NR		
Helicopter transmission manufacturing plant Texas, USA 2004	Personal breathing zone sampling on 2 consecutive days; pre-shift and post-shift exhaled breath sampling	5 in plating department (TWA calculations)	NIOSH method 1025. GC-FID (1 µg/sample) (0.016 ppm [0.08 mg/m³] MDC)	Day 1: 0.073 ppm (0.042–0.097 ppm) [0.37 mg/m³ (0.21–0.488) mg/m³)]	NR	2-BP was a contaminant of a 1-BP vapour degreasing solvent. Mentions ASTM standard of < 0.10% 2-BP.	NIOSH (2006a)
				Day 2: 0.022 ppm (0.017–0.031 ppm) [0.11 mg/m³ (0.086–0.156 mg/m³)]	NR		
Aerospace components manufacturing plant Illinois, USA, 2004	Personal air monitoring	11 using vapour degreasers on 2 consecutive days	GC-FID	ND (all samples)		2-BP was a contaminant of a 1-BP vapour degreasing solvent.	NIOSH (2006b)

Table 1.1 (continued)

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
Printed electronics circuit assembly manufacturing plant California, USA, 2004	Personal breathing zone air (full-shift TWA) and exhaled breath sampling	5 on 2 consecutive days	NIOSH method 1025. GC-FID (0.5 µg/sample) (0.0083 ppm [0.042 mg/m^3] MDC for air; 0.033 ppm [0.166 mg/m^3] MDC for exhaled breath)	ND (all personal breathing zone and exhaled breath samples)	NR	2-BP was a contaminant of a 1-BP cleaning solvent to remove oils and flux. Qualitatively examined skin contact.	NIOSH (2007a)
Dry cleaners New Jersey, USA, 2009	Personal samples (8-h TWA) Stationary samples	14 13	NIOSH method 1025. GC-FID (0.004 ppm [0.020 mg/m^3] TWA)	NR NR (ND–0.02 ppm) [(ND–0.10 mg/m^3)]	NR NR	The cleaning solvent contained 0.1% 2-BP. Area samples were collected in the front of the shop and in the back near the machine.	Blando et al. (2010)
Hydraulic power control component manufacturer, parts degreasing Arizona, USA, 2004	Personal air monitoring (full-shift TWA) Exhaled breath	2 full-shift samples each from 4 workers (n = 8) 25 exhaled breath samples from 5 workers	NIOSH method 1025. GC-FID (0.7 µg/sample) (0.012 ppm [0.060 mg/m^3] MDC for full-shift samples; 0.046 ppm [0.23 mg/m^3] MDC for exhaled breath samples)	Day 1: 0.0030 ppm (ND–0.0069 ppm) [0.016 mg/m^3 (ND–0.035 mg/m^3)] Day 2: 0.00038 ppm (ND–0.0015 ppm) [0.0019 mg/m^3 (ND–0.0075 mg/m^3)] Exhaled breath: all ND	NR	2-BP was not detected or was detected in trace quantities, between LOD and LOQ. The reported values are estimates, which could have considerable variability. 2-BP included in exposure assessment as assumed impurity in degreaser. Qualitative dermal exposure assessment done. Urine analysed for bromide ion and propyl mercapturic acid. Workers sampled on 2 consecutive days.	NIOSH (2007b)[b]

Table 1.1 (continued)

Occupational group/job type/ industry Location and date	Monitoring method	No. of samples	Analytical method (LOD)	Mean (range)	Median	Comments	Reference
Optical prism and assemblies manufacturer, using solvent to clean glass California, USA, 2004	Personal air monitoring (full-shift TWA) Exhaled breath (pre-shift and post-shift)	2 full-shift samples each from 7 workers (n = 14) Pre-shift and post-shift samples from 7 workers (n = 14)	NIOSH method 1025. GC-FID (0.5 µg/sample) (0.0083 ppm [0.042 mg/m^3] MDC for full-shift samples; 0.033 ppm [0.166 mg/m^3] MDC for exhaled breath samples)	Day 1: all ND Day 2: 0.026 ppm (ND–0.028 ppm) [0.131 mg/m^3 (ND–0.141 mg/m^3)] Exhaled breath: all ND	NR	Day 2 average was calculated only with detectable results (n = 3). 2-BP included in exposure assessment as assumed impurity in degreaser. Qualitative dermal exposure assessment done. Urine analysed for bromide ion and propyl mercapturic acid. Workers sampled on 2 consecutive days.	NIOSH (2007c)
Adhesives and coatings manufacturer, using solvent to produce adhesives Ohio, USA, 2004	Personal air monitoring (full-shift TWA) Exhaled breath (pre-shift and post-shift)	2 full-shift samples each from 11 workers (n = 22) Pre-shift and post-shift samples from 7 workers (n = 14)	NIOSH method 1025. GC-FID (0.5 µg/sample) (0.0083 ppm [0.042 mg/m^3] MDC for full-shift samples; 0.033 ppm [0.166 mg/m^3] MDC for exhaled breath samples)	Day 1: 0.19 ppm (ND–0.98 ppm) [0.96 mg/m^3 (ND–4.93 mg/m^3)] Day 2: 0.19 ppm (0.051–1.00 ppm) [0.96 mg/m^3 (0.26–5.03 mg/m^3)] Exhaled breath: all ND	NR	Day 1 average was calculated only with detectable results (n = 10). 2-BP included in exposure assessment as assumed impurity in adhesive. Highest 2-BP concentrations found in workers not near adhesive operations; may be interference or another source. Urine analysed for bromide ion and propyl mercapturic acid. Qualitative dermal exposure assessment done. Workers monitored for 2 full shifts.	NIOSH (2007d)

ASTM, American Society for Testing and Materials; 1-BP, 1-bromopropane; 2-BP, 2-bromopropane; GC-EID, gas chromatography-electron ionization detection; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; GM, geometric mean; GSD, geometric standard deviation; h, hour(s); LOD, limit of detection; LOQ, limit of quantification; MDC, minimum detectable concentration; min, minute; NR, not reported; ND, not detected; ppm, parts per million; TWA, time-weighted average.

[a] Single measurement, not the mean.

[b] The study was unclear on how censored (non-detect) data were used in calculating the average. The average was used instead of the geometric mean. Indicates that the 2-BP data are estimates.

in North Carolina, USA, full-shift exposures to 2-bromopropane ranged from < 0.01 to 0.55 ppm [< 0.05–2.77 mg/m³]; the highest full-shift exposures occurred in the assembly department (NIOSH, 2002a). In 2000, at a furniture cushion manufacturer in North Carolina, USA, full-shift personal exposures ranged from 0.19 to 1.35 ppm [0.96–6.79 mg/m³]; the highest mean exposure occurred among adhesive sprayers (0.66 ppm [3.32 mg/m³]) (NIOSH, 2002b). In 1999, at a furniture cushion manufacturer in North Carolina, USA, full-shift exposures ranged from 0.08 to 0.68 ppm [0.40–3.42 mg/m³]; the highest mean exposures occurred among adhesive sprayers (0.31 ppm [1.56 mg/m³]) (NIOSH, 2003b). In 2001, at the same plant in North Carolina, USA, full-shift exposures ranged from non-detected to < 0.01 ppm [< 0.05 mg/m³] (minimum quantifiable concentration) among workers determined to be "unexposed" on the basis of job task review, and from non-detected to 0.52 ppm [2.62 mg/m³] among exposed workers. The geometric mean exposures remained highest among adhesive sprayers: 0.18 ppm [0.91 mg/m³] (range, 0.06–0.52 ppm [0.30–2.62 mg/m³]) (NIOSH, 2003b). In 2004, at an adhesive manufacturer in Ohio, USA, full-shift exposures ranged from non-detected to 1.0 ppm [5.0 mg/m³], and all pre-shift and post-shift exhaled breath samples were below the LOD (NIOSH, 2007d). The highest 2-bromopropane exposures measured at the adhesive manufacturing facility occurred among workers not working at the adhesive line, and the authors suggested that there may have been analytical interference or an unrecognized additional source of 2-bromopropane exposure (NIOSH, 2007d). [The Working Group noted that the authors reported possible analytical challenges that may make the reported exposure data less reliable.]

In 2000, at a communications component manufacturing plant in Indiana, USA, where exposures occurred during degreasing in a ventilated area, all measured exposures were below the minimum detectable concentration (0.004 ppm [0.020 mg/m³] for full-shift samples; 0.06 ppm [0.30 mg/m³] for task-based samples) (NIOSH, 2001). In 2004, at an optical prism manufacturer in California, USA, where full-shift exposures occurred during degreasing operations, exposures ranged from non-detectable to 0.028 ppm [0.141 mg/m³], and no 2-bromopropane was detected in exhaled breath samples (NIOSH, 2007c). [The Working Group noted that the minimum detectable concentration was calculated using sampling and analytical method information, rather than the achieved laboratory LOD for NIOSH (2007b, c, d), and that censored (non-detected) data were excluded from the average in NIOSH (2007c). Given that measurement of 2-bromopropane was not the primary aim of these studies, because of potential censored data issues the range should be treated as the most reliable data of these 2-bromopropane measurements.] In 2004, at a hydraulic power control component manufacturer in Arizona, USA, where exposures occurred during degreasing, full-shift exposures ranged from non-detected (minimum detectable concentration, 0.012 ppm [0.060 mg/m³] for full-shift samples) to 0.0069 ppm [0.035 mg/m³] (NIOSH, 2007b). [The Working Group noted that these measurements are below the given minimum detectable concentration and may not be accurate.] In 2004 at a helicopter transmission manufacturer in Texas, USA, where 2-bromopropane exposures occurred during degreasing operations, full-shift exposures ranged from 0.017 to 0.097 ppm [0.086 to 0.488 mg/m³] (NIOSH, 2006a). In 2004 at an aerospace component manufacturing plant, where 2-bromopropane exposures were expected to occur during degreasing operations, none of the full-shift samples were above the LOD (NIOSH, 2006b). It was noted in several of the evaluations that dermal exposures were likely but were only evaluated qualitatively (NIOSH 2007a, b, c, d).

In 2009, four dry-cleaning shops in New Jersey, USA, using a dry-cleaning solvent containing 1-bromopropane consented to air sampling. The dry-cleaning solvent used in one of the shops contained approximately 0.1% 2-bromopropane. The highest 2-bromopropane measurement of the 12 stationary air samples (2–5 samples per shop) was 0.02 ppm [0.10 mg/m^3], but 2-bromopropane was not detected in 14 personal air samples (Blando et al., 2010).

In 2011, 3 workers with protective masks in one 2-bromopropane manufacturing plant in Japan had a mean full-shift personal measurement of 2.64 ppm [13.3 mg/m^3] TWA for 8 hours. One worker was exposed to 32 ppm [161 mg/m^3] of 2-bromopropane while filling drums in a 2-bromopropane manufacturing plant. In the filling area, 96 ppm [483 mg/m^3] of 2-bromopropane were measured in one 45-minute stationary monitoring sample. Four workers in two factories using 2-bromopropane had a mean full shift personal measurement of 0.067 ppm [0.34 mg/m^3] TWA of 2-bromopropane (Ministry of Health, Labour and Welfare, Japan, 2016). [The Working Group noted that there was no information about the tasks in which 2-bromopropane was used in these factories.]

1.4.3 Exposure of the general population

No quantitative data on exposure of the general population were available to the Working Group.

1.5 Regulations and guidelines

An occupational exposure level (OEL) for 2-bromopropane was established at 1 ppm [5 mg/m^3] in the Republic of Korea in 1998 (Yu et al., 1999) and in Finland since 2002 (Ministry of Social Affairs and Health, 2020). An OEL for 2-bromopropane was recommended at 1.0 ppm by the Japan Society for Occupational Health in 1999 and revised at 0.5 ppm [2.5 mg/m^3] in 2021 (Nomiyama, 2021). 2-Bromopropane was restricted for use by pregnant workers, workers who have recently given birth or are breast-feeding, and young people (aged < 18 years) on the basis of a reproductive toxicity classification 1A (ECHA, 2022a). 2-Bromopropane is classified as a reproductive toxicant class 1A in the Republic of Korea (Ministry of Employment and Labor notification 2018-62) (Park, 2020) and as group 1 in Japan by the Japan Society for Occupational Health (Nomiyama, 2021).

For the European Union, the European Chemicals Agency banned the use of 2-bromopropane for cosmetics.

1.6 Quality of exposure assessment in key mechanistic studies in humans

The Working Group reviewed two cross-sectional studies (Kim et al., 1996b; Ichihara et al., 1999) that contributed to mechanistic evidence on immunosuppression after exposure to 2-bromopropane. The studies focused on relatively small groups of workers in an electronics plant in the Republic of Korea, who were exposed to 2-bromopropane used in cleaning baths during the production of electronic switches, and workers in a 2-bromopropane production plant in China. The study in the Republic of Korea involved 25 female workers and 8 male workers, all exposed to 2-bromopropane. The study in China involved a total of 25 workers, of whom operators, mixers, boilers, and a laboratory worker were exposed to 2-bromopropane. The study also included salespeople and accountants who were not exposed to 2-bromopropane.

Details on the exposure assessment methods used in the studies are summarized in Table S1.2 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/631).

1.6.1 Exposure assessment methods

Air concentrations of various chemicals used in the tactile switch assembly operation section of the electronics factory in the Republic of Korea were assessed under simulated conditions, as detailed in Section 1.4.2. Fourteen stationary air samples were collected to obtain background levels near each cleaning bath and a few automatic assembly machines for 3 hours. Three short-term (15-minute) air samples were collected in the enclosure around the cleaning baths (KOSHA, 1995; Kim et al., 1996b). Personal breathing zone measurements were not conducted, and there was also no quantification of the dermal exposure that had been reported by the involved workers. The compositions of the different chemical mixtures used in the production process were assessed by chemical analyses of bulk samples. The solvent used in the cleaning baths contained 97.4% 2-bromopropane.

In the 2-bromopropane manufacturing plant in China, air concentrations of 2-bromopropane and 2-propanol were measured with passive samplers for all workers during an entire working day, which lasted 8 hours (Ichihara et al., 1999). The production workers worked in three shifts, and the office workers worked in day shifts. Ambient air sampling of 2-bromopropane with indicator tubes in the breathing zone of the workers was also performed. The authors estimated cumulative exposure by multiplying the result of each worker's single 8-hour TWA measurement of exposure to 2-bromopropane by the duration of employment.

1.6.2 Critical review of exposure assessment methods

The exposure assessment methods used in the study of the workers in the Republic of Korea provided anecdotal evidence of background concentrations of 2-bromopropane and a few co-exposures around the cleaning baths and the automatic assembly machines (Kim et al., 1996b). They also provided estimates of short-term levels in the enclosures around the cleaning baths, where workers had to perform tasks irregularly. From interviews with the workers, it was apparent that dermal exposure of 2-bromopropane was likely as well, because of hand-dipping and not using dermal hand protection. The authors convincingly showed that the personal exposures of the workers involved had been very high (> 10 ppm [> 50 mg/m^3]) and that dermal exposure would have also contributed.

The exposure assessment methods used in the study of the workers in China entailed personal shift-long (8-hour) measurements, which indicated high exposures (> 10–100 ppm [> 50–500 mg/m^3]) for most of the workers in the production area (Ichihara et al., 1999). Clear differences in exposure were seen between the different stages in the production process, with 20-fold higher exposures at the end of the process compared with the beginning of the process. The estimation of cumulative exposure based on a single measurement (with the implicit assumption that it is representative of the whole employment period, which varied between 5 and 69 months) will have led to non-differential misclassification and attenuation of the exposure–outcome associations. A group-based strategy (assigning exposure based on job-specific average exposure) would have prevented bias towards the null of exposure–outcome associations. Also, in this study dermal exposure to 2-bromopropane was not taken into account.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Inhalation

In a subchronic study that used a genetically modified mouse strain and complied with Good Laboratory Practice (GLP), groups of 25 male and 25 female Jic:CB6F1-Tg rasH2@Jcl (rasH2) mice (age, 7–8 weeks) were treated with 2-bromopropane (purity, 99.9%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 26 weeks (JBRC, 2019a, b; also reported by Goto et al., 2023). The concentration in the exposure chambers was set to 0 (clean air; control), 67, 200, or 600 ppm for the control group and the groups at the lowest, intermediate, and highest concentrations, respectively, for males and females and was monitored every 15 minutes. The mean air concentrations (± SD) for these groups were 0.0 ± 0.0, 66.8 ± 1.2, 200.6 ± 3.6, and 599.2 ± 10.0 ppm, respectively. For males, the survival rate of the group at 200 ppm was lower than that of controls starting at week 15. For females, the survival rate in the group at 600 ppm was lower than that of controls starting at week 19 and continued to decrease until study termination. At 26 weeks, survival was 25/25, 25/25, 21/25, and 24/25 for males, and 23/25, 24/25, 24/25, and 19/25 for females, for 0 (control), 67, 200, and 600 ppm, respectively. Male mice at 200 and 600 ppm showed a suppression of body-weight gain throughout the exposure period, whereas male mice at 67 ppm showed a decrease of body-weight gain until week 23. The relative final body weight in males was 98%, 91%, and 91% of the control value for the groups at 67, 200, and 600 ppm, respectively. Female mice at 600 ppm showed a small suppression of body-weight gain throughout the exposure period. The relative final body weight in females was 102%, 96%, and 94% of the control value for the groups at 67, 200, and 600 ppm, respectively. All mice underwent complete necropsy, and all organs and tissues were examined microscopically.

In male mice, there were significant positive trends in the incidence of bronchioloalveolar carcinoma of the lung (P = 0.0226, Peto trend test, prevalence method; P = 0.0347, Cochran–Armitage trend test) and in the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung (P = 0.0312, Peto trend test, prevalence method). There was a significant positive trend in the incidence of haemangioma or haemangiosarcoma (combined) of the subcutis (P = 0.0466, Peto trend test, combined analysis).

In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung (P = 0.0415, Peto trend test, prevalence method). There were significant positive trends in the incidence of malignant lymphoma of the lymph nodes (P = 0.0144, Peto trend test, standard method and combined analysis; P = 0.0186, Cochran–Armitage trend test) and in the incidence of malignant lymphoma of all sites (lymph nodes and thymus) (P = 0.0296, Peto trend test, standard method; P = 0.0073, Peto trend test, combined analysis; P = 0.0094, Cochran–Armitage trend test).

[The Working Group noted that this was a well-described and well-conducted subchronic study that complied with GLP, used multiple concentrations, used both sexes (with respective control groups), and used a genetically modified mouse strain that is highly susceptible to carcinogenesis.]

Table 3.1 Studies of carcinogenicity in rats and transgenic mice exposed to 2-bromopropane

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Jic:CB6F1-Tg rasH2@Jcl (rasH2) (M) 7–8 wk 26 wk JBRC (2019d)	Inhalation (whole-body exposure) Purity, 99.9% Air 0, 67, 200, 600 ppm 6 h/day, 5 days/wk 25, 25, 25, 25 25, 25, 21, 24	*Lung* Bronchioloalveolar carcinoma 0/25, 1/25, 3/25, 4/25	*P* = 0.0226, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis *P* = 0.0347, Cochran–Armitage trend test	*Principal strengths*: well-conducted GLP study; males and females used; multiple concentrations used.
		Bronchioloalveolar adenoma or carcinoma (combined) 3/25, 4/25, 5/25, 8/25	*P* = 0.0312, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Subcutis Haemangioma or haemangiosarcoma (combined) 0/25, 0/25, 1/25, 2/25	*P* = 0.0466, Peto trend test, combined analysis NS, Peto trend test, standard method and Peto trend test, prevalence method NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Jic:CB6F1-Tg rasH2@Jcl (rasH2) (F) 7–8 wk 26 wk JBRC (2019d)	Inhalation (whole-body exposure) Purity, 99.9% Air 0, 67, 200, 600 ppm 6 h/day, 5 days/wk 25, 25, 25, 25 23, 24, 24, 19	*Lung* Bronchioloalveolar adenoma or carcinoma (combined) 4/25, 3/25, 7/25, 8/25 *Lymph nodes* Malignant lymphoma 0/25, 0/25, 0/25, 2/25 *All sites* Malignant lymphoma 1/25, 0/25, 0/25, 4/25	*P* = 0.0415, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test *P* = 0.0144, Peto trend test, standard method *P* = 0.0144, Peto trend test, combined analysis Data not applicable for Peto trend test, prevalence method *P* = 0.0186, Cochran–Armitage trend test *P* = 0.0073, Peto trend test, combined analysis *P* = 0.0296, Peto trend test, standard method NS, Peto trend test, prevalence method *P* = 0.0094, Cochran–Armitage trend test	*Principal strengths*: well-conducted GLP study; males and females used; multiple concentrations used.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a)	Inhalation (whole-body exposure) Purity, ≥ 99.7% Air 0, 67, 200, 600 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 31, 19, 0	*Zymbal gland* Malignant tumours 0/50, 5/50*, 6/50**, 23/50***	$P < 0.0001$, Peto trend test, standard method $P = 0.0010$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0281$, **$P = 0.0133$, ***$P < 0.0001$, Fisher exact test	*Principal strengths*: well-conducted GLP study; multiple concentrations used; males and females used; covered most of the lifespan; adequate number of animals per group; adequate duration of exposure and observation. *Other comments*: lower survival in all treated groups. Historical controls reported by Takanobu et al. (2015): benign tumours of the Zymbal gland, 2/699 (0.3%; range, 0–2.0%); malignant tumours of the Zymbal gland, 2/699 (0.3%; range, 0–2.0%); bronchioloalveolar adenoma, 40/699 (5.7%; range, 2.0–12.0%); basal cell epithelioma of the skin/appendage, 1/699 (0.1%; range, 0–2.0%); keratoacanthoma of the skin/appendage, 25/699 (3.6%; range, 0–14.0%); sebaceous adenoma of the skin/appendage, 1/699 (0.1%; range, 0–2.0%); squamous cell papilloma of the skin/appendage, 8/699 (1.1%; range, 0–4.0%); squamous cell carcinoma of the skin/appendage, 1/699 (0.1%; range, 0–2.0%); adenoma of the large intestine, 1/699 (0.1%; range, 0–2.0%); mucinous adenocarcinoma of the large intestine, 0/699; malignant lymphoma, 1/699 (0.1%; range, 0–2.0%); squamous cell papilloma of the stomach, 1/699 (0.1%; range, 0–2.0%); squamous cell carcinoma of the stomach, 3/699 (0.4%; range, 0–2.0%); adenoma of the preputial gland, 11/699 (1.6%; range, 0–6.0%); fibroma of the subcutis, 68/699 (9.7%; range, 2.0–16.3%); fibrosarcoma of the subcutis, 4/699 (0.6%; range, 0–4.0%); haemangioma of the subcutis, 1/699 (0.1%; range, 0–2.0%); follicular adenoma of the thyroid gland, 8/698 (1.1%; range, 0–4.1%); follicular adenocarcinoma of the thyroid gland, 2/698 (0.3%; range, 0–2.0%); bronchioloalveolar carcinoma, 7/699 (1.0%; range, 0–4.0%); haemangioma (all sites), 1/699 (0.1%; range, 0–2.0%); brain glioma, 4/699 (0.6%; range, 0–4.0%); mononuclear cell leukaemia, 81/699 (11.6%; range, 6.0–20.0%).
		Benign or malignant tumours (combined) 0/50, 5/50*, 7/50**, 25/50***	$P < 0.0001$, Peto trend test, standard method $P = 0.0002$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0281$, **$P = 0.0062$, ***$P < 0.0001$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Skin/appendage* Basal cell epithelioma 0/50, 0/50, 2/50, 3/50	$P = 0.0103$, Peto trend test, standard method $P = 0.0025$, Peto trend test, prevalence method $P = 0.0001$, Peto trend test, combined analysis $P = 0.0298$, Cochran–Armitage trend test	
		Keratoacanthoma 4/50, 5/50, 7/50, 6/50	$P = 0.005$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Sebaceous adenoma 0/50, 1/50, 2/50, 10/50*	$P < 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0006$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		Basal cell carcinoma		
		0/50, 0/50, 0/50, 12/50*	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0001$, Fisher exact test	
		Squamous cell carcinoma or basal cell carcinoma (combined)		
		0/50, 1/50, 0/50, 13/50*	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P < 0.0001$, Fisher exact test	
		Squamous cell papilloma, basal cell epithelioma, sebaceous adenoma, keratoacanthoma, squamous cell carcinoma, or basal cell carcinoma (combined)		
		5/50, 6/50, 9/50, 22/50*	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0001$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Large intestine* Adenoma 0/50, 0/50, 1/50, 3/50	$P = 0.0059$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P = 0.0139$, Cochran–Armitage trend test	
		Adenocarcinoma 0/50, 1/50, 6/50*, 8/50**	$P = 0.0002$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P = 0.0012$, Cochran–Armitage trend test *$P = 0.0133$, **$P = 0.0029$, Fisher exact test	
		Adenoma or adenocarcinoma (combined) 0/50, 1/50, 7/50*, 11/50**	$P < 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0062$, **$P = 0.0003$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Small intestine* Adenocarcinoma 0/50, 0/50, 2/50, 7/50*	$P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P = 0.0435$, Peto trend test, standard method $P = 0.0001$, Cochran–Armitage trend test *$P = 0.0062$, Fisher exact test	
		Lymph nodes Malignant lymphoma 1/50, 0/50, 3/50, 7/50*	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, combined analysis $P = 0.0470$, Peto trend test, prevalence method $P = 0.0013$, Cochran–Armitage trend test *$P = 0.0297$, Fisher exact test	
		Stomach Squamous cell papilloma 0/50, 0/50, 1/50, 4/50	$P < 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P = 0.0032$, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		Squamous cell papilloma or carcinoma (combined)		
		0/50, 0/50, 1/50, 5/50*	$P < 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P = 0.0007$, Cochran–Armitage trend test *$P = 0.0281$, Fisher exact test	
		Preputial gland		
		Adenoma		
		0/50, 0/50, 1/50, 4/50	$P = 0.0032$, Peto trend test, standard method $P = 0.0213$, Peto trend test, prevalence method $P = 0.0003$, Peto trend test, combined analysis $P = 0.0032$, Cochran–Armitage trend test	
		Adenoma or squamous cell papilloma (combined)		
		0/50, 0/50, 1/50, 4/50	$P = 0.0032$, Peto trend test, standard method $P = 0.0213$, Peto trend test, prevalence method $P = 0.0003$, Peto trend test, combined analysis $P = 0.0032$, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		Adenocarcinoma, adenoma, or squamous cell papilloma (combined)		
		0/50, 1/50, 1/50, 4/50	$P = 0.0217$, Peto trend test, prevalence method $P = 0.0068$, Peto trend test, standard method $P = 0.0005$, Peto trend test, combined analysis $P = 0.0149$, Cochran–Armitage trend test	
		Subcutis		
		Haemangioma		
		0/50, 0/50, 3/50, 1/50	$P = 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Fibroma		
		7/50, 5/50, 15/50*, 5/50	$P = 0.0005$, Peto trend test, standard method $P = 0.0003$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis NS, Cochran–Armitage trend test *$P = 0.0448$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		Fibroma or fibrosarcoma (combined)		
		7/50, 5/50, 16/50*, 5/50	$P = 0.0005$, Peto trend test, standard method $P = 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis NS, Cochran–Armitage trend test *$P = 0.0279$, Fisher exact test	
		Thyroid		
		Follicular adenoma		
		0/50, 1/50, 5/50*, 2/50	$P = 0.0007$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test *$P = 0.0281$, Fisher exact test	
		Follicular adenocarcinoma		
		0/50, 3/50, 1/50, 0/50	NS, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		Follicular adenoma or adenocarcinoma (combined)		
		0/50, 4/50, 6/50*, 2/50	$P = 0.0026$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test *$P = 0.0133$, Fisher exact test	
		Lung		
		Bronchioloalveolar adenoma		
		3/50, 7/50, 5/50, 7/50	$P = 0.0011$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Bronchioloalveolar carcinoma		
		1/50, 3/50, 1/50, 2/50	NS, Peto trend test, standard method, Peto trend test, prevalence method, Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Bronchioloalveolar adenoma, squamous cell carcinoma, or bronchioloalveolar carcinoma (combined)		
		4/50, 8/50, 6/50, 9/50	$P = 0.0094$, Peto trend test, standard method $P = 0.0003$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		*All sites* Haemangioma (subcutis and spleen) 0/50, 1/50, 3/50, 2/50	$P < 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Brain Glioma 0/50, 2/50, 4/50, 2/50	$P = 0.0040$, Peto trend test, standard method $P = 0.0046$, Peto trend test, combined analysis NS, Peto trend test, prevalence method NS, Cochran–Armitage trend test	
		Spleen Mononuclear cell leukaemia 10/50, 7/50, 16/50, 4/50	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, combined analysis $P = 0.0297$, Peto trend test, prevalence method NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Pancreas* Islet cell adenoma 3/50, 2/50, 5/50, 1/50	$P = 0.0193$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Islet cell adenoma or islet cell adenocarcinoma (combined) 3/50, 3/50, 7/50, 1/50	$P = 0.0030$, Peto trend test, prevalence method $P = 0.0087$, Peto trend test, combined analysis NS, Peto trend test, standard method NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a)	Inhalation (whole-body exposure) Purity, ≥ 99.7% Air 0, 67, 200, 600 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 43, 36, 25, 0	*Mammary gland* Adenoma 1/50, 0/50, 5/50, 0/50	$P = 0.0056$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	*Principal strengths*: well-conducted GLP study; multiple concentrations used; males and females used; covered most of the lifespan; adequate number of animals per group; adequate duration of exposure and observation. *Other comments*: lower survival in all treated groups. Historical controls reported by Takanobu et al. (2015): adenoma of the mammary gland, 1/550 (0.2%; range, 0–2.0%); adenocarcinoma of the mammary gland, 3/550 (0.5%; range, 0–2.0%); mononuclear cell leukaemia, 66/550 (12.0%; range, 4.0–18.0%); fibroadenoma of the mammary gland, 60/550 (10.9%; range, 4.0–16.0%); adenoma of the large intestine, 0/550; mucinous adenocarcinoma of the large intestine, 0/550; benign tumours of the Zymbal gland, 0/550; malignant tumours of the Zymbal gland, 2/550 (0.4%; range, 0–2.0%); adenoma of the clitoral gland, 11/550 (2.0%; range, 0–4.0%); basal cell epithelioma of the skin/appendage, 0/550; keratoacanthoma of the skin/appendage, 1/550 (0.2%; range, 0–2.0%); sebaceous adenoma of the skin/appendage, 0/550; squamous cell papilloma of the skin/appendage, 2/550 (0.4%; range, 0–2.0%); squamous cell carcinoma of the skin/appendage, 3/550 (0.5%; range, 0–2.0%); trichoepithelioma of the skin/appendage, 1/550 (0.2%; range, 0–2.0%); fibroma of the subcutis, 6/550 (1.1%; range, 0–2.0%); fibrosarcoma of the subcutis, 0/550; adenoma of the uterus, 1/550 (0.2%; range, 0–2.0%); adenocarcinoma of the uterus, 4/550 (0.7%; range, 0–4.0%).
		Adenocarcinoma 0/50, 2/50, 5/50*, 48/50**	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0281$, **$P < 0.0001$, Fisher exact test	
		Fibroadenoma 2/50, 4/50, 13/50*, 1/50	$P = 0.0051$, Peto trend test, standard method $P = 0.0002$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis NS, Cochran–Armitage trend test *$P = 0.0019$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		Adenoma or fibroadenoma (combined)		
		3/50, 4/50, 16/50*, 1/50	$P = 0.0051$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis NS, Cochran–Armitage trend test *$P = 0.0008$, Fisher exact test	
		Adenocarcinoma or adenosquamous carcinoma (combined)		
		0/50, 2/50, 6/50*, 48/50**	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P = 0.0133$, **$P < 0.0001$, Fisher exact test	
		Adenoma, fibroadenoma, adenocarcinoma, or adenosquamous carcinoma (combined)		
		3/50, 6/50, 21/50*, 48/50*	$P < 0.0001$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P < 0.0001$, Cochran–Armitage trend test *$P < 0.0001$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Spleen* Mononuclear cell leukaemia 2/50, 6/50, 10/50*, 1/50	*P* = 0.0042, Peto trend test, standard method *P* = 0.0407, Peto trend test, prevalence method *P* = 0.0008, Peto trend test, combined analysis NS, Cochran–Armitage trend test **P* = 0.0139, Fisher exact test	
		Vagina Squamous cell papilloma 1/50, 2/50, 7/50*, 4/50	*P* = 0.0007, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test **P* = 0.0297, Fisher exact test	
		Squamous cell papilloma or carcinoma (combined) 1/50, 2/50, 8/50*, 4/50	*P* = 0.0005, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test **P* = 0.0154, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Large intestine* Adenoma or adenocarcinoma (combined) 0/50, 0/50, 2/50, 4/50	$P = 0.0001$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P = 0.0076$, Cochran–Armitage trend test	
		Zymbal gland Malignant tumours 0/50, 1/50, 1/50, 4/50	$P = 0.0096$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P = 0.0149$, Cochran–Armitage trend test	
		Benign or malignant tumours (combined) 0/50, 1/50, 1/50, 4/50	$P = 0.0096$, Peto trend test, standard method $P < 0.0001$, Peto trend test, prevalence method $P < 0.0001$, Peto trend test, combined analysis $P = 0.0149$, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Clitoral gland* Adenoma 1/50, 1/50, 4/50, 4/50	*P* = 0.0421, Peto trend test, prevalence method *P* = 0.0297, Peto trend test, combined analysis NS, Peto trend test, standard method NS, Cochran–Armitage trend test	
		Squamous cell papilloma or adenoma (combined) 1/50, 1/50, 4/50, 5/50	*P* = 0.0154, Peto trend test, prevalence method *P* = 0.0104, Peto trend test, combined analysis NS, Peto trend test, standard method *P* = 0.0481, Cochran–Armitage trend test	
		Squamous cell papilloma, adenoma, or adenocarcinoma (combined) 1/50, 1/50, 5/50, 6/50	*P* = 0.0069, Peto trend test, prevalence method *P* = 0.0046, Peto trend test, combined analysis NS, Peto trend test, standard method *P* = 0.0226, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Skin/appendage* Squamous cell papilloma 2/50, 0/50, 0/50, 4/50	$P = 0.0055$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis $P = 0.0501$, Cochran–Armitage trend test	
		Squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or sebaceous adenoma (combined) 4/50, 0/50, 0/50, 5/50	$P = 0.0106$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or squamous cell carcinoma (combined) 4/50, 0/50, 1/50, 5/50	$P = 0.0107$, Peto trend test, prevalence method $P = 0068$, Peto trend test, combined analysis NS, Peto trend test, standard method NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Subcutis* Fibroma 2/50, 1/50, 4/50, 0/50	$P = 0.0407$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Fibroma or fibrosarcoma (combined) 2/50, 1/50, 5/50, 2/50	$P = 0.0258$, Peto trend test, standard method $P = 0.0045$, Peto trend test, prevalence method $P = 0.0003$, Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Uterus Endometrial stromal polyp 9/50, 4/50, 11/50, 8/50	$P = 0.0154$, Peto trend test, prevalence method $P = 0.0111$, Peto trend test, combined analysis NS, Peto trend test, standard method NS, Cochran–Armitage trend test	
		Adenoma or adenocarcinoma (combined) 2/50, 0/50, 1/50, 4/50	$P = 0.0003$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2019a) (cont.)		*Pancreas* Islet cell adenoma 1/50, 0/50, 4/50, 0/50	$P = 0.0163$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		Islet cell adenoma or islet cell adenocarcinoma (combined) 1/50, 1/50, 4/50, 0/50	$P = 0.0290$, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	

F, female; GLP, Good Laboratory Practice; h, hour(s); M, male; NS, not significant; ppm, parts per million; wk, week(s).

3.2 Rat

Inhalation

In a well-conducted chronic toxicity and carcinogenicity study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female F344/DuCrlCrlj rats (age, 6–7 weeks) were treated with 2-bromopropane (purity, ≥ 99.7%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2019c, d; also reported by Senoh et al., 2023). The concentration in the exposure chambers was set to 0 (clean air; control), 67, 200, or 600 ppm for males and females and was monitored every 15 minutes. The mean air concentrations (± standard deviation, SD) for these groups were measured as 0.0 ± 0.0, 67.2 ± 0.3, 200.2 ± 0.6, and 600.9 ± 1.5 ppm, respectively. The survival rates of males and females in the 67 and 200 ppm groups were lower than those in the control group, and none of the males in the 600-ppm group were alive by week 85. At study termination, survival was 38/50, 31/50, 19/50, and 0/50 in males, and 43/50, 36/50, 25/50, and 0/50 in females, for 0 (control), 67, 200, and 600 ppm, respectively. The body weights of males and females in the groups at 67 and 200 ppm remained similar to those of their respective controls. In the groups at 600 ppm, males and females did not survive beyond week 84 and showed a decrease in body-weight gain starting from week 4 for males and from week 5 for females and continuing throughout the exposure period for both males and females. In males, the relative final body weight (except for the 600-ppm group, which was measured at week 82) at 67, 200, and 600 ppm was 101%, 99%, and 69% of the control value, respectively. In females, the relative final body weight (except for the 600-ppm group, which was measured at week 82) at 67, 200, and 600 ppm was 99%, 103%, and 72% of the control value, respectively. All rats underwent complete necropsy, and all organs and tissues were examined microscopically.

In male rats, there were significant increases in tumour incidence for many different tissue types. There was a significant positive trend ($P < 0.0001$, Peto trend test, standard method and combined analysis; $P = 0.0010$, Peto trend test, prevalence method; $P < 0.0001$, Cochran–Armitage trend test) in the incidence of malignant tumours of the Zymbal gland: 0/50, 5/50 (10%), 6/50 (12%), and 23/50 (46%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of malignant tumours of the Zymbal gland was significantly increased in each of the treated groups ($P = 0.0281$, $P = 0.0133$, and $P < 0.0001$ at 67, 200, and 600 ppm, respectively; Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 2/699 (0.3%; range, 0–2.0%). [The Working Group noted that several Peto trend tests were conducted in this study; the Peto test standard method was referred to as death analysis, the Peto test prevalence method was referred to as incidental tumour test, and the Peto test combined analysis was referred to as death analysis plus incidental tumour test. A significant P value in any Peto test was considered relevant for the detection of treatment-related increases in tumour incidence. The Working Group also noted that the data reported by Takanobu et al. (2015) are from control male F344/DuCrlCrlj rats in inhalation studies by the Japan Bioassay Research Center, but these studies were started in 2000–2009, in contrast to the 2-bromopropane study, which started in 2016.]

There was a significant positive trend ($P < 0.0001$, Peto trend test, standard method and combined analysis; $P = 0.0002$, Peto trend test, prevalence method; $P < 0.0001$, Cochran–Armitage trend test) in the incidence of benign or malignant tumours (combined) of the Zymbal gland: 0/50, 5/50 (10%), 7/50 (14%), and 25/50 (50%) for the groups at 0 (control), 67, 200, and

600 ppm, respectively. The incidence of benign or malignant tumours (combined) of the Zymbal gland was significantly increased in each of the treated groups (P = 0.0281, P = 0.0062, and P < 0.0001 at 67, 200, and 600 ppm, respectively; Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 2/699 (0.3%; range, 0–2.0%).

There were significant positive trends in the incidence of tumours of the skin/appendage. Specifically, there was a significant positive trend in the incidence of basal cell epithelioma (P = 0.0103, Peto trend test, standard method; P = 0.0025, Peto trend test, prevalence method; P = 0.0001, Peto trend test, combined analysis; P = 0.0298, Cochran–Armitage trend test). There was a significant positive trend in the incidence of keratoacanthoma (P = 0.005, Peto trend test, prevalence method). There was a significant positive trend (P < 0.0001, Peto trend test, prevalence method; P < 0.0001, Cochran–Armitage trend test) in the incidence of sebaceous adenoma: 0/50, 1/50 (2%), 2/50 (4%), and 10/50 (20%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of sebaceous adenoma was significantly increased at the highest concentration (P = 0.0006, Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 1/699 (0.1%; range, 0–2.0%). There was a significant positive trend (P < 0.0001, Peto trend test, standard method, prevalence method, and combined analysis; P < 0.0001, Cochran–Armitage trend test) in the incidence of basal cell carcinoma: 0/50, 0/50, 0/50, and 12/50 (24%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of basal cell carcinoma was significantly increased at the highest concentration (P = 0.0001, Fisher exact test). There was a significant positive trend (P < 0.0001, Peto trend test, standard method, prevalence method, and combined analysis; P < 0.0001, Cochran–Armitage trend test) in the incidence of squamous cell carcinoma or basal cell carcinoma (combined): 0/50, 1/50 (2%), 0/50, and 13/50 (26%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell carcinoma or basal cell carcinoma (combined) was significantly increased at the highest concentration (P < 0.0001, Fisher exact test). The incidence of basal cell epithelioma, sebaceous adenoma, and squamous cell carcinoma of the skin/appendage in historical controls reported by Takanobu et al. (2015) was 1/699 (0.1%; range, 0–2.0%), 1/699 (0.1%; range, 0–2.0%), and 1/699 (0.1%; range, 0–2.0%), respectively. There was a significant positive trend (P < 0.0001, Peto trend test, standard method, prevalence method, and combined analysis; P < 0.0001, Cochran–Armitage trend test) in the incidence of squamous cell papilloma, basal cell epithelioma, sebaceous adenoma, keratoacanthoma, squamous cell carcinoma, or basal cell carcinoma (combined): 5/50 (10%), 6/50 (12%), 9/50 (18%), and 22/50 (44%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell papilloma, basal cell epithelioma, sebaceous adenoma, keratoacanthoma, squamous cell carcinoma, or basal cell carcinoma (combined) was significantly increased at the highest concentration (P = 0.0001, Fisher exact test). There was a significant positive trend in the incidence of adenoma of the large intestine (P = 0.0059, Peto trend test, prevalence method; P = 0.0139, Cochran–Armitage trend test). There was a significant positive trend (P = 0.0002, Peto trend test, prevalence method; P = 0.0012, Cochran–Armitage trend test) in the incidence of adenocarcinoma of the large intestine: 0/50, 1/50 (2%), 6/50 (12%), and 8/50 (16%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenocarcinoma of the large intestine was significantly increased at 200 and 600 ppm (P = 0.0133 and P = 0.0029, respectively, both Fisher exact test). There was a significant positive trend (P < 0.0001, Peto trend test, prevalence method; P < 0.0001, Cochran–

Armitage trend test) in the incidence of adenoma or adenocarcinoma (combined) of the large intestine: 0/50, 1/50 (2%), 7/50 (14%), and 11/50 (22%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenoma or adenocarcinoma (combined) of the large intestine was significantly increased at 200 and 600 ppm ($P = 0.0062$ and $P = 0.0003$, respectively, both Fisher exact test). The incidence of adenoma and of mucinous adenocarcinoma of the large intestine in historical controls reported by Takanobu et al. (2015) was 1/699 (0.1%; range, 0–2.0%) and 0/699, respectively. There was a significant positive trend ($P = 0.0435$, Peto trend test, standard method; $P < 0.0001$, Peto trend test, prevalence method and combined analysis; $P = 0.0001$, Cochran–Armitage trend test) in the incidence of adenocarcinoma of the small intestine: 0/50, 0/50, 2/50 (4%), and 7/50 (14%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenocarcinoma of the small intestine was significantly increased at the highest concentration ($P = 0.0062$, Fisher exact test). There was a significant positive trend ($P = 0.0470$, Peto trend test, prevalence method; $P < 0.0001$, Peto trend test, standard method and combined analysis; $P = 0.0013$, Cochran–Armitage trend test) in the incidence of malignant lymphoma of the lymph nodes: 1/50 (2%), 0/50, 3/50 (6%), and 7/50 (14%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of malignant lymphoma of the lymph nodes was significantly increased at the highest concentration ($P = 0.0297$, Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 1/699 (0.1%; range, 0–2.0%). There was a significant positive trend ($P < 0.0001$, Peto trend test, prevalence method; $P = 0.0032$, Cochran–Armitage trend test) in the incidence of squamous cell papilloma of the stomach: 0/50, 0/50, 1/50 (2%), and 4/50 (8%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell papilloma of the stomach at the highest concentration exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 1/699 (0.1%; range, 0–2.0%). There was a significant positive trend ($P < 0.0001$, Peto trend test, prevalence method; $P = 0.0007$, Cochran–Armitage trend test) in the incidence of squamous cell papilloma or carcinoma (combined) of the stomach: 0/50, 0/50, 1/50 (2%), and 5/50 (10%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell papilloma or carcinoma (combined) of the stomach was significantly increased at the highest concentration ($P = 0.0281$, Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 3/699 (0.4%; range, 0–2.0%). There were significant positive trends in the incidence of tumours of the preputial gland. Specifically, there were significant positive trends in the incidence of adenoma ($P = 0.0032$, Peto trend test, standard method; $P = 0.0213$, Peto trend test, prevalence method; $P = 0.0003$, Peto trend test, combined analysis; $P = 0.0032$, Cochran–Armitage trend test), in the incidence of adenoma or squamous cell papilloma (combined) ($P = 0.0032$, Peto trend test, standard method; $P = 0.0213$, Peto trend test, prevalence method; $P = 0.0003$, Peto trend test, combined analysis; $P = 0.0032$, Cochran–Armitage trend test), and in the incidence of adenocarcinoma, adenoma, or squamous cell papilloma (combined) ($P = 0.0068$, Peto trend test, standard method; $P = 0.0217$, Peto trend test, prevalence method; $P = 0.0005$, Peto trend test, combined analysis; $P = 0.0149$, Cochran–Armitage trend test). There was a significant positive trend ($P = 0.0005$, Peto trend test, standard method; $P = 0.0003$, Peto trend test, prevalence method; $P < 0.0001$, Peto trend test, combined analysis) in the incidence of fibroma of the subcutis: 7/50 (14%), 5/50 (10%), 15/50 (30%), and 5/50 (10%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of fibroma of the subcutis was

significantly increased at 200 ppm (P = 0.0448, Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 68/699 (9.7%; range, 2.0–16.3%). There was a significant positive trend (P = 0.0005, Peto trend test, standard method; P = 0.0001, Peto trend test, prevalence method; P < 0.0001, Peto trend test, combined analysis) in the incidence of fibroma or fibrosarcoma (combined) of the subcutis: 7/50 (14%), 5/50 (10%), 16/50 (32%), and 5/50 (10%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of fibroma or fibrosarcoma (combined) of the subcutis was significantly increased at 200 ppm (P = 0.0279, Fisher exact test). The incidence in the control group and all treated groups exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 4/699 (0.6%; range, 0–4.0%). There was a significant positive trend (P = 0.0001, Peto trend test, prevalence method) in the incidence of haemangioma of the subcutis: 0/50, 0/50, 3/50 (6%), and 1/50 (2%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of haemangioma of the subcutis at 200 ppm exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 1/699 (0.1%; range, 0–2.0%). There was a significant positive trend (P = 0.0007, Peto trend test, prevalence method) in the incidence of follicular adenoma of the thyroid gland: 0/50, 1/50 (2%), 5/50 (10%), and 2/50 (4%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of follicular adenoma of the thyroid gland was significantly increased at 200 ppm (P = 0.0281, Fisher exact test). There was a significant positive trend (P = 0.0026, Peto trend test, prevalence method) in the incidence of follicular adenoma or adenocarcinoma (combined) of the thyroid gland: 0/50, 4/50 (8%), 6/50 (12%), and 2/50 (4%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of follicular adenoma or adenocarcinoma (combined) of the thyroid gland was significantly increased at 200 ppm (P = 0.0133, Fisher exact test), and the incidence in all treated groups exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 2/698 (0.3%; range, 0–2.0%). There were significant positive trends in the incidence of bronchioloalveolar adenoma of the lung (P = 0.0011, Peto trend test, prevalence method) and in the incidence of bronchioloalveolar adenoma, squamous cell carcinoma, or bronchioloalveolar carcinoma (combined) of the lung (P = 0.0094, Peto trend test, standard method; P = 0.0003, Peto trend test, prevalence method; P < 0.0001, Peto trend test, combined analysis). [The Working Group noted that bronchioloalveolar neoplasms and squamous cell neoplasms of the lung should not be combined (see Brix et al., 2010, and General Remarks). Therefore, the Working Group did not consider combination of bronchioloalveolar neoplasms of the lung and squamous cell carcinoma of the lung to be appropriate for detection of increase in tumour incidence.] There was a significant positive trend (P < 0.001, Peto trend test, prevalence method) in the incidence of haemangioma of all sites (subcutis and spleen): 0/50, 1/50 (2%), 3/50 (6%), and 2/50 (4%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of haemangioma of all sites (subcutis and spleen) at 200 and 600 ppm exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 1/699 (0.1%; range, 0–2.0%). There was a significant positive trend (P = 0.0040, Peto trend test, standard method; P = 0.0046, Peto trend test, combined analysis) in the incidence of glioma of the brain: 0/50, 2/50 (4%), 4/50 (8%), and 2/50 (4%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of glioma at 200 ppm exceeded the upper bound of the range observed in historical controls reported by Takanobu et al. (2015): 4/699 (0.6%; range, 0–4.0%). There was a significant positive trend (P < 0.001, Peto trend test, standard method and

combined analysis; $P = 0.0297$, Peto trend test, prevalence method) in the incidence of mononuclear cell leukaemia of the spleen: 10/50 (20%), 7/50 (14%), 16/50 (32%), and 4/50 (8%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of mononuclear cell leukaemia of the spleen at 200 ppm exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 81/699 (11.6%; range, 6.0–20.0%). There were significant positive trends in the incidence of islet cell adenoma of the pancreas ($P = 0.0193$, Peto trend test, prevalence method) and in the incidence of islet cell adenoma or islet cell adenocarcinoma (combined) of the pancreas ($P = 0.0030$, Peto trend test, prevalence method; $P = 0.0087$, Peto trend test, combined analysis).

In female rats, there was a significant positive trend ($P = 0.0056$, Peto trend test, prevalence method) in the incidence of adenoma of the mammary gland: 1/50 (2%), 0/50, 5/50 (10%), and 0/50 for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenoma of the mammary gland at 200 ppm exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 1/550 (0.2%; range, 0–2.0%). [The Working Group noted that several Peto trend tests were conducted in this study; the Peto test standard method was referred to as death analysis, the Peto test prevalence method was referred to as incidental tumour test, and the Peto test combined analysis was referred to as death analysis plus incidental tumour test. A significant P value in any Peto test was considered relevant for the detection of treatment-related increases in tumour incidence. The Working Group also noted that the data reported by Takanobu et al. (2015) are from control female F344/DuCrlCrlj rats in inhalation studies by the Japan Bioassay Research Center, but these studies were started in 2000–2009, in contrast to the 2-bromopropane study, which started in 2016.]

There was a significant positive trend ($P < 0.0001$, Peto trend test, standard method, prevalence method, and combined analysis; $P < 0.0001$, Cochran–Armitage trend test) in the incidence of adenocarcinoma of the mammary gland: 0/50, 2/50 (4%), 5/50 (10%), and 48/50 (96%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenocarcinoma of the mammary gland was significantly increased at 200 and 600 ppm ($P = 0.0281$ and $P < 0.0001$, respectively, both Fisher exact test), and the incidence in all treated groups exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 3/550 (0.5%; range, 0–2.0%). There was a significant positive trend ($P = 0.0051$, Peto trend test, standard method; $P = 0.0002$, Peto trend test, prevalence method; $P < 0.0001$, Peto trend test, combined analysis) in the incidence of fibroadenoma of the mammary gland: 2/50 (4%), 4/50 (8%), 13/50 (26%), and 1/50 (2%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of fibroadenoma of the mammary gland was significantly increased at 200 ppm ($P = 0.0019$, Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 60/550 (10.9%; range, 4–16.0%). There was a significant positive trend ($P = 0.0051$, Peto trend test, standard method; $P < 0.0001$, Peto trend test, prevalence method and combined analysis) in the incidence of adenoma or fibroadenoma (combined) of the mammary gland: 3/50 (6%), 4/50 (8%), 16/50 (32%), and 1/50 (2%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenoma or fibroadenoma (combined) of the mammary gland was significantly increased at 200 ppm ($P = 0.0008$, Fisher exact test). There was a significant positive trend ($P < 0.0001$, Peto trend test, standard method, prevalence method, and combined analysis; $P < 0.0001$, Cochran–Armitage trend test) in the incidence of adenocarcinoma or adenosquamous carcinoma (combined) of the

mammary gland: 0/50, 2/50 (4%), 6/50 (12%), and 48/50 (96%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenocarcinoma or adenosquamous carcinoma (combined) of the mammary gland was significantly increased at 200 and 600 ppm (P = 0.0133 and P < 0.0001, respectively, both Fisher exact test). There was a significant positive trend (P < 0.0001, Peto trend test, standard method, prevalence method, and combined analysis; P < 0.0001, Cochran–Armitage trend test) in the incidence of adenoma, fibroadenoma, adenocarcinoma, or adenosquamous carcinoma (combined) of the mammary gland: 3/50 (6%), 6/50 (12%), 21/50 (42%), and 48/50 (96%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenoma, fibroadenoma, adenocarcinoma, or adenosquamous carcinoma (combined) of the mammary gland was significantly increased at 200 and 600 ppm (P < 0.0001, Fisher exact test). [The Working Group noted that mammary gland adenoma and mammary gland fibroadenoma should not be combined, because they are thought to arise from different parts of the mammary gland (see Brix et al., 2010). The only exception may occur when an adenoma or a carcinoma arises from a fibroadenoma, and then it should be combined with other adenomas and carcinomas of the mammary gland. The conditions for this exception were not reported for the current study by JBRC (2019c, d). Therefore, the Working Group did not consider combination of mammary gland adenoma and mammary gland fibroadenoma, or combination of mammary gland adenoma, fibroadenoma, adenocarcinoma, and adenosquamous carcinoma, to be appropriate for detection of increase in tumour incidence.]

There was a significant positive trend (P = 0.0042, Peto trend test, standard method; P = 0.0407, Peto trend test, prevalence method; P = 0.0008, Peto trend test, combined analysis) in the incidence of mononuclear cell leukaemia of the spleen: 2/50 (4%), 6/50 (12%), 10/50 (20%), and 1/50 (2%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of mononuclear cell leukaemia of the spleen was significantly increased at 200 ppm (P = 0.0139, Fisher exact test) and exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 66/550 (12%; range, 4–18.0%).

There was a significant positive trend (P = 0.0007, Peto trend test, prevalence method) in the incidence of squamous cell papilloma of the vagina: 1/50 (2%), 2/50 (4%), 7/50 (14%), and 4/50 (8%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell papilloma of the vagina was significantly increased at 200 ppm (P = 0.0297, Fisher exact test). There was a significant positive trend (P = 0.0005, Peto trend test, prevalence method) in the incidence of squamous cell papilloma or carcinoma (combined) of the vagina: 1/50 (2%), 2/50 (4%), 8/50 (16%), and 4/50 (8%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell papilloma or carcinoma (combined) of the vagina was significantly increased at 200 ppm (P = 0.0154, Fisher exact test).

There was a significant positive trend (P = 0.0001, Peto trend test, prevalence method; P = 0.0076, Cochran–Armitage trend test) in the incidence of adenoma or adenocarcinoma (combined) of the large intestine. The incidence of adenoma and of mucinous adenocarcinoma of the large intestine in historical controls reported by Takanobu et al. (2015) was 0/550 for both.

There was a significant positive trend (P = 0.0096, Peto trend test, standard method; P < 0.0001, Peto trend test, prevalence method and combined analysis; P = 0.0149, Cochran–Armitage trend test) in the incidence of malignant tumours of the Zymbal gland. There was a significant positive trend (P = 0.0096, Peto trend test, standard method; P < 0.0001, Peto trend test, prevalence method and combined analysis; P = 0.0149, Cochran–Armitage trend test) in

the incidence of benign or malignant tumours (combined) of the Zymbal gland. The incidence of benign or malignant tumours (combined) of the Zymbal gland at the highest concentration (4/50; 8%) exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 2/550 (0.4%; range, 0–2.0%).

There were significant positive trends in the incidence of tumours of the clitoral gland. Specifically, there were significant positive trends in the incidence of adenoma (P = 0.0421, Peto trend test, prevalence method; P = 0.0297, Peto trend test, combined analysis), in the incidence of squamous cell papilloma or adenoma (combined) (P = 0.0154, Peto trend test, prevalence method; P = 0.0104, Peto trend test, combined analysis; P = 0.0481, Cochran–Armitage trend test), and in the incidence of squamous cell papilloma, adenoma, or adenocarcinoma (combined) (P = 0.0069, Peto trend test, prevalence method; P = 0.0046, Peto trend test, combined analysis; P = 0.0226, Cochran–Armitage trend test). There was a significant positive trend (P = 0.0055, Peto trend test, prevalence method) in the incidence of squamous cell papilloma of the skin/appendage: 2/50 (4%), 0/50, 0/50, and 4/50 (8%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of squamous cell papilloma of the skin/appendage at the highest concentration exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 2/550 (0.4%; range, 0–2.0%). There was a significant positive trend in the incidence of squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or sebaceous adenoma (combined) of the skin/appendage (P = 0.0106, Peto trend test, prevalence method). There was a significant positive trend in the incidence of squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or squamous cell carcinoma (combined) of the skin/appendage (P = 0.0107, Peto trend test, prevalence method; P = 0.0068, Peto trend test, combined analysis). There was a significant positive trend (P = 0.0407, Peto trend test, prevalence method) in the incidence of fibroma of the subcutis: 2/50 (4%), 1/50 (2%), 4/50 (8%), and 0/50 for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of fibroma of the subcutis at 200 ppm exceeded the upper bound of the range observed in historical controls as reported by Takanobu et al. (2015): 6/550 (1.1%; range, 0–2.0%). There was a significant positive trend in the incidence of fibroma or fibrosarcoma (combined) of the subcutis (P = 0.0258, Peto trend test, standard method; P = 0.0045, Peto trend test, prevalence method; P = 0.0003, Peto trend test, combined analysis). There was a significant positive trend in the incidence of endometrial stromal polyps of the uterus (P = 0.0154, Peto trend test, prevalence method; P = 0.0111, Peto trend test, combined analysis). There was a significant positive trend (P = 0.0003, Peto trend test, prevalence method) in the incidence of adenoma or adenocarcinoma (combined) of the uterus: 2/50 (4%), 0/50, 1/50 (2%), and 4/50 (8%) for the groups at 0 (control), 67, 200, and 600 ppm, respectively. The incidence of adenoma and of adenocarcinoma of the uterus in historical controls reported by Takanobu et al. (2015) was 1/550 (0.2%; range, 0–2.0%) and 4/550 (0.7%; range, 0–4.0%), respectively. There were significant positive trends in the incidence of islet cell adenoma of the pancreas (P = 0.0163, Peto trend test, prevalence method) and in the incidence of islet cell adenoma or islet cell adenocarcinoma (combined) of the pancreas (P = 0.0290, Peto trend test, prevalence method).

Regarding non-neoplastic lesions, in male rats, there were increases in the incidence of bronchioloalveolar epithelial hyperplasia of the lung and of extramedullary haematopoiesis in the spleen at the highest concentration. In female rats, there were increases in the incidence and/or severity of the following non-neoplastic lesions: extramedullary haematopoiesis in the spleen (at the intermediate and highest concentrations),

ulcers of the forestomach (at the intermediate and highest concentrations), and hyperplasia of the vagina (at the intermediate concentration).

[The Working Group noted that this was a well-described and well-conducted study that complied with GLP, used multiple concentrations, used both sexes (with respective control groups), had an adequate duration of exposure and observation, and had an adequate number of animals per group. The Working Group also noted that an unusually high degree of carcinogenic activity with regard to incidence, site, and types of tumours was observed in both males and females.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of 2-bromopropane has been assessed in one well-conducted subchronic study that complied with GLP in genetically modified male and female Jic:CB6F1-Tg rasH2@Jcl (rasH2) mice treated by inhalation (JBRC, 2019a, b; also reported by Goto et al., 2023) and in one well-conducted GLP study in male and female F344/DuCrlCrlj rats treated by inhalation (JBRC, 2019c, d; also reported by Senoh et al., 2023).

In the inhalation study that complied with GLP in genetically modified male and female Jic:CB6F1-Tg rasH2@Jcl (rasH2) mice (JBRC, 2019a, b; also reported by Goto et al., 2023), there were significant positive trends in the incidence of bronchioloalveolar carcinoma of the lung and in the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung in male mice. There was a significant positive trend in the incidence of haemangioma or haemangiosarcoma (combined) of the subcutis in male mice. In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung. There were significant positive trends in the incidence of malignant lymphoma of the lymph nodes and in the incidence of malignant lymphoma of all sites.

In the inhalation study that complied with GLP in male and female F344/DuCrlCrlj rats (JBRC, 2019c, d; also reported by Senoh et al., 2023), there were significant increases in tumour incidence for many different tissue types.

In male rats, there was a significant positive trend in the incidence of malignant tumours of the Zymbal gland, and the incidence was significantly increased in all treated groups. There was a significant positive trend in the incidence of benign or malignant tumours (combined) of the Zymbal gland, and the incidence was significantly increased in all treated groups. There were significant positive trends in the incidence of basal cell epithelioma of the skin/appendage and in the incidence of keratoacanthoma of the skin/appendage. There was a significant positive trend in the incidence of sebaceous adenoma of the skin/appendage, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of basal cell carcinoma of the skin/appendage, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of squamous cell carcinoma or basal cell carcinoma (combined) of the skin/appendage, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of squamous cell papilloma, basal cell epithelioma, sebaceous adenoma, keratoacanthoma, squamous cell carcinoma, or basal cell carcinoma (combined) of the skin/appendage, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of adenoma of the large intestine. There was a significant positive trend in the incidence of adenocarcinoma of the large intestine, and the incidence was significantly increased at 200 and 600 ppm. There was a significant positive

trend in the incidence of adenoma or adenocarcinoma (combined) of the large intestine, and the incidence was significantly increased at 200 and 600 ppm. There was a significant positive trend in incidence of adenocarcinoma of the small intestine, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of malignant lymphoma of the lymph nodes, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of squamous cell papilloma of the stomach. There was a significant positive trend in the incidence of squamous cell papilloma or carcinoma (combined) of the stomach, and the incidence was significantly increased at the highest concentration. There was a significant positive trend in the incidence of adenoma of the preputial gland, of adenoma or squamous cell papilloma (combined) of the preputial gland, and of adenocarcinoma, adenoma, or squamous cell papilloma (combined) of the preputial gland. There was a significant positive trend in the incidence of fibroma of the subcutis, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of fibroma or fibrosarcoma (combined) of the subcutis, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of haemangioma of the subcutis. There was a significant positive trend in the incidence of follicular adenoma of the thyroid gland, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of follicular adenoma or adenocarcinoma (combined) of the thyroid gland, and the incidence was significantly increased at 200 ppm. In addition, there was a significant positive trend in the incidence of bronchioloalveolar adenoma of the lung. There was a significant positive trend in the incidence of haemangioma of all sites. There was a significant positive trend in the incidence of glioma of the brain. There was a significant positive trend in the incidence of mononuclear cell leukaemia of the spleen. There were significant positive trends in the incidence of islet cell adenoma of the pancreas and in the incidence of islet cell adenoma or islet cell adenocarcinoma (combined) of the pancreas.

In female rats, there was a significant positive trend in the incidence of adenoma of the mammary gland. There was a significant positive trend in the incidence of adenocarcinoma of the mammary gland, and the incidence was significantly increased at 200 and 600 ppm. There was a significant positive trend in the incidence of fibroadenoma of the mammary gland, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of adenocarcinoma or adenosquamous carcinoma (combined) of the mammary gland, and the incidence was significantly increased at 200 and 600 ppm. There was a significant positive trend in the incidence of mononuclear cell leukaemia of the spleen, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of squamous cell papilloma of the vagina, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of squamous cell papilloma or carcinoma (combined) of the vagina, and the incidence was significantly increased at 200 ppm. There was a significant positive trend in the incidence of adenoma or adenocarcinoma (combined) of the large intestine. There were significant positive trends in the incidence of malignant tumours of the Zymbal gland and in the incidence of benign or malignant tumours (combined) of the Zymbal gland. There were significant positive trends in the incidence of tumours of the clitoral gland. Specifically, there were significant positive trends in the incidence of adenoma, in the incidence of squamous cell papilloma or adenoma (combined), and in the incidence of squamous cell papilloma, adenoma, or adenocarcinoma (combined). There was a significant positive trend in the incidence of

squamous cell papilloma of the skin/appendage. There was a significant positive trend in the incidence of squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or sebaceous adenoma (combined) of the skin/appendage. There was a significant positive trend in the incidence of squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or squamous cell carcinoma (combined) of the skin/appendage. There were significant positive trends in the incidence of fibroma of the subcutis and in the incidence of fibroma or fibrosarcoma (combined) of the subcutis. There were significant positive trends in the incidence of endometrial stromal polyps of the uterus and in the incidence of adenoma or adenocarcinoma (combined) of the uterus. There were significant positive trends in the incidence of islet cell adenoma of the pancreas and in the incidence of islet cell adenoma or islet cell adenocarcinoma (combined) of the pancreas.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Two studies that investigated the toxicokinetics of 2-bromopropane in exposed humans, both in occupational settings, were available to the Working Group (Kawai et al., 1997, 2002).

Kawai et al. (1997) measured urinary metabolites of 2-bromopropane in 5 male workers exposed to 2-bromopropane at a geometric mean concentration of 3 mg/m^3 (geometric SD, 1.47 mg/m^3) and 20 unexposed male workers. The concentration of 2-bromopropane was measured by stationary air sampling at five sites in the workshop, following a grid sampling strategy. End-of-shift urinary concentrations of 2-bromopropane, 2-propanol (isopropyl alcohol), and acetone were measured with headspace GC-FID, and the end-of-shift urinary concentration of bromide ion was measured with GC-ECD after methylation using dimethyl sulfate. No 2-bromopropane or 2-propanol was detected in end-of-shift urine samples. The urinary concentrations of acetone and bromide ion for four exposed workers were within the ranges found for unexposed workers, but for one worker were higher than the upper limits of the ranges for unexposed workers. The worker with the highest urinary concentration was also likely to have the highest exposure because he was in charge of maintenance and frequent checking of a machine that used 2-bromopropane. [The Working Group noted that this study suggests that 2-bromopropane undergoes hydrolysis to produce bromide ion and 2-propanol, which oxidizes to acetone, in the urine of humans. The exposures at this plant (3 mg/m^3 [0.6 ppm]) were lower than the one government OEL of 1 ppm.]

Kawai et al. (2002) investigated the metabolism and excretion of 2-bromopropane with GC-ECD in urine samples from 10 groups (23–54 per group) in China, Japan, and the Republic of Korea. Derived data indicated that the mean metabolic bromide ion concentrations were 5.4 and 6.5 mg/mL in the urine of men and women, respectively, in Japan and ranged from 1.8 to 2.8 mg/mL for four groups in China and from 8 to 12 mg/mL for four groups of women in the Republic of Korea. Regression analyses showed that the urinary bromide concentration was positively associated with intake of marine products and negatively associated with intake of cereals or potato. [The Working Group noted that urinary bromide can be derived from intake of marine products in humans; therefore, it does not necessarily indicate exposure to a brominated compound that includes 2-bromopropane. The Working Group noted that acetone is also not specific as a biomarker for 2-bromopropane exposure.]

4.1.2 Experimental systems

(a) Absorption

Kim et al. (1997) reported that the penetration speeds of 2-bromopropane into the skin of male Crl:SKH-hrBr hairless mice were 4.165 mg/cm^2 per hour as measured with in vitro diffusion cell methods and 3.12 mg/cm^2 per hour as measured with in vivo methods.

(b) Metabolism

In the urine of rats exposed to 2-bromopropane at 0, 500, 1000, or 1500 mg/m^3 for 4 hours, concentrations of acetone and bromide ion increased in a dose-dependent manner (Kawai et al., 1997). Urinary metabolites were analysed in two rats that were fed a diet containing ^{35}S-labelled yeast and then dosed with 2-bromopropane. Traces of radioactive material, with the same R_F value (retention factor; describing migration in the solvent) as isopropyl mercapturic acid, were detected in the ethyl acetate extract of acidified urine excreted in the first 24 hours (Barnsley et al., 1966).

Kaneko et al. (1997) assessed the metabolism of 2-bromopropane by measuring the rate of disappearance of the substrate (2-bromopropane) and the rate of formation of the product (2-propanol). The reaction mixture contained rat hepatic microsomes, nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate, and 2-bromopropane at 0.025–6.4 mM. The double reciprocal plot of the rate of metabolism against the concentration of the substrate indicated at least two sets of the metabolic constants V_{max} (maximal velocity) and K_m (Michaelis constant) in the metabolism of 2-bromopropane: V_{max1} = 0.38 mmol/mg protein per minute, V_{max2} = 1.30 mmol/mg protein per minute, K_{m1} = 0.07 mM, and K_{m2} = 0.32 mM. Calculations based on the formation of the product 2-propanol showed a lower V_{max2} of 1.02 nmol/mg protein per minute and a higher K_{m2} of 0.58 mM.

[The Working Group noted that the difference between the rate of disappearance of the substrate and the rate of formation of the product suggests the presence of metabolic pathways other than the pathway from 2-bromopropane to 2-propanol.]

A study on aerobic degradation of 2-bromopropane by a tropical marine yeast, *Yarrowia lipolytica* NCIM 3589, showed that the first product was 2-propanol, which was further metabolized to 2-propionic acid, eventually leading to the formation of carbon dioxide (Vatsal et al., 2015).

[The Working Group noted that, on the basis of the available data, 2-bromopropane is likely to be hydrolysed to bromide ion and 2-propanol, which is expected to be further oxidized to acetone, as well as being partially conjugated with glutathione.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether 2-bromopropane is electrophilic or can be metabolically activated to electrophiles; is genotoxic; induces oxidative stress; is immunosuppressive; modulates receptor-mediated effects; or causes immortalization. No data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is electrophilic or can be metabolically activated to electrophiles

One study investigating the potential of 2-bromopropane to be electrophilic was available to the Working Group. An excess amount (0.3 mL) of 2-bromopropane was incubated with 2.0 mg of 2′-deoxyguanosine, dissolved in 1.0 mL of phosphate-buffered saline at pH 7.4, and incubated at 37 °C for 16 hours. After removal of the unreacted 2-bromopropane by extraction with diethyl ether, the remaining aqueous

solution was heated at 100 °C for 30 minutes. The high-performance liquid chromatography (HPLC) chromatogram identified one peak corresponding to N^7-isopropyl guanine (Zhao et al., 2002). [Given that the specific gravity of 2-bromopropane is 1.306 g/mL (20/4 °C), the concentration of 2-bromopropane in the reaction solution is 16.3 mM. The Working Group noted that the study showed qualitatively the potential of DNA adduct formation by 2-bromopropane in a cell-free system, although the experimental conditions were not appropriate.]

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

No data in humans exposed to 2-bromopropane were available to the Working Group.

(ii) Human primary cells

Toraason et al. (2006) reported that exposure to 2-bromopropane at 1 mM for 4 and 8 hours, but not at 0.01 or 0.1 mM for 8 hours, significantly increased the comet tail moment, as measured with the alkaline electrophoresis (comet) assay, in primary human leukocytes from an unexposed non-smoking adult male volunteer (Toraason et al., 2006). [The Working Group noted that the comet tail moment was increased by exposure to 2-bromopropane only at the highest dose of 1 mM.]

(b) Experimental systems

(i) Non-human mammals in vivo

Pregnant ICR mice were exposed to 2-bromopropane by intraperitoneal injection with a single dose of 300, 600, 900, or 1800 mg/kg bw on day 0 of gestation. The frequency of micronuclei was evaluated in the embryos removed after cervical dislocation of the mothers on day 3 of gestation (Ishikawa et al., 2001). The exposure to 2-bromopropane increased the number of micronuclei per embryo in a dose-dependent manner. The percentage of embryos with micronuclei increased significantly at 900 and 1800 mg/kg bw. [The Working Group noted that the dose of 2-bromopropane was according to the Organisation for Economic Co-operation and Development (OECD) guideline (OECD, 2016) and that the micronucleus assay was performed as described in Titenko-Holland et al. (1998).]

The frequency of micronuclei did not increase in bone marrow polychromatic erythrocytes of Sprague-Dawley rats exposed to 2-bromopropane by intraperitoneal injection at 125, 250, or 500 mg/kg bw for 6 days per week for 28 days. However, the ratio (percentage) of polychromatic erythrocytes to the total number of erythrocytes was decreased in both male and female rats, suggesting bone marrow depression (Maeng & Yu, 1997). In contrast, the frequency of micronucleated hepatocytes (per 1000 hepatocytes) increased significantly in the liver of partially hepatectomized male Sprague-Dawley rats exposed intraperitoneally to 2-bromopropane at 200, 400, 800, or 1600 mg/kg bw (two injections), compared with the control group (olive oil, 4 mg/kg bw) (Maeng et al., 1996).

(ii) Non-human mammalian cells in vitro

In primary Leydig cells derived from Sprague-Dawley rats and exposed to 2-bromopropane at 0.01, 0.10, or 1 mM for 24 hours, the proportion of cells with undamaged DNA decreased significantly and the proportion with different grades of damaged DNA increased significantly, as measured with the single-cell gel electrophoresis (comet) assay. A total of 450 cells were evaluated in each dose group. Specifically, the percentage of cells with 5–20% and 20–40% of DNA damage was observed starting at 0.01 mM 2-bromopropane, the percentage with 40–90% of DNA damage was observed at concentrations above 0.10 mM, and the percentage with > 90% of DNA damage was observed at 1 mM (Wu et al., 2002).

Chromosomal aberrations were not observed in Chinese hamster lung cells exposed to 2-bromopropane at six different concentrations ranging from 0.077 to 2.46 mg/mL for 6 hours with metabolic activation (with the S9 microsomal mixture) and for 24 hours without metabolic activation (Maeng & Yu, 1997).

(iii) Non-mammalian experimental systems

Exposure to 2-bromopropane, tested at five concentrations (50, 100, 500, 1000, and 5000 µg/plate) in a preliminary assay and at five concentrations (313, 625, 1250, 2500, and 5000 µg/plate) in a second assay, induced mutagenicity in the *Salmonella typhimurium* strain TA100 with metabolic activation with the S9 microsomal mixture in a dose-dependent manner, and in the strain TA1535 with or without metabolic activation. In contrast, mutagenicity was not observed in the *S. typhimurium* strains TA98 or TA1537 or in *Escherichia coli* WP2 *uvrA*, indicating that 2-bromopropane induced mainly base-pair substitution mutations in *S. typhimurium* strains (Maeng & Yu, 1997).

4.2.3 Induces oxidative stress

(a) Humans

(i) Exposed humans

No data in humans exposed to 2-bromopropane were available to the Working Group.

(ii) Human cells in vitro

Flow cytometry-based analysis showed that exposure to 2-bromopropane at 100 µM for 24 hours increased the number of reactive oxygen species (ROS)-positive cells, as indicated by elevated levels of dihydroethidium, in spermatogenic cultures differentiated from human male embryonic stem cells for 10 days (Easley et al., 2015). Induction of oxidative stress was also confirmed by the elevation of ROS levels as observed with live-cell 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) imaging, and by the translocation of nuclear factor erythroid 2-related factor 2 (NRF2), a master regulator of oxidative stress, into the nucleus after exposure to 2-bromopropane at the same concentration [100 µM] for 24 hours. Annexin V flow cytometry-based analysis showed that pretreatment with the antioxidant l-sulforaphane at 1.0 µM significantly improved cell viability in cultures treated with 2-bromopropane at 100 µM for 24 hours (Easley et al., 2015). [The Working Group noted that the DCFH-DA method is known to produce ROS, and that the imaging of vehicle- or H_2O_2-exposed human spermatogenic cells showed the absence or presence of DCFH-DA-positive cells, respectively. In addition, the Working Group noted that the study was correctly performed and well controlled, because the percentage of cell viability was improved by pretreatment with the antioxidant l-sulforaphane.]

(b) Experimental systems

(i) Non-human mammals in vivo

Huang et al. (2009) showed that exposure of male Sprague-Dawley rats to 2-bromopropane by intraperitoneal injection at 1 g/kg bw per day for 7 days induced lipid peroxidation. There were significantly increased levels of 2-thiobarbituric acid-reactive substances (TBARS) in plasma and the epididymis, expressed as nmol/mL of plasma and nmol/mg protein of the epididymis, but not in the testis. 2-Bromopropane also induced a decrease in glutathione-*S*-transferase activity, as measured by the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione, in the testis and the epididymis. Glutathione-*S*-transferase activity was expressed as µmol/minute per mL of plasma and µmol/minute per mg protein of the testis and the epididymis. However, apoptosis, as measured by increased terminal deoxynucleotidyl transferase deoxyuridine phosphate (dUTP) nick end labelling (TUNEL)-positive or caspase-3-positive spermatogenic cells, was observed in the testis.

Pretreatment with 5 mg/kg of the antioxidant melatonin was able to attenuate the 2-bromopropane-induced oxidative damage and apoptosis in the various compartments investigated (Huang et al., 2009).

(ii) Non-human mammalian cells in vitro

Chan (2011) showed that exposure of mouse blastocyst cells to 2-bromopropane at 5 μM increased the fluorescence intensity of DCFH-DA, which indicates the generation of ROS. Pretreatment with 20 μM resveratrol blocked the generation of ROS.

In addition, Wu et al. (2002) showed that exposure of primary Leydig cells derived from Sprague-Dawley rats to 2-bromopropane at 0.1 or 1 mM not only induced DNA damage (as reported in Section 4.2.2(b)(ii)) but also significantly increased malondialdehyde levels (expressed in μmol/mL) and glutathione peroxidase enzymatic activity (expressed in U/mL) and decreased superoxide dismutase enzymatic activity (expressed in U/mL).

4.2.4 Is immunosuppressive

(a) Humans

Several studies reported signs of haematotoxicity (depletion of immune cells), which can be associated with immunosuppression (Smith et al., 2020). In a case-series study in an electronics factory in the Republic of Korea, alterations in haematopoiesis, and hence myelotoxicity, were reported in workers who were highly exposed to 2-bromopropane while working in the tactile switch assembly operation section, where 2-bromopropane was used as a solvent (Kim et al., 1996b; Park et al., 1997). The investigation started after the reporting of amenorrhoea among 16 of the 25 female workers employed in the tactile switch assembly operation section. [Exposure characterizations of the studies, including critical appraisals, are provided in Sections 1.4 and 1.6.] The mean stationary concentration of 2-bromopropane under simulated conditions in the factory was calculated as 12.4 ppm (SD, 3.13 ppm; range, 9.2–19.6 ppm). In contrast, the short-term stationary concentration of 2-bromopropane inside the hood of the cleaning baths was calculated as 4140.7 ppm. The employees performed work there irregularly (with unknown frequency and duration). Workers were suspected to have significant dermal exposures, because they reported unprotected hand-dipping of parts into the 2-bromopropane cleaning solvent. [The Working Group considered that the calculated exposure was probably underestimated, because the assessment was performed only under simulated conditions.]

Kim et al. (1996b) described clinical findings in the exposed workers. Blood samples were collected from 25 female workers and 8 male workers. The measurements comprised blood count, haemoglobin, and a test for bleeding tendency. Among the female workers, 11 women had leukocyte levels (range, 1910–3980 cells/μL) lower than the normal range (4800–10 800 cells/μL), 8 women had erythrocyte levels lower than the normal range (range, 2.8–3.7 × 10^6 cells/μL; lower reference normal value, 4.2 × 10^6 cells/μL), and 9 women had platelet levels lower than the normal range (range, 1.5–10.9 × 10^4 cells/μL; lower reference normal value, 13 × 10^4 cells/μL). All the women who reported amenorrhoea had pancytopenia. Two women with signs of marked pancytopenia (erythrocytes, 2.28–2.57 × 10^6 cells/μL; leukocytes, 1650–1910 cells/μL; platelets, 1.5–1.7 × 10^4 cells/μL) had bone marrow biopsy findings that showed marked hypoplastic marrow (with cellularity of 15% and 25%, respectively). Among the male workers, 3 men had leukocyte levels lower than the normal range (range, 4340–4680 cells/μL) and, among them, one had mild pancytopenia (erythrocytes, 3.53 × 10^6 cells/μL; leukocytes, 4680 cells/μL; platelets, 6.8 × 10^4 cells/μL) (Kim et al., 1996b).

Additional comparative analyses were performed between highly exposed workers and "unexposed worker" groups: (i) workers in a section other than the tactile switch assembly operation section, and (ii) workers who quit the job before 2-bromopropane was introduced as a solvent (Park et al., 1997).

Park et al. (1997) did not report any findings of pancytopenia in the "unexposed worker" groups among 77 workers in the tactile switch processing operation section and the general switch processing operation section, or among the 6 employees who worked in the tactile switch assembly operation section before 2-bromopropane was introduced as a solvent. [Although the exposure assessment had limitations, i.e. the stationary measurement was performed in a simulated exposure setting and no dermal exposure was measured, the Working Group noted that all the workers were clearly exposed to high levels of 2-bromopropane (see Section 1.6) and considered the study particularly informative because it provided evidence of immunosuppressive effects in humans, on the basis of findings of leukopenia and evidence of bone marrow suppression.]

Haematological effects in workers exposed to 2-bromopropane were also studied in a 2-bromopropane production facility in China (Ichihara et al., 1999). A cross-sectional study was performed in 25 workers (11 men and 14 women) employed in December 1996. Exposures to 2-bromopropane were measured in workers directly involved in the production (operators and mixers), those in areas adjacent to production (laboratory worker, repairperson, boiler), and "unexposed" workers (accountants, salespeople, engineer, assistant manager). Median exposures for workers directly involved in production and transfer were 6.77 ppm (for operators) and 6.30 ppm (for mixers). Workers in areas adjacent to production (laboratory worker, repairperson, boiler) were exposed at lower levels. One accountant had a full-shift exposure of 0.88 ppm above the LOD of 0.02 ppm; the exposures in other non-factory-related workers (accountants, salespeople, engineer, assistant manager) were below the LOD on the sampling day. Instantaneous stationary air samples, although they were collected using imprecise detection and measurement methods, indicated median area concentrations for specific production areas, ranging between 4.0 ppm and 88.6 ppm. The medical examination included interviews, blood sample collections, hormone levels, and sperm samples (see also Section 4.2.5). Leukocytes, erythrocytes, haemoglobin, and haematocrit were measured. No workers showed signs of leukocytopenia or pancytopenia, including the 4 women with amenorrhoea or polymenorrhoea. Among the female workers with normal menstruation, leukocyte counts were lower in the 5 female operators (who were exposed to 2-bromopropane) than in the unexposed female workers (3 accountants and 1 analyst) ($P < 0.05$). Leukocyte counts decreased with increasing TWA of 2-bromopropane exposure, although this inverse association was weak. Erythrocyte counts also decreased with increasing TWA of 2-bromopropane exposure ($P < 0.05$). [The exposure assessment method used by the authors (individual-based assessment of exposure based on a single 8-hour TWA personal measurement) will have led to attenuation of the exposure–outcome associations. A group-based approach (in which each worker would have been assigned the median exposure of the job they performed) would have resulted in a stronger and unbiased estimate of the exposure–outcome associations. The Working Group therefore re-analysed the association between exposure to 2-bromopropane and the outcomes leukocyte count and erythrocyte count. For the association between 2-bromopropane and leukocyte count, the group-based exposure assessment would have resulted in an almost 2-fold stronger inverse association ($\beta = -0.1369$, group-based, versus $\beta = -0.0784$, individual-based) with a slightly

stronger statistical significance (P = 0.1294 versus P = 0.2597). For the association between 2-bromopropane and erythrocyte count, the group-based exposure assessment would have resulted in a 2-fold stronger inverse association (β = −0.0796, group-based, versus β = −0.0384, individual-based) with a stronger statistical significance (P = 0.0042 versus P = 0.0874) (Fig. 4.1).]

(b) Experimental systems

Sprague-Dawley rats were exposed orally to 2-bromopropane at 100, 330, or 1000 mg/kg bw per day for 28 consecutive days (Jeong et al., 2002). The rats were immunized intravenously with sheep erythrocytes 4 days before necropsy. Exposure to 2-bromopropane at 1000 mg/kg bw per day significantly reduced body weight, thymus weight, leukocyte count, and platelet count in peripheral blood, and the number of different subpopulations of splenic lymphocytes. In addition, there were dose-dependent decreases in the number of thymocyte subpopulation cells per thymus and in the number of $CD4^+CD8^+$ cells in the thymus, with statistically significant changes at 330 and 1000 mg/kg bw per day. Exposure to 2-bromopropane also induced decreases in the numbers of $CD4^-CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^-$ cells in the thymus and in the numbers of total cells per spleen, antibody-forming cells per spleen, antibody-forming cells per spleen cell, T cells per spleen, T helper cells per spleen, cytotoxic T cells per spleen, and B cells per spleen, with statistically significant changes at 1000 mg/kg bw per day. [The Working Group noted that the study suggested an immunotoxic potential of 2-bromopropane in rats.]

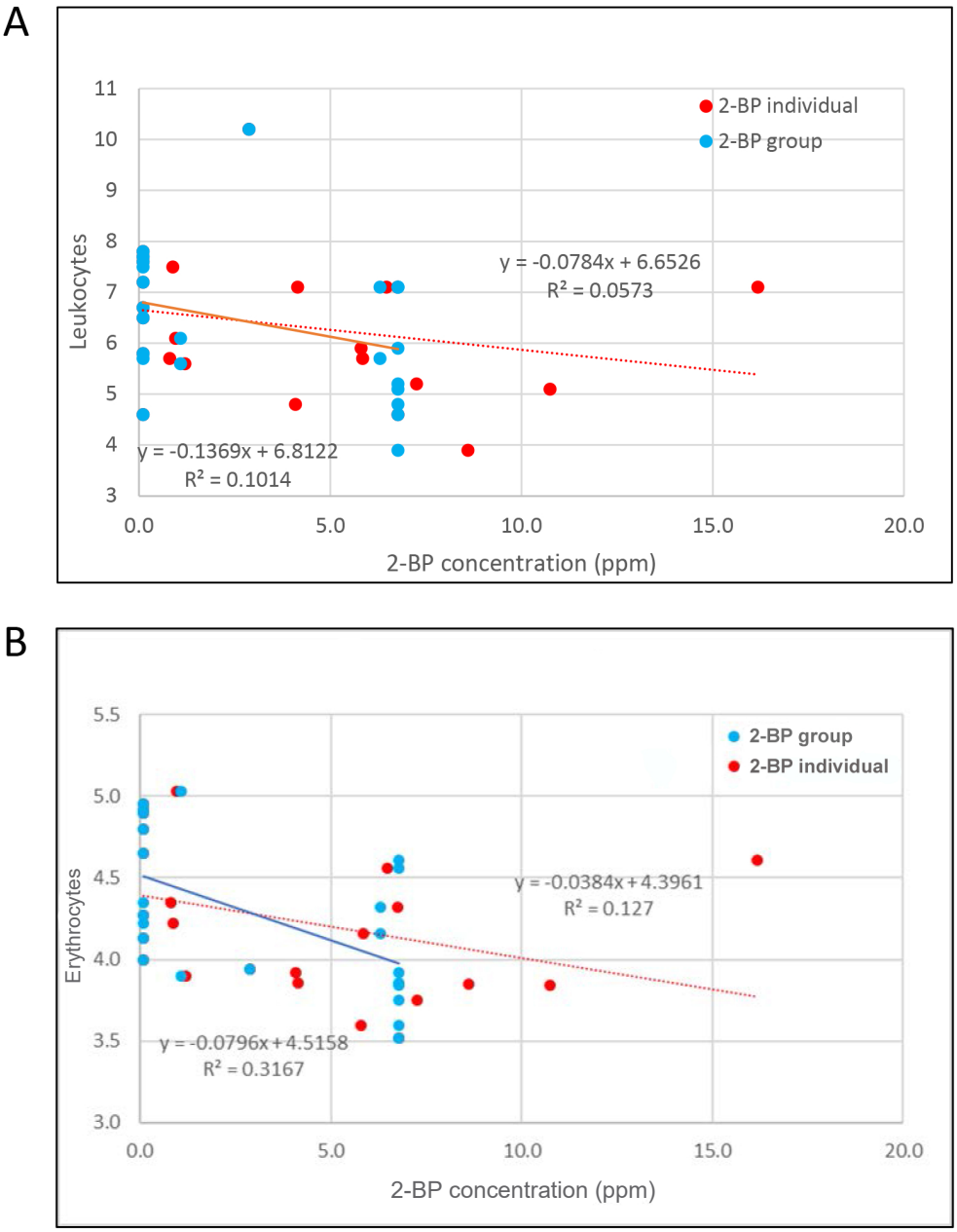
Exposure of CD3-stimulated splenocytes derived from C3H male mice to 2-bromopropane at 10 μM for 24 hours downregulated the expression of the tumour necrosis factor alpha (TNFα) gene but did not alter the expression of the pro-inflammatory cytokines interleukin 6 (IL-6), IL-1, and interferon gamma (IFNγ) (Kim et al., 2002). Similarly, serum IL-6 levels did not increase after single or repeated intraperitoneal injections of 2-bromopropane at 3.5 g/kg bw in C3H male mice. No effect was observed after 24 or 48 hours of 2-bromopropane treatment of CD3-stimulated mice splenocytes (Kim et al., 2003).

Exposure of male Wistar rats to 2-bromopropane by inhalation at 1000 ppm for 9 weeks or at 3000 ppm for 9–11 days significantly decreased the erythrocyte, platelet, and leukocyte counts, and levels of haemoglobin and haematocrit in the peripheral blood (Ichihara et al., 1997). In the same experimental setting, exposure to 2-bromopropane significantly decreased the erythrocyte count at 300 ppm and higher, platelet count at 300 and 1000 ppm, and leukocyte count at 1000 ppm. The highest concentration induced a hypoplastic profile in the bone marrow, causing replacement of fatty spaces, and a decrease in the number of megakaryocytes, but it did not change the ratio of granulocytes to erythrocytes in the bone marrow (Nakajima et al., 1997).

Oral exposure of male Sprague-Dawley rats to 2-bromopropane at 1000 mg/kg bw per day significantly decreased the weight of the spleen after 2 and 4 weeks of exposure and the weight of the thymus after 2, 3, and 4 weeks of exposure, and reduced the peripheral leukocyte count after 3 weeks of exposure. The weight of the spleen did not recover 8 weeks after the end of exposure (Lee et al., 1998).

In a dose-finding study for a carcinogenicity test that complied with GLP, groups of 10 male and 10 female F344/DuCrlCrlj rats (age, 6–7 weeks) were treated with 2-bromopropane (purity, 99.7%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 13 weeks, at concentrations of 0 (clean air; control), 100, 300, 1000, 2000, and 3000 ppm (JBRC, 2016a, b). In the haematology results, there were significant decreases in the erythrocyte count, haemoglobin, haematocrit, platelet count, and leukocyte count, and increases in

Fig. 4.1 Leukopenia and pancytopenia in workers exposed to 2-bromopropane



Regression analysis of 2-bromopropane (2-BP) exposure levels (ppm) and (A) leukocyte and (B) erythrocyte counts ($\times 10^6$/mL) in workers at a production plant in Yixing City, Jiangsu Province, China, as per a group-based approach (blue dots), compared with an individual-based approach (red dots). Data from Ichihara et al. (1999) were re-analysed by the Working Group with the group-based approach. A group-based approach (in which each worker would have been assigned the median exposure of the job they performed) would have resulted in a stronger and unbiased estimate of the exposure–outcome associations. For the association between 2-BP and leukocyte count, the group-based exposure assessment would have resulted in an almost 2-fold stronger inverse association ($\beta = -0.1369$, group-based, versus $\beta = -0.0784$, individual-based). For the association between 2-BP and erythrocyte count, the group-based exposure assessment would have resulted in a 2-fold stronger inverse association ($\beta = -0.0796$, group-based, versus $\beta = -0.0384$, individual-based).
Created by the Working Group.

mean corpuscular volume and mean corpuscular haemoglobin in the male and female groups at 1000 ppm and higher. In addition, there was a significant dose-dependent increase in reticulocyte counts in the male groups at 2000 ppm and higher and in the female groups at 1000 ppm and higher. There was a significant decrease in the absolute and relative weights of the thymus in the male and female groups at 1000 ppm and higher. In addition, there were significant decreases in the absolute and relative weights of the testis and the epididymis in the male groups at 300 ppm and higher. In the histopathology results, there were significant increases in the incidence of decreased haematopoiesis of the bone marrow in the male and female groups at 2000 ppm and higher and in the incidence of atrophy of the thymus and extramedullary haematopoiesis in the spleen in the male and female groups at 1000 ppm and higher. In addition, there were significant increases in the incidence of oedema and tubular atrophy in the testis in the groups at 300 ppm and higher, decreased sperm count in the epididymis in the groups at 1000 ppm and higher, and debris of spermatic elements in the epididymis in all treated groups (JBRC, 2016a, b).

4.2.5 Modulates receptor-mediated effects

(a) Humans

Alterations in hormone levels and myelotoxicity were reported in the two cross-sectional studies that investigated the effects of 2-bromopropane exposure in workers in an electronics factory in the Republic of Korea (Kim et al., 1996b) and in a 2-bromopropane production factory in China (Ichihara et al., 1999). Both studies are also described in Section 4.2.4, and their exposure assessment is reported in Section 1.6. More details on the alterations in hormone levels are given below.

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and testosterone were measured in the population of workers, including 25 women and 8 men, employed in the electronics factory in the Republic of Korea (Kim et al., 1996b). Female workers were followed up at 2 and 7 years after the first investigation (Koh et al., 1998; Yun et al., 2002), and male workers were followed up at 2–3 months (Hong et al., 2002). In the 16 women who reported amenorrhoea (lasting 2–14 months), FSH levels were elevated (range, 27.8–136.7 mIU/mL) compared with reference normal values (1.9–11.9 mIU/mL); this is indicative of ovarian failure. In 14 of the 16 women, LH levels were also elevated (range, 12.9–48.7 mIU/mL) above normal values (1.9–11.9 mIU/mL). Prolactin levels were within the normal range, and estradiol levels were lower (< 13.6 pg/mL) than normal values (30–120 pg/mL). Koh et al. (1998) reported the results of ovarian biopsy in 6 of the women with amenorrhoea and confirmed the diagnosis of ovarian failure. Two of the 16 women with amenorrhoea recovered their ovarian function after 24 months from the last exposure (Koh et al., 1998). At the 7-year follow-up of these 16 women, 10 women recovered from the amenorrhoea only after hormone replacement therapy (Yun et al., 2002). Serum levels of FSH and LH decreased but remained elevated above the normal ranges both in women who recovered from amenorrhoea (FSH mean, 88.29; SD, 24.69 mIU/mL; LH mean, 26.45; SD, 11.79) and in those who did not recover from amenorrhoea (FSH mean, 76.68; SD, 27.98; LH mean, 26.45; SD, 11.79). Among the 8 male workers, FSH levels (13.5–19 mIU/mL) were towards the upper end of the normal range, and LH, prolactin, and testosterone levels were within the normal range (Kim et al., 1996b). Signs of oligospermia, azoospermia, and reduced sperm motility were also reported in 4, 2, and 5 men, respectively.

Hong et al. (2002) followed up these men at 2–3 months after the initial examination and found that FSH and LH levels remained towards the upper end of the normal range (FSH range

in men, 9.6–74.4 mIU/mL; LH range in men, 40.6–4.8 mIU/mL).

Serum levels of FSH and LH were also measured in workers in the 2-bromopropane factory in China (Ichihara et al., 1999). In addition, estradiol was measured in women and testosterone was measured in men. In men, levels of FSH and LH were towards the upper end of the normal range, and testosterone levels were within the normal range. In women, FSH, LH, and estradiol levels were within the normal range. In a regression analysis, LH, FSH, estradiol, and testosterone levels were not associated with values of individual TWA 2-bromopropane concentration. [The Working Group noted that almost all female workers with amenorrhoea had higher FSH and LH levels and lower estradiol levels, and that male workers with severely decreased sperm indices had higher FSH levels and lower testosterone levels, suggesting that an increase in FSH or LH levels results from reduced sex hormone production in the ovary or the testis.]

(b) Experimental systems

(i) Non-human mammals in vivo

Wu et al. (1999a) investigated the male reproductive toxicity of 2-bromopropane in sexually mature and immature male Sprague-Dawley rats. Mature and immature rats treated for 5 days per week for 5–7 weeks with subcutaneous injection of 2-bromopropane at 600 and 1800 mg/kg bw had significantly reduced serum testosterone levels. At the highest dose of 1800 mg/kg bw, there was a significant increase in *β-LH* gene expression in the pituitary gland. [The Working Group interpreted the increased gene expression of *β-LH* as being a result of positive feedback due to decreased serum testosterone level.]

Male Wistar rats were exposed to 2-bromopropane by inhalation for 8 hours per day for 7 days per week at 0, 300, or 1000 ppm for 9 weeks or at 3000 ppm for 9–11 days (Ichihara et al., 1997). Exposure to 2-bromopropane decreased in a dose-dependent manner the epididymal sperm count and motility and the number of erythrocytes and platelets at 300 ppm or higher. Histopathological examination showed a decrease in spermatogenic cells at 300 ppm and depletion of spermatogenic cells at 1000 and 3000 ppm, but Sertoli cells remained. In female Wistar rats exposed to 2-bromopropane by inhalation at 100, 300, or 1000 ppm for 8 hours per day, 7 days per week, for 9 weeks, the vaginal smear test showed that the number of normal estrous cycles decreased at 300 and 1000 ppm, and the histopathological study showed dose-dependent atresia of ovarian follicle accompanied by decreased numbers of normal atresia and growing follicles at 300 and 1000 ppm (Kamijima et al., 1997).

Four groups of 5 female ICR mice each were exposed to 2-bromopropane at 0, 500, 1000, or 2000 mg/kg bw by intraperitoneal injection, 8 times at intervals of 2 or 3 days for 17 days (Sekiguchi & Honma, 1998). Pregnant mare's serum gonadotropin and human chorionic gonadotropin were injected on day 15 and day 17 of 2-bromopropane injection to induce superovulation, and the liver, uterus, and oviduct were removed on autopsy. Exposure to 2-bromopropane at 2000 mg/kg bw did not change the body weight or liver weight but decreased the weight of the uterus. However, 2-bromopropane decreased the numbers of ovulated ova in a dose-dependent manner, with a significant change at 1000 and 2000 mg/kg bw.

Omura et al. (1999) investigated target cells of 2-bromopropane in the testis of Wistar rats by intraperitoneal injection of 2-bromopropane at 1335 mg/kg bw for 1–5 days and found that 2-bromopropane targets spermatogonia. Takeuchi et al. (2004) investigated the developmental effects of exposure to 2-bromopropane by inhalation on pups of Sprague-Dawley rats. Adult female rats were exposed to 2-bromopropane at 0, 125, 250, 500, or 1000 ppm for 6 hours per day, 7 days per

week, during 2 weeks of the pre-mating period, during the mating period until copulation, and during days 0–19 of gestation. After parturition, the dams were allowed to breastfeed their pups until postnatal day 4. No signs indicating maternal toxicity, such as abnormal clinical signs or body-weight loss, were observed. Exposure to 2-bromopropane at 1000 ppm significantly decreased the number of pups, although the number of implantations was not decreased. The weight or survival of pups was not affected by exposure to 2-bromopropane until postnatal day 4. The study showed that exposure to 2-bromopropane induced fetal lethality in the post-implantation period (Takeuchi et al., 2004).

Kim et al. (2004a) investigated the effects on embryo-fetal development of maternal exposure to 2-bromopropane in pregnant ICR mice treated by subcutaneous injection at 0, 500, 1000, or 1500 mg/kg per day on days 6–17 of gestation. Caesarean sections were carried out on all dams on day 18 of gestation, and the fetuses were examined for external, visceral, and skeletal abnormalities. A dose-dependent decrease in fetal body weight and an increase in the incidence of fetal malformations and of ossification delay were found.

Kim et al. (2004b) investigated the effects on embryo-fetal development of maternal exposure to 2-bromopropane in pregnant Sprague-Dawley rats treated by subcutaneous injection at 0, 250, 500, or 1000 mg/kg bw per day on days 6–19 of gestation. An increase in the number of fetal deaths, a decrease in litter size, a decrease in fetal body weight, and an increase in the incidence of fetal malformations were observed at 1000 mg/kg bw per day, which induced maternal toxicity such as an increase in the incidence of abnormal signs, a suppression of body weight and body-weight gain, and a decrease in food intake. Minimal developmental toxicity, including decreased fetal body weight and increased fetal ossification delay, was observed at 500 mg/kg bw per day, but no adverse effects on dams or fetal development were observed at 250 mg/kg bw per day (Kim et al., 2004b).

[The Working Group noted that developmental effects were observed at the level that induced maternal toxicity in mice and rats.]

(ii) Non-human mammalian cells in vitro

Exposure of primary Leydig cells derived from male Sprague-Dawley rats to 2-bromopropane at 0.01 or 0.1 mM did not induce a detectable change in the secretion of testosterone during 24 hours of treatment, but exposure at 1 mM decreased the secretion of testosterone after 12 hours of treatment (Wu et al., 1999b). [The Working Group noted that the 1 mM concentration of 2-bromopropane induced cytotoxicity.]

[The Working Group acknowledged that several of the effects observed after exposure to 2-bromopropane and reported in this section were also consistent with reproductive toxicity mediated through receptor modulation. However, the Working Group deemed it relevant to include evidence reporting on measured changes in blood levels of hormones, i.e. LH, estradiol, and testosterone, including studies in experimental systems in vivo, in line with the evidence on cancers in experimental animals (see Section 3) in relevant target organs (e.g. the uterus, mammary gland, and thyroid).]

4.2.6 Causes immortalization

Human cells in vitro

Exposure to 2-bromopropane significantly increased spheroid formation in various human colorectal adenocarcinoma cells at non-cytotoxic concentrations: 0.01–1 μM in CSC221 cells, 0.05–1 μM in DLD1 cells, 0.01–5 μM in Caco2 cells, and 0.1–1 μM in HT29 cells (Cho et al., 2017). After 72 hours of exposure to 2-bromopropane, the cancer stem cell markers *ALDH-1*, *CD133*, *LGR-5*, and *MSI-1* increased at the mRNA and protein levels, and *CD44* and *BMI-1* increased at the mRNA levels in CSC221 cells.

In addition, 2-bromopropane enhanced the activation of promoters associated with cancer stem cell markers, such as TOPflash and glioma-associated oncogene homologue zinc finger protein (Gli). 2-Bromopropane increased the mRNA expression of signalling molecules such as Gli-1, Gli-2, Smoothened (SMO), and β-catenin. [The Working Group noted that 2-bromopropane increased the stemness of cancer cells. However, the Working Group questioned the relevance of the test system and the markers.]

4.3 Evaluation of high-throughput in vitro toxicity screening data

2-Bromopropane was tested in high-throughput toxicity screening assays under the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2019). Chemical samples were procured at high purity, prepared in dimethyl sulfoxide stock solutions at a concentration of about 20 mM, and tested over a period of several years in biochemical and cellular bioassays measuring a wide variety of biological end-points. In addition, chemical analysis of the samples was done in high-throughput fashion at an early and a late stage of the sample testing lifetime, as described in Tice et al. (2013). Data on testing results from the concentration–response testing design for all end-points were analysed for significant activity, and an active/inactive "hit call" was made for each response, together with a potency value (Filer et al., 2017). For all active calls, individual concentration–response curves were examined to ensure that biologically meaningful activity was detected. Bioassay end-points were mapped, where possible, to the key characteristics of carcinogens using the "kc-hits" software (the key characteristics of carcinogens – high-throughput screening discovery tool, available from https://gitlab.com/i1650/kc-hits; Reisfeld et al., 2022) to aid in providing mechanistic insights (Chiu et al., 2018). The detailed results are available in the supplementary material for this volume (Annex 2, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/631) and are briefly summarized below.

The testing results for 2-bromopropane high-throughput toxicity testing in the CompTox Chemicals Dashboard encompassed 235 assay end-points, of which 111 were mapped to the key characteristics of carcinogens. The cytotoxicity limit based on a panel of cellular cytotoxicity and viability assays was estimated to be > 1 mM (US EPA, 2022a). 2-Bromopropane was inactive in all but 2 of the 111 assays. The active hit calls were in two viability assays mapped to the key characteristic "alters cell proliferation, cell death, or nutrient supply", but both results were flagged for low efficacy and activity only at the highest testing concentration of 79.2 μM. [The Working Group considered this as weak evidence of activity for this key characteristic.]

The chemical analysis of a dimethyl sulfoxide stock solution used in testing was graded "Fns", indicating that no 2-bromopropane was detected (NIH, 2022). 2-Bromopropane has an experimental vapour pressure of 216 mm Hg [at 25 °C] (US EPA, 2022b). [The Working Group concluded that this high volatility may have led to little or no presence of the chemical in the biological assays.]

5. Summary of Data Reported

5.1 Exposure characterization

2-Bromopropane is synthesized by heating 2-propanol (isopropyl alcohol) together with hydrogen bromide. It also occurs as an impurity of commercial-grade 1-bromopropane used in vapour degreasing, historically at concentrations of 0.1–0.2% but nowadays to a maximum of

0.05%. For other uses of 1-bromopropane, such as adhesives, no maximum level of 2-bromopropane impurity in 1-bromopropane has been set. Historically the production volume for 2-bromopropane has been low. It was originally produced in Japan and the USA. Currently at least 13 manufacturers in China are known, but production volumes are unknown.

Occupational exposure to 2-bromopropane can occur via the respiratory and/or dermal route during its production and use as a cleaning or dry-cleaning agent or solvent, and in the production and application of adhesives. Historical evidence of very high personal exposure comes from studies in a plant producing 2-bromopropane and in an electronics plant where it has been used as a cleaning agent. Occupational exposure to 2-bromopropane has also occurred because of its presence as an impurity of 1-bromopropane, which since the 1990s has been used as a substitute for ozone-depleting and other solvents.

There are no available data on exposure of the general population to 2-bromopropane.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with 2-bromopropane caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in males and females of a single species (rat) in one study that complied with Good Laboratory Practice (GLP).

2-Bromopropane was administered by inhalation in one study that complied with GLP, in male and female F344/DuCrlCrlj rats. In male rats, 2-bromopropane caused an increase in the incidence of the following tumours: malignant tumours of the Zymbal gland and benign or malignant tumours (combined) of the Zymbal gland; malignant tumours and appropriate combinations of malignant or benign tumours of the skin/appendage: specifically, basal cell carcinoma of the skin/appendage, squamous cell carcinoma or basal cell carcinoma (combined) of the skin/appendage, and squamous cell papilloma, basal cell epithelioma, sebaceous adenoma, keratoacanthoma, squamous cell carcinoma, or basal cell carcinoma (combined) of the skin/appendage; adenocarcinoma of the large intestine and adenoma or adenocarcinoma (combined) of the large intestine; adenocarcinoma of the small intestine; malignant lymphoma of the lymph nodes; squamous cell papilloma or carcinoma (combined) of the stomach; adenocarcinoma, adenoma, or squamous cell papilloma (combined) of the preputial gland; fibroma or fibrosarcoma (combined) of the subcutis; follicular adenoma or adenocarcinoma (combined) of the thyroid gland; glioma of the brain; mononuclear cell leukaemia of the spleen; and islet cell adenoma or islet cell adenocarcinoma (combined) of the pancreas.

In female rats, 2-bromopropane caused an increase in the incidence of the following tumours: adenocarcinoma of the mammary gland and adenocarcinoma or adenosquamous carcinoma (combined) of the mammary gland; mononuclear cell leukaemia of the spleen; squamous cell papilloma or carcinoma (combined) of the vagina; adenoma or adenocarcinoma (combined) of the large intestine; malignant tumours of the Zymbal gland and benign or malignant tumours (combined) of the Zymbal gland; squamous cell papilloma, adenoma, or adenocarcinoma (combined) of the clitoral gland; squamous cell papilloma, trichoepithelioma, basal cell epithelioma, keratoacanthoma, or squamous cell carcinoma (combined) of the skin/appendage; fibroma or fibrosarcoma (combined) of the subcutis; adenoma or adenocarcinoma (combined) of the uterus; and islet cell adenoma or islet cell adenocarcinoma (combined) of the pancreas.

5.4 Mechanistic evidence

The only evidence of absorption, distribution, metabolism, and excretion in humans and rats in vivo comes from concentrations of acetone and bromide ion in the urine after inhalation exposure to 2-bromopropane; however, these markers are unreliable.

One experimental study showed skin penetration of 2-bromopropane in hairless mice in vivo and in vitro. Data on the metabolism of 2-bromopropane are available from acellular systems with rat hepatic microsomes, suggesting that 2-bromopropane is hydrolysed to bromide ion and 2-propanol, which is expected to be further oxidized to acetone, as well as the presence of other metabolic pathways. Urinary excretion of isopropyl mercapturic acid in rats dosed with 2-bromopropane suggests that 2-bromopropane is partially conjugated with glutathione.

Data were available for 2-bromopropane for the following key characteristics of carcinogens: "is genotoxic", "induces oxidative stress", "is immunosuppressive", and "modulates receptor-mediated effects".

There is consistent and coherent evidence that 2-bromopropane exhibits key characteristics of carcinogens.

2-Bromopropane is genotoxic. No data were available in humans exposed to 2-bromopropane. There is consistent and coherent evidence for the genotoxicity of 2-bromopropane in experimental systems. In one study using the comet assay in primary human leukocytes, 2-bromopropane induced DNA damage. 2-Bromopropane caused a dose-dependent increase in micronucleus formation in mouse embryos and in rat liver, but not in polychromatic erythrocytes in rat bone marrow. 2-Bromopropane increased the frequency of DNA damage in primary Leydig cells derived from rats but did not cause chromosomal aberrations in Chinese hamster lung cells with or without metabolic activation. 2-Bromopropane was mutagenic in the *Salmonella typhimurium* strain TA100 with metabolic activation in a dose-dependent manner and in the strain TA1535 with or without metabolic activation but did not induce mutagenicity in *S. typhimurium* strains TA98 or TA1537 or in *Escherichia coli* WP2 *uvrA*, indicating that 2-bromopropane induced base-pair substitution mutations in *Salmonella* strains.

2-Bromopropane induces oxidative stress. No data were available in humans or in human primary cells exposed to 2-bromopropane. There is consistent and coherent evidence for induction of oxidative stress by 2-bromopropane in experimental systems. In one study in spermatogenic cells differentiated from human embryonic stem cells, 2-bromopropane increased levels of reactive oxygen species and translocation of NRF2 into the nucleus. In rats, 2-bromopropane increased levels of 2-thiobarbituric acid-reactive substances in plasma and the epididymis and decreased glutathione levels in the testis and the epididymis. Exposure to 2-bromopropane increased the generation of reactive oxygen species in mouse blastocyst cells, and increased malondialdehyde levels and glutathione peroxidase activity and decreased superoxide dismutase activity in Leydig cells derived from rats.

2-Bromopropane is immunosuppressive. The evidence in exposed humans is suggestive. One study among workers at an electronics factory who were exposed to solvents containing 2-bromopropane showed that 2-bromopropane induced pancytopenia in blood and hypoplastic bone marrow. Another study, among workers manufacturing 2-bromopropane, showed an inverse association between 2-bromopropane exposure level and both leukocyte and erythrocyte cell counts. The evidence is consistent and coherent in experimental systems. In rats, exposure to 2-bromopropane caused dose-dependent decreases in thymus weight and cellularity, leukocyte count, and various subpopulations of lymphocytes in the spleen and the thymus, haematotoxicity, and significant evidence of

decreased haematopoiesis of the bone marrow. In addition, a decrease in the T-cell-dependent antibody response was observed.

There is suggestive evidence that 2-bromopropane modulates receptor-mediated effects. In one study among workers at an electronics factory who were exposed to 2-bromopropane-containing solvents, amenorrhoea was seen in female workers with high levels of follicle-stimulating hormone and luteinizing hormone and low levels of estradiol. In the same electronics factory, azoospermia or oligospermia was observed in male workers. In a second study, among workers manufacturing 2-bromopropane, findings for modulation of receptor-mediated effects were largely negative. In rats, exposure to 2-bromopropane resulted in reduced serum testosterone levels and significantly increased expression of the β-luteinizing hormone (β-LH) gene in the pituitary gland.

For the key characteristics "is electrophilic or can be metabolically activated to electrophiles", "induces chronic inflammation", and "causes immortalization", there was a paucity of available data.

2-Bromopropane was found to be mostly without effects relevant to the key characteristics of carcinogens in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, although the 2-bromopropane testing solution was considered problematic for use in high-throughput assays.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of 2-bromopropane.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-bromopropane.

6.3 Mechanistic evidence

There is *strong evidence* that 2-bromopropane exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

2-Bromopropane is *probably carcinogenic to humans (Group 2A).*

6.5 Rationale

The Group 2A evaluation for 2-bromopropane is based on *sufficient evidence* for cancer in experimental animals and *strong mechanistic evidence* in experimental systems, supported by suggestive mechanistic evidence in exposed humans. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of malignant neoplasms in males and females of a single species (rat) in one study that complied with Good Laboratory Practice. In addition, an increase in the incidence of appropriate combinations of benign and malignant neoplasms was observed in males and females in this study. Also, an unusually high degree of carcinogenic activity with regard to incidence, site, and types of tumours was observed in both males and females. The evidence regarding cancer in humans was *inadequate* because no studies were available.

There was *strong* mechanistic evidence of several key characteristics of carcinogens (genotoxicity, induction of oxidative stress, and immunosuppression) in experimental systems. There was suggestive evidence of immunosuppression

and of modulation of receptor-mediated effects in two studies of small numbers of workers exposed to 2-bromopropane. Although no experimental studies were available in human primary cells or tissues, the Working Group concluded that a Group 2A evaluation was appropriate, given the unusually high degree of carcinogenic activity observed in the animal bioassays and the consistent and coherent evidence that 2-bromopropane exhibits key characteristics of carcinogens, in particular immunosuppression, across mammalian species and in vitro systems, supported by suggestive evidence of immunosuppression and of modulation of receptor-mediated effects in exposed humans.

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BUTYL METHACRYLATE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 97-88-1 (NCBI, 2022)

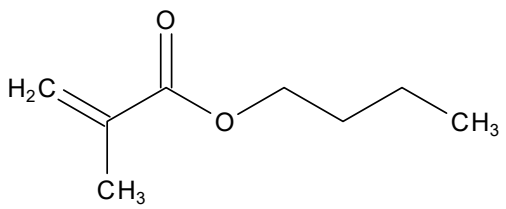
EC/List No.: 202-615-1 (NCBI, 2022)

IUPAC systematic name: butyl 2-methyl-prop-2-enoate (NCBI, 2022)

Synonyms: *n*-butyl methacrylate; butyl 2-methacrylate; 2-methyl-butylacrylate; butyl 2-methyl-2-propenoate; 2-propenoic acid, 2-methyl-, butyl ester; methacrylic acid, butyl ester; *n*BMA (NCBI, 2022).

1.1.2 Structural and molecular information

Chemical structure:



Molecular formula: $C_8H_{14}O_2$ (NCBI, 2022)

Relative molecular mass: 142.20 (NCBI, 2022).

1.1.3 Chemical and physical properties

Description: clear colourless liquid with faint characteristic odour of esters (NCBI, 2022)

Boiling point: 160–163 °C (NCBI, 2022; Royal Society of Chemistry, 2022)

Melting point: –50 to –75.0 °C (NCBI, 2022; Royal Society of Chemistry, 2022)

Flash point: 48.5–52 °C at 101.3 kPa (ECHA, 2022a, IFA, 202 (2)

Density: 0.894 g/mL at 20 °C (NCBI, 2022; Royal Society of Chemistry, 2022)

Vapour pressure: 3 hPa at 20 °C (IFA, 2022)

Solubility: 360 mg/L at 25 °C in water; soluble in ethyl ether and ethanol (ECHA, 2022a; NCBI, 2022)

Octanol/water partition coefficient (P): log K_{ow} = 2.88 (NCBI, 2022; Royal Society of Chemistry, 2022)

Stability: readily polymerized, which can be caused by heat, moisture, or oxidants (NCBI, 2022)

[The Working Group used a conversion factor of 1 ppm ≈ 5.91 mg/m^3 at 20 °C and 1.013 hPa (ECETOC, 1998).]

1.1.4 Technical grade and impurities

Butyl methacrylate of high purity (~99%) is available commercially from several vendors (ECETOC, 1997). Bulk methacrylates are subject to spontaneous polymerization unless a stabilizer is added. Polymerization inhibitors include hydroquinone, monomethyl ether of hydroquinone, or 2-(1,1-dimethylethyl)-4,6-dimethylphenol at 10–100 ppm or a total of < 0.1% by weight (ECETOC, 1998; OECD, 2007).

Typical impurities include methacrylic acid (CAS No. 79-41-4) or methyl methacrylate (CAS No. 80-62-6) (depending on the esterification route used for synthesis), the unreacted butanol, and water (ECETOC, 1998; NCBI, 2022).

1.2 Production and use

1.2.1 Production process

Butyl methacrylate can be manufactured in several ways. One method is via direct esterification of methacrylic acid or transesterification of methyl methacrylate with butanol (ECETOC, 1998; Bauer, 2000). Another method is the catalytic oxidation of isobutylene followed by esterification with butanol. Finally, acetone can be reacted with hydrocyanic acid and esterified in sulfuric acid with butanol (Bisesi, 1994).

1.2.2 Production volume

Butyl methacrylate has been classified by the Organisation for Economic Co-operation and Development as a High Production Volume chemical (OECD, 2007). Companies in China, Germany, Japan, the Republic of Korea, and the USA produce butyl methacrylate, although specific production amounts could not be pinpointed (OECD, 2007; Business Research Insights, 2021). Between 10 000 and 100 000 tonnes per year are manufactured in and/or imported to the European Economic Area (ECHA, 2022b). In the USA, 20 million to < 100 million pounds [~9100–45 000 tonnes] were produced or imported in 2019 (US EPA, 2020).

1.2.3 Uses

Butyl methacrylate is a monomer used to create acrylic polymers and is used in a variety of products worldwide. It is used in coatings, polyvinyl chloride plastics, polypropylene non-woven materials, glues, caulks or other sealants, inks and paints, pesticides, and healthcare materials, among others. The butyl group on the methacrylic ester adds flexibility to the resulting materials. Butyl methacrylate is also used in textile emulsions, leather creation, and paper finishing (ECETOC, 1998; Urban et al., 2006; Gantrade, 2018; Dow, 2020).

These materials are used in a variety of industries. In Denmark, Finland, Norway, and Sweden in 2011–2020, butyl methacrylate was used most frequently in wholesale trade and repair of motor vehicles; the manufacture of other transport equipment, furniture, and non-furniture wood and cork products; and the repair and installation of machinery and equipment (SPIN, 2023). The highest-volume uses in these countries in 2000–2020 were in the manufacture of chemicals and chemical preparations (such as paints, lacquers, varnishes, adhesives, and binders), specialized construction activities, furniture manufacture, repair of machinery and equipment, and wholesale and retail trade and repair of motor vehicles (SPIN, 2023).

Butyl methacrylate monomer has been used to create monolithic columns for gas chromatographic analysis of parabens (Carrasco-Correa et al., 2015). The monomer is used directly in only a few consumer products; it has been included in nail polish, and possibly in nail extension and nail hardener products (Kanerva et al., 1996; Sainio et al., 1997; Cosmetic Ingredient Review Expert Panel, 2005; Ceballos et al., 2019), and in fragrances at an estimated worldwide use of < 1 tonne per year in 2015 (Api et al., 2020). In

addition, some dental products and joint replacement cement may include butyl methacrylate monomer (Cautilli and Hozack, 1994; Urban et al., 2006). Food-grade plastics can contain butyl methacrylate (ECETOC, 1998).

1.3 Detection and quantification

Methodologies for the collection, detection, and quantification of butyl methacrylate in air, water, and consumer products have been developed and used in research. [The Working Group did not identify butyl methacrylate sample collection and analytical methods that have undergone validation by authoritative bodies or consensus organizations.] The characteristic self-polymerization of acrylates, particularly at high temperatures, poses challenges when developing sampling and analysis methods.

1.3.1 Air

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) suggested that National Institute for Occupational Safety and Health (NIOSH) method 1450 (esters I) can be adopted to sample for butyl methacrylate using activated carbon media followed by gas chromatography-flame ionization detection (GC-FID) (ECETOC, 1998).

A few documents and studies describe practical sample collection and analysis of butyl methacrylate. Two older reports described dosing a cartridge with Tenax GC sorbent material for thermal desorption and analysis via GC (US EPA, 1984; ECETOC, 1997). Another report described active sample collection from air using activated charcoal media (NIOSH, 1981). Butyl methacrylate can be analysed in these media via GC or high-performance liquid chromatography (HPLC) and mass spectrometry (MS).

One study evaluated a methodology for sampling and analysis of four methacrylate compounds using activated carbon media, desorbed using carbon bisulfide and analysed using GC-FID; the minimum quantifiable concentration in a 3 L air sample was 0.07 mg/m^3, and samples were stable at room temperatures for at least a week (Rong et al., 2019).

1.3.2 Water

Butyl methacrylate has been measured in aqueous solutions via direct GC or HPLC. Ultraviolet detection has been used after chromatographic separation (ECETOC, 1997). If polymer is present, solvent extraction or headspace analysis should be undertaken first (ECETOC, 1997). The limit of detection (LOD) with a GC-C18 reversed-phase column was reported to be 0.05 mg/L in ecotoxicological tests, although the detector was not reported by ECETOC (1997).

1.3.3 Soil, sediment, and consumer products

Although the Working Group did not identify validated methods for measuring butyl methacrylate in soil, sediments, biosolids, or consumer products, several methods have been developed and used for specific applications. ECETOC suggested that residual monomer in products, aqueous polymer emulsions, or other materials can be analysed using headspace analysis after extraction using a low-volatility solvent (ECETOC, 1997). Residual butyl methacrylate has been measured in water-based polymer emulsions using headspace GC-MS, with an LOD of 1.4 mg/kg (Petha et al., 2017), in dental acrylic resins (LOD, 0.295 µg/mL; Urban et al., 2006), and in food-contact plastics using HPLC after methanol extraction (LOD, 0.03 mg/kg; Qiu et al., 2021). A nail hardener consumer product was analysed for the presence of monomer via GC-MS (Kanerva et al., 1996).

1.3.4 Human biomarkers

The Working Group was not able to identify human biomarkers that have been validated for exposure to butyl methacrylate. HPLC and GC methods have reportedly been used to analyse for the presence of monomer in biological media (in blood, urine, amniotic fluids, liver, and lung tissue) (ECETOC, 1998). [The Working Group noted that specific methodological details were not found in the report from ECETOC (1998).]

HPLC with ultraviolet detection has been used to measure butyl methacrylate and the metabolite methacrylic acid in biological samples (blood, liver) after butyl acetate extraction (Jones, 2002).

1.4 Occurrence and exposure

1.4.1 Occurrence

(a) Air, water, and soil

In 2010–2020 in Japan, estimated releases of butyl methacrylate into the air averaged [5204 kg] and ranged from 9383 kg in 2014 to 2011 kg in 2020 (Japan Ministry of the Environment, 2023). The chemical and warehousing industries reported the largest releases to the atmosphere, and only the chemical industry reported releases to water systems (Japan Ministry of the Environment, 2021). ECETOC reported estimates of environmental releases in 1994 in the European Union (< 0.3 tonnes to air and < 0.15 tonnes to water), but more recent estimates were not identified (ECETOC, 1998). Unreacted butyl methacrylate monomer is not expected to accumulate in environmental media, because of its short half-life in air and water (ECETOC, 1997). In 2011, a Japanese nationwide survey found butyl methacrylate in air samples from 2 of the valid 14 sites, at levels up to 37 ng/m^3 (LOD, 8.7 ng/m^3) (Japan Ministry of the Environment, 2012). Butyl methacrylate was not detected in the surface water of 14 sampled sites (7 rivers, 5 coastal and 2 offshore sites; LOD, 12 ng/m^3). In Japan in 2007, a total of 68 facilities reported butyl methacrylate releases: 67 reported releases in air (total, 4645 kg) and 5 in water (total, 1907 kg) (Japan Ministry of the Environment. 2007). In 2009, the corresponding values for a total of 62 facilities were 62 (total, 3125 kg) and 3 (total, 1703 kg), reporting releases in air and water, respectively (Japan Ministry of the Environment, 2009).

(b) Consumer products and food

Migration of unreacted butyl methacrylate into food from packaging is expected to be low (ECETOC, 1998). One study analysed food-grade plastics for the presence of butyl methacrylate, which was not found above the LOD (0.03 mg/kg) (Qiu et al., 2021).

In a method-development study, residual butyl methacrylate monomer was extracted from acrylic resins used in dental applications using 2 mL of methanol; the mean concentration of extracted monomer from a 100 mg specimen ranged from 160.56 µg/mL to 277.87 µg/mL (Urban et al., 2006).

In 1995 in Finland, butyl methacrylate was detected in small amounts in 6 of 42 [25%] nail polish samples tested; concentrations ranged from 0.014% to 0.067% (Sainio et al., 1997).

1.4.2 Occupational exposure

The exposure routes for butyl methacrylate are via inhalation, dermal exposure, and ingestion. Although the vapour pressure of butyl methacrylate is lower than that of other methacrylates, inhalation is likely to be the primary route of exposure in the workplace, with contributions from dermal exposure and accidental ingestion in some settings. Dermal absorption is likely if skin exposure occurs (ECETOC, 1998).

According to the National Occupational Exposure Survey (NOES) conducted by NIOSH from 1981 to 1983 (NIOSH, 1988), workers in

the USA who were potentially exposed to butyl methacrylate were represented in the following industries, from most to least: miscellaneous manufacturing, chemicals and allied products, instruments and related products, machinery, rubber and miscellaneous plastic products, printing and publishing, wholesale trade and durable goods, paper and allied products, special trade contractors, health services, fabricated metal products, and trucking and warehousing (NIOSH, 1983) [10 001 exposed workers were reported; the Working Group estimated a confidence interval of 5400–14 600].

Occupational exposure in air in different jobs and industries is summarized in Table 1.1.

In France, the Institut national de recherche et de sécurité pour la prévention des accidents du travail et des maladies professionnelles (INRS) provided butyl methacrylate exposure data for 2000–2020 by industry and occupation (INRS, 2022). The data for the three most studied occupations for butyl methacrylate sampling in these data are included in Table 1.1. The highest single personal exposure measurement (90 mg/m^3) was among equipment operators in the chemical industry in the manufacture of adhesives (INRS, 2022). By industry, the chemical manufacturing industry had the highest mean exposure (n = 26; 14 mg/m^3) (INRS, 2022).

The highest measured concentrations reported were measured in a paint-manufacturing plant in China in 2017, where mean concentrations ranged from 6.7 mg/m^3 in a warehouse to 57.3 mg/m^3 on a reaction line and in inspection (Ding, 2019).

In 1981, stationary measurements were taken while a facsimile machine was running (about 1 hour daily) in an office space where administrative staff worked; concentrations ranged from 0.13 mg/m^3 to 0.29 mg/m^3 and were considered low by the authors of the report (NIOSH, 1981). Exposures to butyl methacrylate have occurred among office and machine repair technicians because of its presence in facsimile machine paper. [The Working Group noted that it is not clear to what extent paper containing butyl methacrylate continues to be used.]

The cross-sectional portion of one study (group B, Raymond, 1996) evaluated butyl methacrylate exposure among facsimile machine repair workers by measuring breathing-zone total particulate; this sampling methodology is not a standard approach and measures a surrogate rather than the specific agent (for analytical methods, see Section 1.3.1). [The Working Group noted that the authors included conflicting data about the composition of facsimile machine fumes; unpublished data indicated that two thirds of machine particulate emitted is butyl methacrylate, but data in an appendix indicated that butyl methacrylate comprises one third of machine particulate. Vapour (gas) exposures were not measured, but the article also provided conflicting information about the butyl methacrylate content of emitted gases. Sales and administrative workers were classified as not exposed, because the machine tasks were not performed in their workplace; it is not clear whether they participated in air monitoring.] In a case series with seven technicians (group A), employees were determined to be exposed given their work as a machine repair technician combined with use of butyl methacrylate-containing paper. In a follow-up with 32 employees (group C), the workers were classified as not exposed (or less exposed), given their work doing some repair tasks (less frequently than technicians in group A and group B) and the gradual discontinuation of acrylate-containing paper. No quantitative exposure assessment was done for group A or group C (Raymond, 1996).

For shipbuilding work in Finland, Engström et al. (1990) measured personal exposures of up to 0.14 mg/m^3 during outfitting work using an epoxyester-based primer.

An ECETOC report contained butyl methacrylate exposure data for full and partial shifts for several tasks at a monomer production facility

Table 1.1 Occupational exposure to butyl methacrylate measured in air samples

Occupational group/job type Location and date	Monitoring method	No. of samples Type of sampling	Analytical method (LOD)	Mean (range) mg/m³	Median (IQR)	Comments	Reference
Monomer production and/or handling							
Monomer production/laboratory City unknown, 1992–1993	NR	NR NR/area	NR (NR)	0.06 (NR)	–	4–8 h	ECETOC (1998)
	NR	NR NR/area	NR (NR)	NR (< 0.5–0.14)	–	5 min to 1 h	
Monomer production/task NR City unknown, 1992	NR	NR Area	NR (NR)	0.4 (NR)	–	4–8 h	ECETOC (1998)
Monomer production/filling City unknown, 1992–1993	NR	NR	NR (NR)	0.05 (NR)	–	4–8 h	ECETOC (1998)
	NR	NR	NR (NR)	NR (ND to < 0.11)	–	5 min to 1 h	
Monomer production/other operations City unknown, 1992–1994	NR	NR	NR (NR)	0.02 (NR)	–	4–8 h	ECETOC (1998)
Polymerization/storage and distribution City unknown, 1992–1994	NR	NR	NR (NR)	0.32 (NR)	–	4–8 h	ECETOC (1998)
Polymerization/block City unknown, 1992–1994	NR	NR	NR (NR)	0.29 (NR)	–	4–8 h	ECETOC (1998)
Polymerization/block City unknown, 1992–1994	NR	NR	NR (NR)	1.49 (NR)	–	5 min to 1 h	ECETOC (1998)
Paint manufacturer/MG[c] reaction line Guangzhou, China, 2017	NR	3 NR	GC (0.1 mg/m³)	34.7 (NR)	–	Duration, NR	Ding (2019)
Paint manufacturer/replacement kettle Guangzhou, China, 2017	NR	3 NR	GC (0.1 mg/m³)	23.8 (NR)	–	Duration, NR	Ding (2019)
Paint manufacturer/emulsification Guangzhou, China, 2017	NR	3 NR	GC (0.1 mg/m³)	43.2 (NR)	–	Duration, NR	Ding (2019)
Paint manufacturer/AC[c] reaction line Guangzhou, China, 2017	NR	3 NR	GC (0.1 mg/m³)	57.3 (NR)	–	Duration, NR	Ding (2019)
Paint manufacturer/inspection Guangzhou, China, 2017	NR	3 NR	GC (0.1 mg/m³)	57.3 (NR)	–	Duration, NR	Ding (2019)
Paint manufacturer/warehouse Guangzhou, China, 2017	NR	3 NR	GC (0.1 mg/m³)	6.7 (NR)	–	Duration, NR	Ding (2019)
Operator of chemical industry devices France, 2010–2020	NR	22 PBZ	NR (NR)	15 (< LOQ[b]–90)	2.0 (< LOQ–23)	Duration, NR	INRS (2022)

Table 1.1 (continued)

Occupational group/job type Location and date	Monitoring method	No. of samples Type of sampling	Analytical method (LOD)	Mean (range) mg/m³	Median (IQR)	Comments	Reference
Non-production workplaces or unknown							
Facsimile machine operation Jamaica, New York, USA, 1981	Activated charcoal tube	5 Area	NR (NR)	0.21 (0.13–0.29)	0.21 (NR)	Partial shift (6 h in total)	NIOSH (1981)
Shipbuilding hull construction City unknown, 1990 or earlier	Amberlite XAD-2 tubes	1 PBZ	GC with FID	0.020	NA	30–60 min	Engström et al. (1990)
Shipbuilding outfitting work City unknown, 1990 or earlier	Amberlite XAD-2 tubes	9 PBZ	GC with FID	NR (NR–0.14)	0.030 (NR)	30–60 min	Engström et al. (1990)
Facsimile machine repair New York, New York, USA, before 1996	NR	1 PBZ	NR (NR)	0.60[a]	NA	6 h	Raymond (1996)
Facsimile machine repair Dallas, Texas, USA, before 1996	NR	NR PBZ	NR (NR)	NR (0.14–0.40)[a]	NR (NR)	128–420 min	Raymond (1996)
Moulder/laminator France, 2000–2020	NR	28 PBZ	NR (NR)	1.6 (< LOQ[b]–37)	0.20 (0.098–0.30)	Duration, NR	INRS (2022)
Operator of an adhesives application device France, 2010–2020	NR	11 PBZ	NR (NR)	3.9 (< LOQ[b]–37)	< LOQ (< LOQ to < LOQ)	Duration, NR	INRS (2022)

FID, flame ionization detection; GC, gas chromatography; h, hour(s); IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; min, minute(s); NA, not applicable; ND, not detectable; NR, not reported; PBZ, personal breathing zone.

[a] This measurement was for total particulate, used as a surrogate for butyl methacrylate exposure.

[b] LOQ was not reported for Institut national de recherche et de sécurité (INRS) data.

[c] The meaning of the abbreviations "AC" and "MG" was not available to the Working Group.

and at a polymerization facility. The highest short-term (5 minutes to 1 hour) exposure at the monomer production facility (0.14 mg/m^3) was recorded in a laboratory, and the highest short-term (5 minutes to 1 hour) exposure at the polymerization facility (1.49 mg/m^3) was during block polymerization (ECETOC, 1998).

Peak levels of butyl methacrylate were detected but not quantified during joint implant cement removal during a hip surgery revision and were below the LOD of the method used (0.01 mg/sample) (Cautilli & Hozack, 1994).

[The Working Group noted that exposure assessment data were not found for workers in several industries with known use of butyl methacrylate, such as furniture manufacturing and repair, construction, vehicle repair and manufacturing, and dental care.]

1.4.3 Exposure of the general population

One government report from Japan published estimated exposures for the general population based on butyl methacrylate releases and environmental sampling data in air and water. The report estimated a maximum exposure of 0.32 µg/m^3 based on a plume–puff model and reported releases in 2010 (Japan Ministry of the Environment, 2021). The same report estimated maximum ingestion exposures of 0.00048 µg/kg per day using data for public freshwater sources and 0.0088 µg/kg per day using water discharge data (Japan Ministry of the Environment, 2021).

The general population may be exposed to residual monomer from new dental fillings, food packaging, and cosmetic products (see Section 1.4.1b).

In 2016 and 2017, 18 air samples (personal and static) were collected in 7 nail salons in Boston (USA). Butyl methacrylate was not detected, but ethyl methacrylate and methyl methacrylate were detected with median values of 24 µ/m^3 and 190 µ/m^3, respectively (Ceballos et al., 2019). In a similar study in 17 nail salons in Michigan (USA), butyl methacrylate was not detected in the 68 air samples analysed, but ethyl methacrylate and methyl methacrylate were detected with mean values of 75 µ/m^3 and 4820 µ/m^3, respectively (Zhong et al., 2019).

The Working Group did not identify published data on biomonitoring of butyl methacrylate.

1.5 Regulations and guidelines

1.5.1 Occupational exposure limits

Governments in the following seven countries have established 8-hour time-weighted average occupational exposure limits for butyl methacrylate: Canada, Denmark, Latvia, Norway, Poland, Romania, and Sweden (see Table 1.2). The limits for time-weighted average for an 8-hour workday or a 40-hour workweek range from 30 mg/m^3 in Latvia to 300 mg/m^3 in Sweden. Four of these countries also have short-term exposure limits (for 15 minutes), which range from 250 mg/m^3 to 450 mg/m^3. Under the European Union directive to protect young people, workers younger than 18 years may not be exposed to butyl methacrylate in the workplace, because of the potential for sensitization (ECHA, 2022c).

As of 1998, companies that produce butyl methacrylate have adopted internal occupational exposure limits at or near 50 ppm [296 mg/m^3], with a short-term exposure limit of 75 ppm [443 mg/m^3] (ECETOC, 1998). [The Working Group noted that there are no details available on how the private limits were derived.]

Derived no-effect levels (DNELs) are available for butyl methacrylate and are already used as part of the chemical safety assessment in registration dossiers prepared by registrants under the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation (ECHA, 2022d).

Table 1.2 Occupational exposure limits for butyl methacrylate

Country	8-hour TWA[a] (mg/m³)	Short-term, 15 minutes (mg/m³)	Reference
Latvia	30	Not available	Republic of Latvia (2007)
Norway	59	Not available	Ministry of Labour and Social Inclusion (2022)
Poland	100	300	Central Institute for Labour Protection - National Research Institute (2023)
Denmark	145	290	Labour Supervision (2007)
Romania	150	250	Ministry of Labour and Social Protection (2002)
Canada	290	Not available	WorkSafeBC (2023)
Sweden	300	450	Swedish Work Environment Authority (2018)

TWA, time-weighted average.
[a] Some limits were issued in parts per million. They were converted to mg/m³ using normal temperature and pressure: 20 °C and 101 325 Pa.

1.5.2 Consumer products

In the USA, butyl methacrylate has been identified as an indirect food additive (Substances for Use as Basic Components of Single and Repeated Use Food Contact Surfaces) as a component of polymeric adhesives, paper, paperboard, and plastics in contact with food; the monomer itself has not been identified as an additive directly to food (US FDA, 2022). The European Food Safety Authority panel on food contact materials, enzymes, flavourings, and processing aids concluded that the intended use of a (butyl acrylate, butyl methacrylate, methyl methacrylate) copolymer in rigid polyvinyl chloride at a maximum level of 1% weight per weight (w/w) and in polylactic acid at a maximum level of 5% w/w is not of safety concern for the consumer (EFSA, 2011). Based on this, the European Commission established that copolymers containing butyl methacrylate, when intended to be used on plastic materials and articles intended to come into contact with food, are limited to be used in rigid polyvinyl chloride at a maximum level of 1% or 2%, depending on the copolymer (European Commission, 2011).

1.6 Quality of exposure assessment in key mechanistic studies in humans

The Working Group reviewed one study in exposed humans. It contained a case series (group A, $n = 7$), a cross-sectional study (group B, $n = 18$), and a follow-up of workers after the product of concern was mostly discontinued, meaning that the butyl methacrylate was eliminated from the facsimile transceiver process (group C, $n = 32$). The small cross-sectional study (group B, $n = 18$) was dedicated to the identification of pulmonary and immunological changes among a subset of facsimile machine repair workers (group A) (Raymond, 1996). Details on the exposure assessment are summarized in Table S1.3 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/631), and exposure levels are described in Section 1.4.2 and Table 1.1.

1.6.1 Exposure assessment methods

Group A: Exposure was determined by job role as a facsimile machine repair technician concurrent with use of acrylate-containing paper and reported exposure to facsimile machine fumes during testing. The facsimile paper used

was made with butyl methacrylate polymer and emitted butyl methacrylate when it was "burned" during facsimile receipt or testing. No specific information about the paper and ingredients was provided. The duration and number of repairs was described to be proportional to symptom intensity, according to informal, non-systematic reporting from patients.

Group B: In this study, workers were identified as exposed ($n = 6$) if they worked as facsimile machine repair technicians and unexposed ($n = 12$) if they worked as administration or sales personnel for the same company in a separate building 2 miles [3.2 km] away.

For the repair technicians, a surrogate for butyl methacrylate exposure was measured through breathing-zone sampling of particulate in air during machine repair and testing. Particle sampling and analysis by gravimetry was used as a surrogate for butyl methacrylate exposure. The particles were sampled during 2.4–7 hours in the breathing zone during machine repair and testing. The results were presented as a time-weighted average. The article cited unpublished data suggesting that fresh facsimile particulate emissions contained about two thirds butyl methacrylate and vapour emissions contained about one third butyl methacrylate. However, a table in the appendix presented data showing the opposite (that emitted particulate contained one third butyl methacrylate and emitted vapours contained two thirds butyl methacrylate). [The Working Group noted that this inconsistency adds further difficulty to interpreting the non-specific air measurements.] Emitted fumes and vapours also contain smaller amounts of ethane, propane, butane, and other unidentified compounds (called "miscellaneous" by the authors). Vapour phase was not sampled in this study, probably resulting in exposure underestimation. A questionnaire was presented to workers to ask about symptoms, age, and smoking status, but it did not cover work-related information (e.g. machine repair and testing performed) or non-occupational exposure sources. The authors did not report demographics for the exposed and unexposed groups separately but reported that they had similar age and smoking status. Mechanical ventilation conditions available were listed (e.g. ventilation rate and availability of local exhaust ventilation) but were not considered or discussed further.

For the unexposed control group, it is unclear whether they were included in the air monitoring campaign (reported as background monitoring) or whether exposure was assumed to be zero. They worked in a building 2 miles [3.2 km] from the machine repair site, and the workers reported having little contact with those activities, so they were likely to be correctly classified as unexposed.

Group C: This group had less-frequent exposure to machine emissions, because their duties were broader than machine repair and testing. In addition, workers' exposures to emissions containing butyl methacrylate decreased in frequency as use of acrylate-containing paper was discontinued and phased out as machines came in for repair. However, residual exposure to low levels still occurred during the testing of machines because some contained the discontinued paper.

1.6.2 Quality of exposure assessment methods

The quality of exposure assessment for butyl methacrylate in this study was only moderate for the workers in group B and was poor for group A and group C. The primary limitation is the use of particle sampling and analysis by gravimetry as a surrogate for butyl methacrylate exposure, rather than characterization and measurement of butyl methacrylate directly in both fume and vapour fractions. In Section 1.3, several possible methods for measuring butyl methacrylate are described. Although butyl methacrylate had been found in air samples in previous studies where facsimile machine operation occurred, the

approach followed in this study does not enable an evaluation of the exposure to butyl methacrylate or an identification of other fume and vapour emission components (NIOSH, 1981). No exposure measurements were taken after the acrylate-containing paper had been discontinued, although residual butyl methacrylate exposure may have occurred because of the use of older machines and residual acrylate-containing paper stock. Another limitation was the lack of information about the variability of exposures within and between workers across shifts and/or job tasks. A single measurement was taken for each worker and was used in subsequent linear regression.

Exposures for group C were the least detailed, because the description was anecdotal and exposure was changing during this follow-up. No details were provided about the frequency or the magnitude of exposures, so the workers cannot be classified as exposed or unexposed.

Overall, the exposure assessment was useful to understand that there was likely to be butyl methacrylate exposure during facsimile machine repair (particularly for group A and group B), but with high uncertainties about the magnitude of exposures (in particulate and vapour forms) and the mixtures of chemicals present in the emissions.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

Studies of carcinogenicity in mice and rats exposed to butyl methacrylate were limited to inhalation studies conducted by the Japan Bioassay Research Center (JBRC, 2018a, b, c, d; also reported by Furukawa et al., 2023). The results of these studies are summarized in Table 3.1.

3.1 Mouse

In a well-conducted study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6D2F$_1$/Crl mice (age, 6–7 weeks) were treated with butyl methacrylate (purity, > 99.8%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2018a, b; also reported by Furukawa et al., 2023). The concentration in the exposure chambers was set to 0 (clean air, control), 8, 30, or 125 ppm for males and females and was monitored every 15 minutes. The mean air concentrations were the target values, and the coefficients of variation were within 1.3%. At week 104, the survival rates of males at 8 and 125 ppm were lower than those of the control group. Survival at study termination was 45/50, 35/50, 41/50, and 37/50 for males and 36/50, 32/50, 33/50, and 33/50 for females at 0 (control), 8, 30, and 125 ppm, respectively. Body-weight gain of male mice at the highest concentration was significantly decreased from week 3 of exposure until week 82, compared with controls. The relative final body weight in males was 102%, 103%, and 100% of the control value at 8, 30, and 125 ppm, respectively. Food consumption of male mice at the highest concentration was significantly decreased from week 3 of exposure until week 102, compared with the control value. Body-weight gain of female mice at the highest concentration was significantly decreased from week 3 of exposure until week 70, compared with controls. The relative final body weight in females was 102%, 101%, and 100% of the control value at 8, 30, and 125 ppm, respectively. Food consumption of female mice at the highest concentration was significantly decreased from week 1 of exposure until week 70, compared with the control value. All mice underwent complete

Table 3.1 Studies of carcinogenicity in mice and rats exposed to butyl methacrylate

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence (%)	Significance	Comments
Full carcinogenicity Mouse, B6D2F$_1$/Crl (M) 6–7 wk 104 wk JBRC (2018a)	Inhalation (whole-body exposure) Purity, ≥ 99.8% Air 0, 8, 30, 125 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 45, 35, 41, 37	*Liver* Hepatocellular adenoma 23/50 (46%), 27/50 (54%), 30/50 (60%), 24/50 (48%)	$P = 0.0255$, Peto trend test, standard method NS, Peto trend test, prevalence method NS, Peto trend test, combined analysis NS, Cochran–Armitage trend test	*Principal strengths*: well-conducted GLP study; covered most of lifespan; males and females used; multiple concentrations used; adequate number of animals per group. *Other comments*: lower survival in low-dose and high-dose group. *Historical controls*: hepatocellular adenoma, 20.1% (range, 8–36%); histiocytic sarcoma (all sites), 7.8% (2–12%) (reported by Furukawa et al., 2023).
		Hepatocellular carcinoma 12/50 (24%), 13/50 (26%), 16/50 (32%), 10/50 (20%)	NS	
		Hepatocellular adenoma or carcinoma (combined) 32/50 (64%), 33/50 (66%), 41/50 (82%)*, 31/50 (62%)	*$P = 0.0352$, Fisher exact test NS, Peto trend test, standard method NS, Peto trend test, prevalence method NS, Peto trend test, combined analysis NS, Cochran–Armitage trend test	
		All sites Histiocytic sarcoma 0/50, 1/50 (2%), 1/50 (2%), 3/50 (6%)	$P = 0.0219$, Peto trend test, standard method $P = 0.0393$, Peto trend test, combined analysis NS, Peto trend test, prevalence method NS, Cochran–Armitage trend test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence (%)	Significance	Comments
Full carcinogenicity Mouse, B6D2F$_1$/Crl (F) 6–7 wk 104 wk JBRC (2018a)	Inhalation (whole-body exposure) Purity, ≥ 99.8% Air 0, 8, 30, 125 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 36, 32, 33, 33	*Pituitary gland* Adenoma: anterior lobe 3/50 (6%), 1/50 (2%), 4/50 (8%), 6/50 (12%) *All sites* Haemangiosarcoma 1/50 (2%), 2/50 (4%), 2/50 (4%), 4/50 (8%)	P = 0.0439, Peto trend test, combined analysis NS, Peto trend test, standard method NS, Peto trend test, prevalence method NS, Cochran–Armitage trend test P = 0.0318, Peto trend test, prevalence method NS, Peto trend test, standard method NS, Peto trend test, combined analysis NS, Cochran–Armitage trend test	*Principal strengths*: well-conducted GLP study; covered most of lifespan; males and females used; multiple concentrations used; adequate number of animals per group. *Historical controls*: adenoma of the anterior lobe of the pituitary gland, 12.8% (range, 4–20%); haemangiosarcoma (all sites), 3% (range, 0–6%) (reported by Furukawa et al. (2023).
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2018d)	Inhalation (whole-body exposure) Purity, ≥ 99.8% Air 0, 30, 125, 500 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 41, 36, 28	*Spleen* Mononuclear cell leukaemia 8/50 (16%), 8/50 (16%), 11/50 (22%), 14/50 (28%) *Subcutis* Fibroma 4/50 (8%), 5/50 (10%), 3/50 (6%), 6/50 (12%)	P = 0.0050, Peto trend test, standard method P = 0.0146, Peto trend test, combined analysis NS, Peto trend test, prevalence method NS, Cochran–Armitage trend test P = 0.0264, Peto trend test, standard method NS, Peto trend test, prevalence method NS, Peto trend test, combined analysis NS, Cochran–Armitage trend test	*Principal strengths*: well-conducted GLP study; covered most of lifespan; males and females used; multiple concentrations used; adequate number of animals per group. *Other comments*: lower survival in high-dose group. *Historical controls*: mononuclear cell leukaemia of the spleen, 61/649 (9.4%; range, 4–14%); fibroma of the subcutis, 75/649 (11.6%; range, 6–16%); interstitial cell tumour of the testis, 531/649 (81.8%; range, 72–98%).

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence (%)	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6–7 wk 104 wk JBRC (2018d) (cont.)		*Testis* Interstitial cell tumour, benign 43/50 (86%), 48/50 (96%), 44/50 (88%), 48/50 (96%)	*P* = 0.0316, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis; NS, Cochran–Armitage trend test	
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6–7 wk 104 wk JBRC (2018d)	Inhalation (whole-body exposure) Purity, ≥ 99.8% Air 0, 30, 125, 500 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 39, 38, 37, 37	*Mammary gland* Fibroadenoma 6/50 (12%), 4/50 (8%), 6/50 (12%), 9/50 (18%)	*P* = 0.0349, Peto trend test, prevalence method NS, Peto trend test, standard method NS, Peto trend test, combined analysis NS, Cochran-Armitage trend test	*Principal strengths*: well-conducted GLP study; covered most of lifespan; males and females used; multiple concentrations used; adequate number of animals per group. *Historical controls*: fibroadenoma of the mammary gland, 75/650 (11.5%; range, 6–20%); C-cell adenoma or C-cell carcinoma (combined) of the thyroid gland, 84/650 (12.9%; range, 2–26%).
		Thyroid C-cell adenoma 4/50 (8%), 3/50 (6%), 9/50 (18%), 6/50 (12%)	NS	
		C-cell carcinoma 0/50, 0/50, 2/50 (4%), 2/50 (4%)	NS	
		C-cell adenoma or carcinoma (combined) 4/50 (8%), 3/50 (6%), 11/50 (22%)*, 8/50 (16%)	**P* = 0.0453, Fisher exact test NS, Peto trend test, prevalence method Data not applicable for Peto trend test, standard method or Peto trend test, combined analysis NS, Cochran–Armitage trend test	

F, female; GLP, Good Laboratory Practice; h, hour(s); M, male; NS, not significant; ppm, parts per million; wk, week(s).

necropsy, and all organs and tissues were examined microscopically.

In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma of the liver (P = 0.0255, Peto trend test, standard method); the incidence of 23/50 (46%), 27/50 (54%), 30/50 (60%), and 24/50 (48%) for the groups at 0 (control), 8, 30, and 125 ppm, respectively, exceeded the upper bound of the range observed in historical controls (average, 20.1%; range, 8–36%) from this laboratory at all doses. [The Working Group noted that several Peto trend tests were conducted in this study; the Peto test standard method was referred to as death analysis, the Peto test prevalence method was referred to as incidental tumour test, and the Peto test combined analysis was referred to as death analysis plus incidental tumour test. A significant P value in any Peto test was considered relevant for the detection of treatment-related increases in tumour incidence. The Working Group also noted that the mouse strain and the diet used in this study were different from those in historical controls. In this study, the laboratory changed the mouse strain from the previous strain ($B6D2F_1$/Crlj) to $B6D2F_1$/Crl, using a new production system (International Genetic Standardization), even though the two strains are similar. In addition, a low-protein diet was used instead of the diet previously used in the studies comprising historical controls.] The incidence of hepatocellular adenoma or carcinoma (combined) of the liver of 32/50 (64%), 33/50 (66%), 41/50 (82%), and 31/50 (62%) for the groups at 0 (control), 8, 30, and 125 ppm, respectively, was significantly increased (P = 0.0352, Fisher exact test) in the group at 30 ppm compared with controls. [The Working Group considered that the lack of a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) at the highest concentration may be due to the suppression of body-weight gain during the experimental period.] There was a significant positive trend in the incidence of histiocytic sarcoma of all sites (including the subcutis, liver, epididymis, or peritoneum) (P = 0.0219, Peto trend test, standard method; P = 0.0393, Peto trend test, combined analysis); the incidence of 0/50, 1/50 (2%), 1/50 (2%), and 3/50 (6%) for the groups at 0 (control), 8, 30, and 125 ppm, respectively, was within the range observed in historical controls (average, 7.8%; range, 2–12%) from this laboratory.

In female mice, there was a significant positive trend in the incidence of adenoma of the anterior lobe of the pituitary gland (P = 0.0439, Peto trend test, combined analysis); the incidence of 3/50 (6%), 1/50 (2%), 4/50 (8%), and 6/50 (12%) for the groups at 0 (control), 8, 30, and 125 ppm, respectively, was within the range observed in historical controls (average, 12.8%; range, 4–20%) from this laboratory for the intermediate and highest dose. [The Working Group noted that neither the incidence of carcinoma nor the incidence of hyperplasia in the anterior lobe of the pituitary gland was significantly increased.] There was a significant positive trend in the incidence of haemangiosarcoma of all sites (including the spleen, subcutis, liver, uterus, bone marrow, peritoneum, or retroperitoneum) (P = 0.0318, Peto trend test, prevalence method); the incidence of 1/50 (2%), 2/50 (4%), 2/50 (4%), and 4/50 (8%) for the groups at 0 (control), 8, 30, and 125 ppm, respectively, exceeded the upper bound of the range observed in historical controls (average, 3.0%; range, 0–6%) from this laboratory at the highest concentration.

There were no increases in the incidence of the non-neoplastic lesions in sites where tumour incidence was considered increased. [The Working Group noted this was a well-described and well-conducted study that complied with GLP, used multiple concentrations, used both sexes, used an adequate number of animals per group, and had an adequate duration of exposure and observation.]

3.2 Rat

In a well-conducted study of chronic toxicity and carcinogenicity that complied with GLP, groups of 50 male and 50 female F344/DuCrlCrlj rats (age, 6–7 weeks) were treated with butyl methacrylate (purity, > 99.8%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2018c, d; also reported by Furukawa et al., 2023). The concentration in the exposure chambers was set to 0 (clean air, control), 30, 125, or 500 ppm for males and females and was monitored every 15 minutes. The mean air concentrations were the target values, and the coefficients of variation were within 1.0%. At 104 weeks, the survival rate of males at the highest concentration was lower than that of the control group. Survival at study termination was 38/50, 41/50, 36/50, and 28/50 for males and 39/50, 38/50, 37/50, and 37/50 for females at 0 (control), 30, 125, and 500 ppm, respectively. Body-weight gain of male rats at the highest concentration was significantly decreased in the early exposure period and from week 70 to the end of the study, compared with controls. The relative final body weight in males was 99%, 99%, and 94% of the control value for 30, 125, and 500 ppm, respectively. Food consumption of male rats at the highest concentration was significantly decreased from week 78 of exposure until week 98, compared with the control value. Body-weight gain of female rats at the highest concentration was significantly decreased in the early exposure period and from week 42 to the end of the study, compared with controls. The relative final body weight in females was 98%, 104%, and 92% of the control value at 30, 125, and 500 ppm, respectively. Food consumption of female rats at the highest concentration was significantly decreased from week 62 of exposure until week 102, compared with the control value. All rats underwent complete necropsy, and all organs and tissues were examined microscopically.

In male rats, there was a significant positive trend in the incidence of mononuclear cell leukaemia of the spleen (P = 0.0050, Peto trend test, standard method; P = 0.0146, Peto trend test, combined analysis); the incidence of 8/50 (16%), 8/50 (16%), 11/50 (22%), and 14/50 (28%) for the groups at 0 (control), 30, 125, and 500 ppm, respectively, exceeded the upper bound of the range observed in historical controls (average, 9.4%; range, 4–14%) from this laboratory at all doses. [The Working Group noted that several Peto trend tests were conducted in this study; the Peto test standard method was referred to as death analysis, the Peto test prevalence method was referred to as incidental tumour test, and the Peto test combined analysis was referred to as death analysis plus incidental tumour test. A significant P value in any Peto test was considered relevant for the detection of treatment-related increases in tumour incidence.] There was a significant positive trend in the incidence of fibroma of the subcutis (P = 0.0264, Peto trend test, standard method); the incidence of 4/50 (8%), 5/50 (10%), 3/50 (6%), and 6/50 (12%) for the groups at 0 (control), 30, 125, and 500 ppm, respectively, was within the range observed in historical controls (average, 11.6%; range, 6–16%) from this laboratory. There was a significant positive trend in the incidence of benign interstitial cell tumour of the testis (P = 0.0316, Peto trend test, prevalence method); the incidence of 43/50 (86%), 48/50 (96%), 44/50 (88%), and 48/50 (96%) for the groups at 0 (control), 30, 125, and 500 ppm, respectively, was within the range observed in historical controls (average, 81.8%; range, 72–98%) from this laboratory. [The Working Group noted that interstitial cell tumour is a common spontaneous tumour with a high incidence in Fischer 344 rats.]

In female rats, there was a significant positive trend in the incidence of fibroadenoma of the mammary gland (P = 0.0349, Peto trend test, prevalence method); the incidence of 6/50 (12%), 4/50 (8%), 6/50 (12%), and 9/50 (18%) for

the groups at 0 (control), 30, 125, and 500 ppm, respectively, was within the range observed in historical controls (average, 11.5%; range, 6–20%) from this laboratory. The incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland of 4/50 (8%), 3/50 (6%), 11/50 (22%), and 8/50 (16%) for the groups at 0 (control), 30, 125, and 500 ppm, respectively, was significantly increased (P = 0.0453, Fisher exact test) in the group at 125 ppm compared with controls. The incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland was within the range observed in historical controls (average, 12.9%; range, 2–26%) from this laboratory.

For both male and female mice, there were no increases in the incidence of non-neoplastic lesions at sites at which tumour incidence was considered to be increased. [The Working Group noted that this was a well-described and well-conducted study that complied with GLP, used multiple concentrations, used both sexes, used an adequate number of animals per group, and had an adequate duration of exposure and observation.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of butyl methacrylate has been assessed in one well-conducted inhalation study that complied with GLP in male and female B6D2F$_1$/Crl mice (JBRC, 2018a, b; also reported by Furukawa et al., 2023) and in one well-conducted inhalation study that complied with GLP in male and female F344/DuCrlCrlj rats (JBRC, 2018c, d; also reported by Furukawa et al., 2023).

In the inhalation study that complied with GLP in male and female B6D2F$_1$/Crl mice, there was a significant positive trend in the incidence of hepatocellular adenoma of the liver in males. The incidence of hepatocellular adenoma or carcinoma (combined) of the liver was significantly increased in males at 30 ppm. There was a significant positive trend in the incidence of histiocytic sarcoma of all sites. In female mice, there was a significant positive trend in the incidence of adenoma of the anterior lobe of the pituitary gland. There was a significant positive trend in the incidence of haemangiosarcoma of all sites (JBRC, 2018a, b; also reported by Furukawa et al., 2023).

In the inhalation study that complied with GLP in male and female F344/DuCrlCrlj rats, there was a significant positive trend in the incidence of mononuclear cell leukaemia of the spleen in males. There was a significant positive trend in the incidence of fibroma of the subcutis and of benign interstitial cell tumour of the testis. In female rats, there was a significant positive trend in the incidence of fibroadenoma of the mammary gland. The incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland was significantly increased in the group at 125 ppm compared with controls (JBRC, 2018c, d; also reported by Furukawa et al., 2023).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on absorption of butyl methacrylate in humans were limited to one study that used human epidermis samples in an in vitro system (Jones, 2002). *n*-Butyl methacrylate (100 µL/cm^2) was absorbed through the epidermis, with a mean rate of absorption of 76.7 µg/cm^2 per hour and a total amount absorbed of 2% over a 24-hour period. Data on distribution, metabolism, and excretion of butyl methacrylate in humans were not available to the Working Group.

4.1.2 Experimental systems

(a) Absorption

An in vitro system using Wistar rat epidermis and whole (viable) Fischer 344 rat skin was used to evaluate absorption of *n*-butyl methacrylate [butyl methacrylate] in the skin (Jones, 2002). *n*-Butyl methacrylate (100 µL/cm^2) was absorbed through the skin. The rat epidermis was about 20 times as permeable as the human epidermis. The mean rate of absorption and the total amount absorbed were 1540 µg/cm^2 per hour and 18% over 24 hours, respectively, for Wistar rat epidermis and 40.9 µg/cm^2 per hour and 0.4% over 10 hours, respectively, for Fischer 344 rat skin. [Based on the study by Jones (2002), the difference in absorption between whole rat skin and epidermis may be attributable to first-pass hydrolysis of butyl methacrylate in the dermis, which was excised from the epidermis in the other absorption tests (rat and human). First-pass hydrolysis could be expected to be lower in human skin than in rat skin. The study reported that for whole rat skin absorption, methacrylic acid but not butyl methacrylate was detected in the receptor chamber and suggested that all the compound absorbed underwent first-pass metabolism in the skin. However, the Working Group considered that this hypothesis was not corroborated by enough evidence. In addition, the Working Group noted that the exact dose applied on the epidermis in the rat study was not clearly reported.]

(b) Distribution

White outbred male rats intraperitoneally injected with 6.7 mmol/kg body weight of radiolabelled butyl methacrylate ([1-^{14}C-butyl] methacrylate) showed radioactivity in the liver, kidney, heart, brain, and plasma, with the highest levels in the liver and kidney and the lowest levels in the brain (Svetlakov et al., 1989). The highest levels of radioactivity were reached in 2 hours and were sustained for 12 hours. [The Working Group noted that the strain of rat was not given.]

(c) Metabolism

ECHA (2022b) reported that *n*-butyl methacrylate [butyl methacrylate] is rapidly hydrolysed by carboxylesterases found in tissues. The half-life of *n*-butyl methacrylate was about 8 minutes, and 99.7% was removed by first-pass metabolism in the rat liver. Butyl methacrylate was completely metabolized to methacrylic acid in the rat skin (Jones, 2002). [The Working Group noted that it is unclear which reference(s) in ECHA (2022b) were used for these data.]

Kotlovskiĭ et al. (1985, 1987, 1988) conducted a series of studies investigating the effects of butyl methacrylate on liver microsomes from rats. Butyl methacrylate interacted with the haemoprotein of liver microsomes obtained from white outbred male rats, with an absorption maximum at 388 nm and minimum at 421–425 nm. [The Working Group noted that the rat strains were not reported.] Butyl methacrylate also stimulated oxygen consumption by liver microsomes (Kotlovskiĭ et al., 1985). In liver microsomes obtained from phenobarbital-induced rats, cytochrome P450 was inactivated only in the presence of butyl methacrylate and NADPH (nicotinamide adenine dinucleotide phosphate, reduced form), but butyl methacrylate alone did not inactivate cytochrome P450. Inactivation of P450 did not occur in vivo. [The Working Group noted that the rat strains were not reported.] A minor subfraction (48 kD) of microsomal protein was reduced by butyl methacrylate (Kotlovskiĭ et al., 1987). [The Working Group noted that the molecular weight standard was used, but no positive control was mentioned and no western blot images were shown.] In liver microsomes isolated from control Wistar rats, butyl methacrylate was hydrolysed to butanol at a rate of 55 ± 11 nmol butanol/1 mg protein per minute (Kotlovskiĭ et al., 1988). [The Working Group noted that sex was not mentioned.] The enzymatic nature of

the alcohol formation reaction was confirmed by the fact that preliminary incubation of rat liver microsomes at 100 °C for 3 minutes prevented the appearance of butanol. [The Working Group noted that the strain of rat was mentioned only in the 1988 study.]

[The Working Group acknowledged that methacrylic acid is a possible metabolite of butyl methacrylate; however, there is not enough evidence to show the metabolic pathway of methacrylic acid in mammalian species in vivo.]

4.2. Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether butyl methacrylate is genotoxic; induces oxidative stress; or induces chronic inflammation. No data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Three studies in experimental systems were available to the Working Group (Waegemaekers & Bensink, 1984; Zeiger et al., 1987; Fediukovich et al., 1988).

Waegemaekers & Bensink (1984) assessed the mutagenicity of 27 acrylate esters, including butyl methacrylate, in the *Salmonella* microsome assay. None of these acrylate esters were mutagenic in the standard Ames assay with TA1535, TA1537, TA1538, TA98, and TA100, both with and without Aroclor 1254-induced or phenobarbital-induced S9 microsomes mix.

Zeiger et al. (1987) reported the results and data from the testing of 255 chemicals for their ability to induce mutations in *Salmonella*. All chemicals were tested, in the presence or absence of liver S9 microsomes from Aroclor-induced male Sprague-Dawley rats and Syrian hamsters, in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and/or TA97. The test result for butyl methacrylate was negative.

Fediukovich et al. (1988) reported that butyl methacrylate failed to induce chromosomal aberrations in rat bone marrow cells. The lack of chromosomal aberrations in vivo suggests that butyl methacrylate does not induce changes in chromosome structure or number.

In the European Chemicals Agency (ECHA) brief profile (ECHA, 2022a), butyl methacrylate is listed as negative regarding genetic toxicity because no adverse effects were observed in vitro or in vivo. [The Working Group had no access to original studies from the profile summary, only to the ECHA conclusion. Overall, the Working Group concluded that the studies available do not support the genotoxicity of butyl methacrylate.]

4.2.2 Induces oxidative stress

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

One study in vitro was available to the Working Group (McCarthy et al., 1994). In this study, the reactivity of several acrylates with glutathione was investigated (reactivity with deoxyribonucleosides was tested only for ethyl acrylate) using glutathione or rat erythrocytes followed by the measurement of free thiol. In the cell-free system, butyl methacrylate did not react with glutathione. Butyl methacrylate was not tested in erythrocytes. [The Working Group noted that the decreased reactivity of methacrylates may be due to a combination of electronic and steric factors introduced by the α-methyl group. The

quality of the study was considered acceptable. This study did not support the hypothesis that butyl methacrylate induces oxidative stress.]

4.2.3 Induces chronic inflammation

(a) Humans

One original study in exposed humans (Raymond, 1996) and one review article on methyl methacrylate and respiratory sensitization (Borak et al., 2011), which also mentioned butyl methacrylate, were available to the Working Group.

Raymond (1996) reported results of a case-series study and a cross-sectional study in technicians repeatedly exposed to facsimile machine fumes and suggested a link between exposure to butyl methacrylate-bearing facsimile fumes and inflammation. In the cross-sectional study, all technicians who had daily contact with facsimile machine fumes (0.14–0.40 mg/m^3 of air) had increased serum immunoglobulin E (IgE) levels (mean ± standard error of the mean, 202 ± 69 U/mL; normal, < 41 U/mL) compared with administrative and sales staff members. IgE and fume levels were positively correlated (r = 0.83). In addition, exposure to fumes caused lung crackles in four of six technicians who were evaluated, whereas the technicians who were not exposed had no crackles. [The Working Group noted that the crackles suggested that butyl methacrylate fumes may have caused inflammation in terminal airway units.] In the case-series study, respiratory reactions and increased levels of blood immunoglobulins (IgE, IgM) among workers with repeated exposure to such airborne emissions of facsimile machine fumes were reported. Exposed workers reported sore throat, fever, lymphadenopathy, chest tightness, dry cough, and dyspnoea, which improved after reassignment. Although chest radiographs were normal, some workers had lung crackles and spirometric abnormalities. Reassignment away from the exposure was followed by improvement of most abnormalities. In a follow-up observation after withdrawal of butyl methacrylate-containing paper, 15 of 32 technicians had increased serum concentrations of total IgE at the time of their initial evaluation. A full set of four serial IgE determinations was available in 10 of these 15 technicians; the final mean value after 21 months of follow-up was lower than both the initial mean and the maximal value ($P < 0.05$).

[The Working Group noted that, because of its limited volatility, butyl methacrylate was associated with a low inhalation toxicity to the lung; however, when it is used as a component of electrosensitive paper, as in the facsimile process, butyl methacrylate could be given off as a fume.] Fume concentrations were evaluated gravimetrically; however, the levels of butyl methacrylate were not evaluated. [The Working Group noted that the authors referred to a previous, unpublished analysis of these emissions from the facsimile machines, which had shown that butyl methacrylate comprised about one third of the vapour phase and more than two thirds of the particulate phase of freshly generated fumes. However, this was considered insufficient to determine exposure variability, because no exposure monitoring specific to butyl methacrylate was performed (see also the exposure assessment review and critique in Section 1, Table S1.3, in Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/631). The Working Group also noted that the study by Raymond (1996) had several limitations, as also discussed by the authors, including a small number of exposed subjects and no pre-exposure physical examinations.]

Methyl methacrylate is a respiratory irritant and dermal sensitizer, whereas its respiratory sensitization potential remains controversial; occupational asthma has been reported in a small number of case reports (Borak et al., 2011). Concerning butyl methacrylate, in the review of Borak et al. (2011), a case study of occupational

asthma and allergic rhinitis due to xerographic toner was reported (Wittczak et al., 2003). The case involved a female secretary aged 44 years who had a 2-year history of rhinorrhoea, dyspnoea, and coughing attacks that occurred 15–20 minutes after making photocopies using xerographic toner containing "polystyrene-*n*-butyl methacrylate, polystyrene-*n*-butyl acrylate, etc.".

[The Working Group noted that these studies may suggest the involvement of butyl methacrylate in the chronic lung inflammatory response under certain circumstances, because health conditions improved after workers were reassigned. However, no evidence of unresolved inflammation supporting persistence of the effect was reported.]

(b) Experimental systems

The Japan Bioassay Research Center conducted 13-week dose-finding inhalation toxicity studies (JBRC, 2015a, b, c, d) of butyl methacrylate in mice and rats for a 104-week carcinogenicity study.

In a dose-finding study for a carcinogenicity test, groups of 10 male and 10 female B6D2F$_1$/Crlj mice (age, 6–7 weeks) were treated with butyl methacrylate (purity, 99.8%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 13 weeks, at concentrations of 0 (clean air, control), 31, 63, 125, 250, and 500 ppm (JBRC, 2015a, b).

After a 13-week exposure period, one male mouse exposed to 31 ppm accidently died. In histopathology, there was no significant increase in findings suggesting chronic inflammation, whereas regeneration, atrophy, necrosis and eosinophilic change of olfactory epithelium, respiratory metaplasia of gland, and eosinophilic change of respiratory epithelium of nasal cavity were observed in male and female mice exposed to 500 ppm. Similar alterations were found to decrease in male mice exposed to 63 ppm and female mice exposed to 31 ppm.

In a dose-finding study for a carcinogenicity test, groups of 10 male and 10 female F344/DuCrlCrlj rats (age, 6–7 weeks) were treated with butyl methacrylate (purity, 99.8%) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for 13 weeks, at concentrations of 0 (clean air, control), 63, 125, 250, 500, and 1000 ppm (JBRC, 2015c, d). There was a significant decrease in absolute and relative weights of the thymus in the group of male rats exposed to 1000 ppm. In histopathology, there was no significant increase in findings suggesting chronic inflammation, whereas epithelial cell degeneration and regeneration in response to stimulation of the nasal cavity were observed. Inflammation of the nasal cavity was observed in a small number of females, but it was not statistically significant compared with controls. [The Working Group noted that in well-conducted studies in experimental animals, butyl methacrylate has shown some nasal irritation (eosinophilic change) and alterations of nasal epithelium, which, however, did not result in a tumour in the nasal cavity in 104-week carcinogenicity studies of mice and rats. See also Sections 3.1 and 3.2.]

4.3 Evaluation of high-throughput in vitro toxicity screening data

Butyl methacrylate was tested in high-throughput toxicity screening assays under the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2019). Chemical samples were procured at high purity, prepared in dimethyl sulfoxide stock solutions at a concentration of about 20 mM, and tested over a period of several years in biochemical and cellular bioassays measuring a wide variety of biological end-points. In addition, chemical analysis of the samples was done in high-throughput fashion at an early and a late stage of the sample testing lifetime, as

described in Tice et al. (2013). Data on testing results from the concentration–response testing design for all end-points were analysed for significant activity, and an active/inactive "hit call" was made for each response, together with a potency value (Filer et al., 2017). For all active calls, individual concentration–response curves were examined to ensure that biologically meaningful activity was detected. Bioassay end-points were mapped, where possible, to the key characteristics of carcinogens using the "kc-hits" software (the key characteristics of carcinogens – high-throughput screening discovery tool, available from https://gitlab.com/i1650/kc-hits; Reisfeld et al., 2022) to aid in providing mechanistic insights (Chiu et al., 2018). The detailed results are available in the supplementary material for this volume (Annex 2, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/631) and are briefly summarized below.

The testing results for butyl methacrylate high-throughput toxicity in the CompTox Chemicals Dashboard encompassed 235 assay end-points, of which 111 were mapped to the key characteristics of carcinogens. The cytotoxicity limit based on a panel of cellular cytotoxicity and viability assays was estimated to be > 1 mM (US EPA, 2022). Only 4 of the mapped end-points indicated positive results, and all were flagged with multiple curve-fitting warnings. [The Working Group did not consider these to be biologically relevant responses.]

The analysis of a stock solution of butyl methacrylate in dimethyl sulfoxide showed the presence of the parent compound at both an early and a late time point in the solution lifetime, although the concentration was listed as 5–30% of expected (NIH, 2022).

5. Summary of Data Reported

5.1 Exposure characterization

Butyl methacrylate is a High Production Volume chemical that is used to create polymers in a variety of products worldwide. It is used in coatings, polyvinyl chloride plastics, polypropylene non-woven materials, glues, caulks or other sealants, inks and paints, pesticides, and health-care materials, among others.

Occupational exposures may occur in the manufacture of chemicals (including butyl methacrylate); the manufacture of paints, coatings, adhesives, and plastics; construction; furniture manufacturing; textile manufacturing; printing and publishing; maritime vessel repair; health and dental care; and personal-care services. The highest exposures were found in paint and adhesive manufacturing. Exposure can occur via all routes, but inhalation is considered the most significant. Seven countries have established limits for occupational exposure to butyl methacrylate in air. Exposure of workers younger than 18 years is restricted in the European Union. Biomonitoring methodologies have not been established.

For the general population, exposure can occur via contaminated air and water, via food contained in butyl methacrylate-containing plastics, and in personal-care and health-care products. Butyl methacrylate has been measured in nail polishes and lacquers and in dental and joint replacement polymers. However, few exposure measurement data for the general population were available. A limit on the migration of methacrylate acids into food from plastic containers has been set by the European Commission.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with butyl methacrylate caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species (mouse and rat).

Butyl methacrylate was administered by inhalation in one study that complied with Good Laboratory Practice in male and female B6D2F_1/Crl mice. In males, butyl methacrylate caused an increase in the incidence of hepatocellular adenoma or carcinoma (combined) of the liver and histiocytic sarcoma of all sites. In females, butyl methacrylate caused an increase in the incidence of haemangiosarcoma of all sites.

Butyl methacrylate was administered by inhalation in one study that complied with Good Laboratory Practice in male and female F344/DuCrlCrlj rats. In males, butyl methacrylate caused an increase in the incidence of mononuclear cell leukaemia of the spleen. In females, butyl methacrylate caused an increase in the incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland.

5.4 Mechanistic evidence

The available data on absorption, metabolism, and excretion of butyl methacrylate in humans are scarce. Only one study on the absorption of butyl methacrylate in humans was available; this study demonstrated dermal absorption in human epidermis samples in an in vitro system. Skin absorption was shown in one in vitro study using epidermis and whole skin of rats. Butyl methacrylate was distributed to the liver, kidney, heart, brain, and plasma of rats. Butyl methacrylate was shown to be hydrolysed to butanol in rat liver microsomes in one study and to methacrylic acid in a second study in rat skin in vitro. No studies on the excretion of butyl methacrylate in rodents were available.

Few mechanistic data were available for butyl methacrylate regarding the key characteristics of carcinogens "is genotoxic", "induces oxidative stress", and "induces chronic inflammation".

There were no mechanistic studies in humans with exposure specifically attributable to butyl methacrylate only.

Regarding genotoxicity, butyl methacrylate did not induce chromosomal aberrations in rat bone marrow cells. In addition, butyl methacrylate gave negative results for gene mutagenicity in the presence and absence of metabolic activation in two well-conducted studies using the Ames assay with various *Salmonella typhimurium* strains.

Butyl methacrylate did not induce oxidative stress in one study in a cell-free system in which butyl methacrylate did not react with glutathione.

There was one study in workers repeatedly exposed for up to 18 months to facsimile machine fumes containing butyl methacrylate; it showed increased levels of blood immunoglobulins (IgE, IgM) and respiratory symptoms (sore throat, chest tightness, dry cough, and dyspnoea). After task reassignment or substitution of butyl methacrylate-free paper, the levels of IgE decreased but remained higher than normal levels. In addition, there was evidence of inflammation in one report of occupational asthma and rhinitis after exposure to facsimile machine fumes. These data could indicate the involvement of butyl methacrylate in chronic lung inflammation.

No data were available regarding butyl methacrylate and the other key characteristics of carcinogens.

Butyl methacrylate was found to be mostly without effects relevant to the key characteristics of carcinogens in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA,

although the butyl methacrylate testing solution was considered problematic for use in high-throughput assays.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of butyl methacrylate.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of butyl methacrylate.

6.3 Mechanistic evidence

There is *inadequate* mechanistic evidence.

6.4 Overall evaluation

Butyl methacrylate is *possibly carcinogenic to humans (Group 2B).*

6.5 Rationale

The Group 2B evaluation for butyl methacrylate is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in males and females of two species (rat and mouse) in two studies that complied with Good Laboratory Practice. The evidence regarding cancer in humans was *inadequate*, because no studies were available. There was also *inadequate* mechanistic evidence.

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DIMETHYL HYDROGEN PHOSPHITE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 868-85-9 (IARC, 1990; Royal Society of Chemistry, 2022)

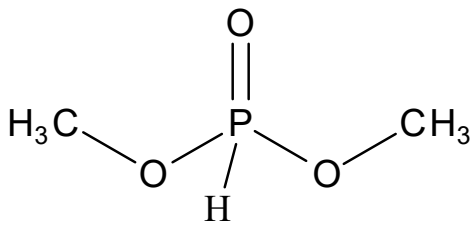
EC/List No.: 212-783-8 (ECHA, 2022)

IUPAC systematic name: dimethyl phosphonate (IARC, 1990; Royal Society of Chemistry, 2022)

Synonyms: dimethyl acid phosphite; phosphonic acid, dimethyl ester; dimethyl phosphite; bis(hydroxymethyl)phosphine oxide; DMHP; TL 585; hydrogen dimethyl phosphite; *O,O*-dimethyl phosphonate; dimethoxyphosphine oxide; phosphorous acid dimethyl ester (IARC, 1990; NCBI, 2022; Royal Society of Chemistry, 2022).

1.1.2 Structural and molecular information

Chemical structure:



Molecular formula: $C_2H_7O_3P$ (IARC, 1990; Royal Society of Chemistry, 2022)

Relative molecular mass: 110.05 (IARC, 1990).

1.1.3 Chemical and physical properties

Description: colourless liquid with mild odour (IARC, 1990)

Boiling-point: 170–171 °C (IARC, 1990; Royal Society of Chemistry, 2022)

Melting-point: less than −60 °C (OECD, 2004; Royal Society of Chemistry, 2022)

Flash-point: 70 °C at 101.3 kPa (OECD, 2004; ECHA, 2022; Royal Society of Chemistry, 2022)

Density: 1.2 g/mL (IARC, 1990; Royal Society of Chemistry, 2022)

Vapour pressure: 1.35 hPa at 20 °C (IFA, 2022a)

Solubility: soluble in water; miscible with most organic solvents (IARC, 1990)

Octanol/water partition coefficient (P): log K_{ow} = −1.2 (OECD, 2004; Royal Society of Chemistry, 2022)

Stability: hydrolyses in water with a half-life of ~10 days at 25 °C and 19 days at 20 °C; basic conditions accelerate hydrolysis (IARC, 1990).

[The Working Group used a conversion factor of 1 ppm ≈ 4.5 mg/m³ at 20 °C and 1.013 hPa (IARC, 1990).]

1.1.4 Technical grade and impurities

Dimethyl hydrogen phosphite of high purity (~99%) is available commercially from several vendors. Trace levels of monomethyl phosphonate, dimethyl methyl phosphonate, trimethyl phosphate, and methanol have been reported in the technical product (OECD, 2004; IARC, 1990).

1.2 Production and use

1.2.1 Production process

Dimethyl hydrogen phosphite is manufactured by the reaction of phosphorous trichloride with methanol or with sodium methoxide (IARC, 1990). Methyl chloride can be used as a catalyst (NCBI, 2022). It can also be synthesized by heating diethyl phosphite in methanol (Balint et al., 2013).

1.2.2 Production volume

The global production capacity for dimethyl hydrogen phosphite was estimated to be 3000–15 000 tonnes for about 10 producers in 2002, with an estimated production of 1000–5000 tonnes in each of western Europe, USA, and the rest of the world (OECD, 2004).

Dimethyl hydrogen phosphite is listed as a High Production Volume chemical; more than 1 million pounds [450 tonnes] were produced or imported into the USA in 1990 and 1994. The aggregated production volume of this chemical in the USA was reported as 500 000 to 1 million pounds [230–450 tonnes] in 2013 and 2014 (US EPA, 2016), 1 to 10 million pounds [450–4500 tonnes] in 2016 and 2017, and 1 to 2 million pounds [4500–9100 tonnes] in 2018 and 2019 (NCBI, 2022).

Since 2002, dimethyl hydrogen phosphite has been manufactured on an industrial scale in western Europe only at a single chemical plant in Leverkusen, Germany (OECD, 2004). Dimethyl hydrogen phosphite is also produced in China (Chemical Book, 2022). [The Working Group noted that the number of manufacturers varies according to different sources.]

1.2.3 Uses

Dimethyl hydrogen phosphite is used as an intermediate in the manufacture of adhesives, lubricants, organophosphate pesticides, and herbicides (such as glyphosate), as a stabilizer in oil and plaster, as a steel corrosion inhibitor in combination with pyrocatechol, and in pharmaceuticals (α-aminophosphonates, which are medicinally important phosphorus analogues of amino acids) (OECD, 2004; Varga & Keglevich, 2021; NCBI, 2022).

Dimethyl hydrogen phosphite is used as a reactive flame retardant (in combination with guanidine and formaldehyde) in textile finishing (IARC, 1990; OECD, 2004). It is also used to increase fire resistance in cellulosic textiles, acrolein-grafted polyamide fibres, and gamma-irradiated polyethylene (NCBI, 2022).

Dimethyl hydrogen phosphite can be converted by chemical synthesis to nerve gases (it is a schedule 3B precursor to dimethyl methylphosphonate) (OECD, 2004; OPCW, 2023).

1.3 Detection and quantification

Capillary gas chromatography-flame ionization detection (GC-FID) has been applied to the analysis of dimethyl hydrogen phosphite in aqueous solutions under simulated physiological conditions. The method had a linear calibration curve over a range of 10 to 1000 ng. High-performance liquid chromatography (HPLC) coupled with radioactivity detection was also used to analyse dimethyl hydrogen phosphite

and its degradation products. (Nomeir et al., 1988; IARC, 1990).

More recently, a gas chromatography-mass spectrometry (GC-MS) method was reported for the analysis of dimethyl hydrogen phosphite sprayed over indoor dust particles in a controlled laboratory experiment. The mass spectrometer was operated in selected-ion monitoring (SIM) mode with electron impact ionization (EI) (Favela et al., 2012).

[The Working Group noted that dimethyl hydrogen phosphite has been measured in the air in a flame-retardant manufacturing plant (see Section 1.4.2), but no information was available on the analytical method used.] There were no data available on the use of these or other methods for the detection and quantification of dimethyl hydrogen phosphite in human tissues.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

No data on environmental occurrence were available to the Working Group.

1.4.2 Occupational exposure

Given the fact that dimethyl hydrogen phosphite has been used as a flame retardant on nylon 6 fibres, as a chemical intermediate in the production of pesticides and in lubricant additives and adhesives (IARC, 1990), workers engaged in manufacturing these products are expected to have been exposed. The National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES) of 1981–1983 estimated that chemical technicians were potentially exposed to dimethyl hydrogen phosphite in the USA [1822 exposed workers were reported; the Working Group estimated a confidence interval of 1184–2460] (NIOSH, 1983).

Data on occupational exposure levels were available from a study published in 1985 (US EPA, 1985). The study in which air monitoring of dimethyl hydrogen phosphite was conducted concerned a manufacturing facility where flame retardants were produced in Charleston, South Carolina, USA. The maximum partial-shift worker exposure was 1.9 ppm [8.6 mg/m^3], and the average exposure was 0.22 ppm [0.99 mg/m^3] (measurement duration, 3–4 hours). For the 8-hour time-weighted average (TWA), these levels were estimated to be 1.1 ppm [4.95 mg/m^3] and 0.16 ppm [0.72 mg/m^3], respectively.

1.4.3 Exposure of the general population

No data on exposure of the general population (including biomonitoring levels) were available to the Working Group.

1.5 Regulations and guidelines

A quantitative limit for exposure to dimethyl hydrogen phosphite occurring in the workplace was found only for Romania (8-hour limit value of 12 mg/m^3) (IFA, 2022a). The United States Environmental Protection Agency (US EPA) has derived acute exposure guideline levels (AEGLs) that are used by emergency planners and responders as guidance in dealing with rare, usually accidental, releases of chemicals into the air (US EPA, 2022a). For dimethyl hydrogen phosphite, interim AEGLs have been available since 2010 (US EPA, 2010) (see Table 1.1).

Because dimethyl hydrogen phosphite can potentially be used in the production of nerve gas, production and export are stringently controlled under the Wassenaar Arrangement, which was signed by 42 countries (Wassenaar Arrangement Secretariat, 2022).

Table 1.1 Acute exposure guideline levels for airborne dimethyl hydrogen phosphite, proposed by the US EPA

Classification	10 minutes	30 minutes	60 minutes	4 hours	8 hours
AEGL-1 Notable discomfort, irritation, or certain asymptomatic non-sensory effects; however, the effects are not disabling and are transient and reversible upon cessation of exposure	Not recommended[a]	Not recommended[a]	Not recommended[a]	Not recommended[a]	Not recommended[a]
AEGL-2 Irreversible or other serious, long-lasting adverse health effects, or an impaired ability to escape	120 ppm (540 mg/m^3)	120 ppm (540 mg/m^3)	95 ppm (430 mg/m^3)	60 ppm (270 mg/m^3)	39 ppm (180 mg/m^3)
AEGL-3 Life-threatening health effects or death	190 ppm (850 mg/m^3)	190 ppm (850 mg/m^3)	150 ppm (670 mg/m^3)	96 ppm (430 mg/m^3)	63 ppm (280 mg/m^3)

AEGL, acute exposure guideline level; ppm, parts per million; US EPA, United States Environmental Protection Agency.
[a] Not recommended due to insufficient data.
Data from US EPA (2022a).

According to the European Globally Harmonized System Classification and Labelling of Chemicals (GHS), dimethyl hydrogen phosphite is classified as "suspected of causing genetic effects" (H341) and "suspected of causing cancer" (H351) (both in Hazard Category 2) (IFA, 2022b).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

In previous evaluations, the *IARC Monographs* programme concluded that there was *limited evidence* in experimental animals for the carcinogenicity of dimethyl hydrogen phosphite (IARC, 1990, 1999).

Studies of carcinogenicity with dimethyl hydrogen phosphite in experimental animals are summarized in Table 3.1.

3.1 Mouse

In a well-conducted study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6–8 weeks) were treated with dimethyl hydrogen phosphite (purity, 97–98%; impurity, 1% trimethyl phosphate) at a dose of 0 (vehicle control-corn oil only), 100, or 200 mg/kg body weight (bw) per day by daily gavage in corn oil (dosing volume, 4.0 mL/kg) 5 days per week for 103 weeks (NTP, 1985; also reported by Dunnick et al., 1986). At study termination, survival was 42/50, 33/50, and 32/50 in males, and 39/50, 37/50, and 34/50 in females, for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. There was a trend for decreased survival in the males (P = 0.018, life-table trend test) with the survival rate being significantly lower (P = 0.029, life-table test) at the higher dose than in vehicle controls. No differences in survival were observed in treated female mice compared with vehicle controls. Body weights of male mice at the higher dose ranged from 5% to 10% lower than those of mice in the vehicle control group between 28 weeks

Table 3.1 Studies of carcinogenicity in mice and rats exposed to dimethyl hydrogen phosphite

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, $B6C3F_1$ (M) 6–8 wk 103 wk NTP (1985)	Oral administration (gavage) Purity, 97–98% (impurity, trimethyl phosphate, 1%) Corn oil 0, 100, 200 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 42, 33, 32	No significant increase in tumour incidence in treated animals		*Principal strengths*: GLP study; males and females used; covered most of the lifespan; adequate number of animals per group; high quality of gross descriptions and microscopic examinations; multiple-dose study; appropriate statistics. *Other comments*: male mice at the higher dose had significantly lower survival.
Full carcinogenicity Mouse, $B6C3F_1$ (F) 6–8 wk 103 wk NTP (1985)	Oral administration (gavage) Purity, 97–98% (impurity, trimethyl phosphate, 1%) Corn oil 0, 100, 200 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 39, 37, 34	*Liver* Hepatocellular adenoma 0/50, 6/49* (12%), 3/50 (6%)	*P = 0.016, incidental tumour and life-table tests; P = 0.012, Fisher exact test NS, Cochran–Armitage trend test, incidental tumour and life-table trend tests	*Principal strengths*: adequate number of animals per group; high quality of gross descriptions and microscopic examinations; multiple-dose study; appropriate statistics; GLP study; males and females used; covered most of the lifespan. *Historical controls*: hepatocellular adenoma: laboratory, 4/148 (2.7% ± 2.4%; range, 0–4%); NTP studies, 47/1176 (4.0% ± 2.6%; range, 0–10%); hepatocellular adenoma or carcinoma (combined): laboratory, 7/148 (4.7% ± 3.0%; range, 2–8%); NTP studies, 80/1176 (6.8% ± 3.4%; range, 2–14%).
		Hepatocellular carcinoma 2/50, 0/49, 0/50	NS	
		Hepatocellular adenoma or carcinoma (combined) 2/50, 6/49, 3/50	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7 wk 103 wk NTP (1985)	Oral administration (gavage) Purity, 97–98% (impurity, trimethyl phosphate, 1%) Corn oil 0, 100, 200 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 39, 29, 23	*Lung* Bronchioloalveolar adenoma 0/50, 0/50, 5/50*	$P = 0.004$, life-table trend test; $P = 0.017$, incidental tumour trend test; $P = 0.006$, Cochran–Armitage trend test *$P = 0.018$, life-table test; *$P = 0.028$, Fisher exact test; NS, incidental tumour test	*Principal strengths*: GLP study; males and females used; covered most of the lifespan; adequate number of animals per group; multiple-dose study; high quality of gross descriptions and microscopic examinations; appropriate statistics. *Other comments*: male rats at the higher dose had significantly lower survival. *Historical controls*: lung bronchioloalveolar adenoma: laboratory, 2/150 (1.3% ± 1.2%; range, 0–2%); NTP studies, 34/1143 (3.0% ± 1.9%; range, 0–6%); lung bronchioloalveolar carcinoma: laboratory, 3/150 (2.0% ± 0.0%; range, NR); NTP studies, 16/1143 (1.4% ± 1.5%; range, 0–6%); lung bronchioloalveolar adenoma or carcinoma (combined): laboratory, 5/150 (3.3% ± 1.2%; range, 2–4%); NTP studies, 50/1143 (4.4% ± 2.4%; range, 0–8%); lung squamous cell carcinoma: laboratory, 0%; NTP studies, 2/1143 (0.2% ± 0.58%; range, 0–2%); forestomach squamous cell papilloma: laboratory, 0/147; NTP studies, 2/1114 (0.002%) [range, NR]; forestomach squamous cell carcinoma: laboratory, 0/147, NTP studies, 0/1114 [range, NR]; forestomach squamous cell papilloma or carcinoma (combined): laboratory, 0/147; NTP studies, 2/1114 (0.002%) [range, NR].
		Bronchioloalveolar carcinoma 0/50, 1/50, 20/50*	$P < 0.001$, life-table trend test; $P < 0.001$, incidental tumour trend test; $P < 0.001$, Cochran–Armitage trend test *$P < 0.001$, life-table test; *$P < 0.001$, incidental tumour test; *$P < 0.001$, Fisher exact test	
		Bronchioloalveolar adenoma or carcinoma (combined) 0/50, 1/50, 24/50*	$P < 0.001$, life-table trend test, $P < 0.001$, incidental tumour trend test; $P < 0.001$, Cochran–Armitage trend test *$P < 0.001$, life-table test; *$P < 0.001$, incidental tumour test; *$P < 0.001$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7 wk 103 wk NTP (1985) (cont.)		Squamous cell carcinoma		
		0/50, 0/50, 5/50*	*P* = 0.004, life-table trend test; *P* = 0.034, incidental tumour trend test; *P* = 0.006, Cochran–Armitage trend test **P* = 0.020, life-table test; **P* = 0.028, Fisher exact test; NS, incidental tumour test	
		Forestomach		
		Squamous cell papilloma		
		0/50, 1/50 (2%), 3/50 (6%)	*P* = 0.032, life-table trend test; *P* = 0.052, incidental tumour trend test; *P* = 0.037, Cochran–Armitage trend test NS, life-table test, incidental tumour test, Fisher exact test	
		Squamous cell carcinoma		
		0/50, 0/50, 3/50 (6%)	*P* = 0.023, life-table trend test; NS, incidental tumour trend test; *P* = 0.037, Cochran–Armitage trend test NS, life-table test, incidental tumour test, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7 wk 103 wk NTP (1985) (cont.)		Squamous cell papilloma or carcinoma (combined) 0/50, 1/50, 6/50*	*P* = 0.002, life-table trend test; *P* = 0.006, incidental tumour trend test; *P* = 0.005, Cochran–Armitage trend test **P* = 0.006, life-table test; **P* = 0.025, incidental tumour test; **P* = 0.013, Fisher exact test	
Full carcinogenicity Rat, F344/N (F) 7 wk 103 wk NTP (1985)	Oral administration (gavage) Purity, 97–98% (impurity, trimethyl phosphate, 1%) Corn oil 0, 50, 100 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 40, 33, 32	*Lung* Bronchioloalveolar carcinoma 0/50, 1/49, 3/50 (6%)	*P* = 0.047, life-table and incidental tumour trend tests; NS, Cochran–Armitage trend test NS, life-table test, incidental tumour test, Fisher exact test	*Principal strengths*: GLP study, studies in both males and females, covers most of the lifespan, adequate number of animals per group, multiple dose study, high quality of gross descriptions and microscopic examinations, appropriate statistics. *Historical controls*: lung bronchioloalveolar carcinoma: laboratory, 1/150 (0.7% ± 1.2%; range, 0–2%); NTP studies, 10/1142 (0.9% ± 1.3%; range, 0–4.2%); forestomach squamous cell papilloma or carcinoma (combined): NR.
		Forestomach Squamous cell papilloma 0/50, 0/50, 1/48	[NS]	
		Squamous cell carcinoma 0/50, 0/50, 1/48	[NS]	
		Squamous cell papilloma or carcinoma (combined) 0/50, 0/50, 2/48	[NS]	

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NR, not reported; NS, not significant; NTP, National Toxicology Program; wk, week(s).

and study termination. Body weights of treated females were similar to those of mice in the vehicle control group throughout the study. The results of histopathological examination were reported for all major tissues and gross lesions.

In female mice, the incidence of hepatocellular adenoma was 0/50, 6/49 (12%), and 3/50 (6%) for the groups for the groups at 0 (control), 100, and 200 mg/kg bw, respectively, and was significantly increased (P = 0.012, Fisher exact test; P = 0.016, life-table test; P = 0.016, incidental tumour test) in the group at the lower dose, exceeding the upper bound of the range observed in historical controls from this laboratory – 4/148 (2.7 ± 2.4%); range, 0–4.0% – and from National Toxicology Program (NTP) studies – 47/1176 (4.0 ± 2.6%); range, 0–10%. The incidence of hepatocellular adenoma or carcinoma (combined) was 2/50 (4%), 6/49 (12%), and 3/50 (6%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. At the lower dose, the incidence exceeded the upper bound of the range observed in historical controls from this laboratory – 7/148 (4.7 ± 3.0%); range, 2–8% – but not from the concurrent NTP studies – 80/1176 (6.8 ± 3.4%); range, 2–14%. [The Working Group noted that hepatocellular carcinoma was observed in two animals in the control group and in none of the treated animals.] In male mice, there were no significant treatment-related effects on the incidence of any tumour.

For both male and female mice, there were no increases in the incidence of non-neoplastic lesions at sites at which tumour incidence was considered to be increased.

[The Working Group noted that this was a well-conducted study that complied with GLP, both sexes were used, the duration of exposure and observation was adequate, an adequate number of animals per group was used, and the descriptions of gross and microscopic examinations were of high quality. The Working Group noted that the impurity trimethyl phosphate had been reported to show clear evidence of carcinogenicity in male Fischer 344 rats (NCI, 1978). However, the Working Group considered that the presence of 1% trimethyl phosphate did not significantly contribute to the results of the present study.]

3.2 Rat

In a well-conducted study of chronic toxicity and carcinogenicity that complied with GLP, groups of 50 male and 50 female F344/N rats (age, about 7 weeks) were treated by gavage with dimethyl hydrogen phosphite (purity, 97–98%; impurity, 1% trimethyl phosphate; mixed in corn oil). Male rats were given doses of 0 (vehicle control, corn oil only), 100, or 200 mg/kg bw per day (dosing volume, 4.0 mL/kg), 5 days per week for 103 weeks (NTP, 1985; also reported by Dunnick et al., 1986). Female rats were given doses of 0 (vehicle control, corn oil only), 50, or 100 mg/kg bw per day under similar conditions. At study termination, survival was 39/50, 29/50, and 23/50 in males, and 40/50, 33/50, and 32/50 in females, in the control group and groups at the lower and higher dose, respectively; the survival in males at the higher dose was significantly lower (P = 0.008, life-table test) than that in vehicle controls, and there was a significant trend observed (P = 0.009, life-table trend test). Decreased survival in treated male rats was considered to be attributable to an increase in the incidence of chronic interstitial pneumonia. In treated females, survival was not significantly affected. Mean body weights of male rats at the higher dose were observed to be 10–15% lower than those of the vehicle controls from 24 weeks to the end of the study. Mean body weights of male rats at the lower dose and female rats at the lower and higher dose were similar to those of the vehicle controls. The results of histopathological examination were reported for all major tissues and gross lesions.

In male rats, there was a significant positive trend (P = 0.004, life-table trend test; P = 0.017, incidental tumour trend test; P = 0.006, Cochran–Armitage trend test) in the incidence of bronchioloalveolar adenoma: 0/50, 0/50, and 5/50 (10%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence of bronchioloalveolar adenoma was significantly increased at the higher dose (P = 0.018, life-table test; P = 0.028, Fisher exact test) and exceeded the upper bound of the range observed in historical controls from this laboratory – 2/150 (1.3 ± 1.2%); range, 0–2% – and from NTP studies – 34/1143 (3.0 ± 1.9%); range, 0–6%. There was a significant positive trend (P < 0.001, life-table trend test; P < 0.001, incidental tumour trend test; P < 0.001, Cochran–Armitage trend test) in the incidence of bronchioloalveolar carcinoma: 0/50, 1/50 (2%), and 20/50 (40%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence of bronchioloalveolar carcinoma was significantly increased at the higher dose (P < 0.001, life-table test; P < 0.001, incidental tumour test; P < 0.001, Fisher exact test) and exceeded the incidence observed in historical controls from this laboratory – 3/150 (2 ± 0%); range, not reported – and from NTP studies – 16/1143 (1.4 ± 1.5%); range, 0–6%. There was a significant positive trend (P < 0.001, life-table trend test; P < 0.001, incidental tumour trend test; P < 0.001, Cochran–Armitage trend test) in the incidence of bronchioloalveolar adenoma or carcinoma (combined): 0/50, 1/50 (2%), and 24/50 (48%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased at the higher dose (P < 0.001, life-table test; P < 0.001, incidental tumour test; P < 0.001, Fisher exact test) and exceeded the upper bound of the range observed in historical controls from this laboratory – 5/150 (3.3 ± 1.2%); range, 2–4% – and from NTP studies – 50/1143 (4.4 ± 2.4%); range, 0–8%. There was a significant positive trend (P = 0.004, life-table trend test; P = 0.034, incidental tumour trend test; P = 0.006, Cochran–Armitage trend test) in the incidence of squamous cell carcinoma of the lung: 0/50, 0/50, and 5/50 (10%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence of squamous cell carcinoma of the lung was significantly increased at the higher dose (P = 0.020, life-table test; P = 0.028, Fisher exact test) and exceeded the upper bound of the range observed in historical controls from this laboratory (0%) and from NTP studies – 2/1143 (0.2 ± 0.58%); range, 0–2%. There was a significant positive trend (P = 0.032, life-table trend test; P = 0.052, incidental tumour trend test; P = 0.037, Cochran–Armitage trend test) in the incidence of squamous cell papilloma of the forestomach: 0/50, 1/50 (2%), and 3/50 (6%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence of squamous cell papilloma of the forestomach in the groups at the lower and higher dose exceeded the incidence in historical controls from this laboratory (0/147) and from NTP studies – 2/1114 (0.002%). There was a significant positive trend (P = 0.023, life-table trend test; P = 0.037, Cochran–Armitage trend test) in the incidence of squamous cell carcinoma of the forestomach: 0/50, 0/50, and 3/50 (6%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence in the group at the higher dose exceeded the incidence in historical controls from this laboratory (0/147) and from NTP studies (0/1114). There was a significant positive trend (P = 0.002, life-table trend test; P = 0.006, incidental tumour trend test; P = 0.005, Cochran–Armitage trend test) in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach: 0/50, 1/50 (2%), and 6/50 (12%) for the groups at 0 (control), 100, and 200 mg/kg bw, respectively. The incidence of squamous cell papilloma or carcinoma (combined) of the forestomach was significantly increased at the higher dose (P = 0.006, life-table test; P = 0.025, incidental tumour test; P = 0.013, Fisher exact test). The incidence in the groups at

the lower and higher dose exceeded the incidence in historical controls from this laboratory (0/147) and from NTP studies – 2/1114 (0.002%).

In female rats, there was a significant positive trend (P = 0.047, life-table trend test; P = 0.047, incidental tumour trend test) in the incidence of bronchioloalveolar carcinoma: 0/50, 1/49 (2%), 3/50 (6%) for the groups at 0 (control), 50, and 100 mg/kg bw, respectively. The incidence in the group at the higher dose exceeded the upper bound of the range observed in historical controls from this laboratory – 1/150 (0.7 ± 1.15%); range, 0–2% – and from NTP studies – 10/1142 (0.9 ± 1.34%); range, 0–4%.

Regarding non-neoplastic lesions, the incidence of lesions of the lung and forestomach was considered to be treatment-related in males and females. In the lung, the incidence of alveolar epithelial hyperplasia, adenomatous hyperplasia, and chronic interstitial pneumonia was significantly increased in male and female rats at the higher dose. The incidence of squamous metaplasia of the lung was also increased in male rats at the higher dose. In the forestomach, the incidence of hyperplasia and hyperkeratosis was significantly increased in males at the higher dose. The incidence of forestomach hyperplasia was also significantly increased in females at the higher dose. [The Working Group noted that this was a well-conducted study that complied with GLP, both sexes were used, the duration of exposure and observation was adequate, there was an adequate number of animals per group, and the descriptions of gross and microscopic examinations were of high quality. The Working Group noted that the impurity trimethyl phosphate had been reported to show clear evidence of carcinogenicity in male F344 rats (NCI, 1978). However, the Working Group considered that the presence of 1% trimethyl phosphate did not significantly contribute to the results of the present study.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of dimethyl hydrogen phosphite has been assessed in one well-conducted study that complied with GLP in male and female B6C3F$_1$ mice (NTP, 1985; also reported by Dunnick et al., 1986), and in one well-conducted GLP study in male and female F344/N rats (NTP, 1985; also reported by Dunnick et al., 1986) treated by oral administration (gavage).

In the GLP study in male and female B6C3F$_1$ mice treated by gavage (NTP, 1985; also reported by Dunnick et al., 1986), there was a significant increase in the incidence of hepatocellular adenoma in females at the lower dose. In male mice, there were no significant treatment-related effects on the incidence of any tumour.

In the GLP study in male and female F344/N rats treated by gavage (NTP, 1985; also reported by Dunnick et al., 1986), there was a significant positive trend in the incidence of bronchioloalveolar carcinoma, and incidence was significantly increased at the higher dose. There was a significant positive trend in the incidence of bronchioloalveolar adenoma in males, and incidence was significantly increased at the higher dose. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined), and incidence was significantly increased at the higher dose. There was a significant positive trend in the incidence of squamous cell carcinoma of the lung, and incidence was significantly increased at the higher dose. There was a significant positive trend in the incidence of squamous cell papilloma of the forestomach and a significant positive trend in the incidence of squamous cell carcinoma of the forestomach. There was a significant positive trend in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach, and incidence was significantly increased at the higher dose. In female rats, there was a significant positive

trend in the incidence of bronchioloalveolar carcinoma.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on the absorption, distribution, metabolism, and excretion of dimethyl hydrogen phosphite in humans were not available to the Working Group.

4.1.2 Experimental systems

The scientific literature on the absorption, distribution, metabolism, and excretion of dimethyl hydrogen phosphite was limited to one study conducted by the NTP (NTP, 1985; Nomeir & Matthews, 1997). This study consisted of in vivo and in vitro experimental components. Distribution and metabolism data were obtained using radioactivity measurements. Male F344/N rats and male B6C3F$_1$ mice were treated by gastric intubation with [^{14}C]-labelled dimethyl hydrogen phosphite (in corn oil; volume, 4 mL/kg bw) at a dose ranging from 10 to 200 mg/kg bw. Dimethyl hydrogen phosphite was readily and almost entirely (approximately 98%) absorbed in the gastrointestinal tract in both rats and mice, and widely distributed in the tissues of both rodent species. In the rats, 24 hours after administration of a dose of 10, 100, or 200 mg/kg bw, the liver and kidneys contained the highest levels of [^{14}C]-labelled dimethyl hydrogen phosphite equivalents (liver, 8.5 ± 1.0 to 165.0 ± 25.0 µg/g wet tissue; kidney, 6.8 ± 1.3 to 175 ± 40.0 µg/g wet tissue), followed by the forestomach, spleen, small intestine, and lung. The lowest concentrations of [^{14}C]dimethyl hydrogen phosphite equivalents were found in the brain, adipose tissue, muscle, and testes (range, 1.2–25 µg/g wet tissue). The concentrations of dimethyl hydrogen phosphite in tissues were approximately proportional to the administered dose. The pattern of distribution at later time points (2, 5, and 10 days) after administration of a single dose at 200 mg/kg bw was similar to that observed at 24 hours. The rate of clearance at these later time points was markedly decreased compared with that at 24 hours. Concentrations of dimethyl hydrogen phosphite in all tissues increased with the number of daily doses at 200 mg/kg bw (one, two, and five) administered.

The tissue distribution pattern for dimethyl hydrogen phosphite in male mice at 1, 2, and 5 days after administration of a single dose at 200 mg/kg bw was similar to that observed in male rats. Tissue concentrations were substantially lower in mice than in rats. The metabolism of dimethyl hydrogen phosphite in vivo was analysed in urine samples by high-performance liquid chromatography. In rats and mice and at all doses administered, dimethyl hydrogen phosphite was metabolized to monomethyl hydrogen phosphite, which was excreted in the urine. The single methyl group removed during the metabolism of dimethyl hydrogen phosphite to monomethyl hydrogen phosphite was further oxidized to carbon dioxide (CO_2), which was released in the expired air.

The in vitro metabolism of dimethyl hydrogen phosphite was investigated using microsomal fractions from the liver, lung, kidney, forestomach, and glandular stomach of the treated rats. Dimethyl hydrogen phosphite was metabolized to formaldehyde in a concentration-dependent manner. This reaction required the presence of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form). Microsomes from the liver, lung, and kidney all demonstrated a similar level of metabolic activity, which was higher than that of microsomes from the forestomach and glandular stomach. [The Working Group noted that metabolism to formaldehyde

in vivo was not reported in this study, although this was imputed on the basis of the results with microsomal fractions in vitro.] Excretion of dimethyl hydrogen phosphite was rapid in both rats and mice, with most being released within the first 24 hours after dose administration. At the doses studied (10, 100, and 200 mg/kg bw) in rats, most of the [^{14}C]dimethyl hydrogen phosphite-related radiolabel was excreted as CO_2 in expired air (49–57%) and as monomethyl hydrogen phosphite in the urine (28–38%), with faeces containing only 2%. After dosing, elimination continued in expired air for approximately 12 hours and in the urine for up to 24 hours. The dose level did not affect the rate or route of elimination. Repeated administration of a 200 mg/kg bw dose daily for 5 days had little effect on metabolism to CO_2 or elimination in the urine.

In mice treated with dimethyl hydrogen phosphite at a dose of 200 mg/kg bw, 49% was excreted in the urine, 44% as CO_2 in expired air, 2.5% as organic volatiles, and 1–2% in the faeces.

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether dimethyl hydrogen phosphite is genotoxic. Sparse data, mostly derived from the NTP report (NTP, 1985), were also available on whether dimethyl hydrogen phosphite induces oxidative stress; induces chronic inflammation; or alters cell proliferation, cell death, or nutrient supply. No data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

Male B6C3F$_1$ mice were injected intraperitoneally on three consecutive days with dimethyl hydrogen phosphite at a dose of 250 or 500 mg/kg bw (Shelby et al., 1993). Bone marrow smears were prepared 24 hours after the third treatment and used for the micronucleus test assay. An initial test showed a significant ($P < 0.001$, ANOVA trend test) increase in the frequency of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes scored at 500 mg/kg bw (6.1) compared with controls (2.1). The repeat test did not show a significant ($P = 0.078$, ANOVA trend test) increase (the frequency of micronucleated polychromatic erythrocytes was 2.7 in controls versus 4.2 at the higher dose). [The Working Group noted that although a reproducible, statistically significant trend was not seen between the two tests, the study results were deemed to show adequate evidence of an effect. It was also noted that a re-evaluation of these data, reported in the NTP database Chemical Effects in Biological Systems (CEBS), indicated significance ($P < 0.001$, one-tailed Pearson chi-squared test) by the pairwise test (NTP, 2018).]

(ii) Non-human mammalian cells in vitro

See Table 4.2.

McGregor et al. (1988) tested the mutagenic potential of dimethyl hydrogen phosphite in the forward mutation assay in L5178Y *Tk*$^{+/-}$ mouse lymphoma cells. Cells (6×10^6 per culture; two cultures per concentration) were exposed to dimethyl hydrogen phosphite at five concentrations for 4 hours, with and without metabolic activation (post-mitochondrial 9000 × *g* supernatant fractions of liver homogenates, S9), and then cultured for 2 days before testing. In the presence of S9, dimethyl hydrogen phosphite was assessed at concentrations up to 2500 µg/mL;

Table 4.1 Genetic and related effects of dimethyl hydrogen phosphite in non-human mammals in vivo

End-point	Assay	Species, strain (sex)	Tissue	Results[a]	Dose (LOED or HID)	Route, duration, dosing regimen	Reference
Micronucleus formation	Micronucleus assay	Mouse, $B6C3F_1$ (M)	Bone marrow (smears)	+ –	500 mg/kg bw, 1st test 500 mg/kg bw, 2nd test	Intraperitoneally; 250 and 500 mg/kg bw for 3 days.	Shelby et al. (1993)

bw, body weight; HID, highest ineffective dose; LOED, lowest observed effective dose; M, male.
[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of dimethyl hydrogen phosphite in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation (forward mutations)	Mouse, L5178Y *Tk*$^{+/-}$ lymphoma cells	–	+	2100 mg/mL	Five-concentration range up to 2200 mg/mL (–S9), for 4 hours. Five-concentration range up to 2500 mg/mL (+S9), for 4 hours. Decreases in pH observed.	McGregor et al. (1988)
Chromosomal aberrations	Hamster, Chinese, ovary cells (CHO)	+	+	250 µg/mL	Concentration range, 0.0–5000 µg/mL. Chromosome fusion at the highest dose.	Gulati et al. (1989)
Sister-chromatid exchange	Hamster, Chinese, ovary cells (CHO)	+	+	1600 µg/mL	Concentration range, 0.0–4000 µg/mL.	Gulati et al. (1989)
Unscheduled DNA synthesis	Rat, F344, Aroclor-pretreated adult males, primary hepatocytes	+	NA	0.01 µL/mL	Treatment concentrations, 0.01–5.0 µL/mL. Cytotoxicity was observed at the highest concentration in Aroclor-pretreated hepatocytes and at the three highest concentrations in 3-MC-pretreated hepatocytes.	Shaddock et al. (1990)
	Rat, F344, 3-MC-pretreated adult males, primary hepatocytes	+	NA	0.025 µL/mL		

CHO, Chinese hamster ovary; HIC, highest ineffective concentration; LEC, lowest effective concentration; 3-MC, 3-methylcholanthrene; NA, not applicable; S9, 9000 × *g* supernatant.
[a] +, positive; –, negative.

a significant response was obtained with the lowest observed effective concentration (LOEC) of 2100 µg/mL. In the absence of S9, dimethyl hydrogen phosphite was tested at up to 2200 µg/mL because of poor growth during the expression period. No significant mutagenic response was observed at any concentration in the absence of metabolic activation. [The Working Group noted that the requirement for S9 in order to obtain a significant response suggested that dimethyl hydrogen phosphite needed to be metabolically activated to induce mutagenicity. The Working Group also noted that dimethyl hydrogen phosphite caused reductions in pH levels in the culture medium either in the presence or absence of S9. However, it was concluded that, in this study, the reduction of pH did not alter the mutagenic effect of dimethyl hydrogen phosphite.]

Dimethyl hydrogen phosphite was tested for induction of sister-chromatid exchange (concentration range, 0.0–4000 µg/mL) and chromosomal aberration (concentration range, 0.0–5000 µg/mL) in Chinese hamster ovary cells (Gulati et al., 1989). Sister-chromatid exchange and chromosomal aberration were induced in both the presence and absence of S9. Sister-chromatid exchange was observed at concentrations of 250–4000 µg/mL and chromosomal aberration at 1600–5000 µg/mL. In most cells treated with dimethyl hydrogen phosphite at 5000 µg/mL, all 21 chromosomes were fused together.

A study using primary hepatocyte cultures derived from livers of adult male Fischer 344 rats pretreated with hepatic mixed-function oxidase inducers, Aroclor and 3-methylcholanthrene, showed that dimethyl hydrogen phosphite induced a significant increase in unscheduled DNA synthesis in rats pretreated with Aroclor (0.01–2.5 µL/mL) or with 3-methylcholanthrene (0.025–0.250 µL/mL) (Shaddock et al., 1990).

(c) Non-mammalian systems

See Table 4.3.

Dimethyl hydrogen phosphite was not mutagenic in the *Salmonella typhimurium* assay system when tested in strains TA98, TA100, TA1535, and TA1537 at 100–10 000 µg/plate, with or without metabolic activation with S9 from livers of Aroclor 1254-induced Sprague-Dawley rats or Syrian hamsters.

When administered via feeding (650 ppm) or injection (1500 ppm), dimethyl hydrogen phosphite did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (NTP, 1985).

Woodruff et al. (1985) tested the ability of dimethyl hydrogen phosphite to induce sex-linked recessive lethal mutations in *D. melanogaster* generations after mating 24-hour-old Canton-S males fed with dimethyl hydrogen phosphite (650 ppm) for 3 days, or 72-hour-old (adult) males treated by injection (1500 ppm). Both routes of exposure gave negative results.

4.2.2 Induces oxidative stress

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

Daily treatment with dimethyl hydrogen phosphite (200 mg/kg bw, via gavage) of male Fischer 344 rats for 4, 5, or 6 weeks showed no effect on the activities of superoxide dismutase or glutathione *S*-transferase in the soluble fraction of the liver, lung, kidney, forestomach, or glandular stomach (Nomeir & Uraih 1988).

4.2.3 Induces chronic inflammation

(a) Humans

No data were available to the Working Group.

Table 4.3 Genetic and related effects of dimethyl hydrogen phosphite in non-mammalian experimental systems

Test system (species, strain)	Assay	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Drosophila melanogaster	Sex-linked recessive lethal test	Recessive lethal mutations	–	NA	650 ppm by feeding		NTP (1985)
			–	NA	1500 ppm by injection		
Drosophila melanogaster	Sex-linked recessive lethal test	Sex-linked recessive lethal mutations	–	NA	650 ppm by feeding		Woodruff et al. (1985)
			–	NA	1500 ppm by injection		
Salmonella typhimurium	Ames bacterial reverse mutation test	Gene mutation			100–10 000 µg/plate	Cytotoxicity observed at the highest concentration.	NTP (1985)
TA98 (frameshift +1)			–	–S9 (rat); –S9 (hamster)			
TA98, TA1538 (frameshift +1)			–	–S9 (rat); –S9 (hamster)			
TA1535 (base substitution, at GC)			–	–S9 (rat); –S9 (hamster)			
TA100 (base substitution, at GC)			–	–S9 (rat); –S9 (hamster)			
TA1537 (frameshift –1)			–	–S9 (rat); –S9 (hamster)			

HIC, highest ineffective concentration; LEC, lowest effective concentration, NA, not applicable; ppm, parts per million; S9, 9000 × *g* supernatant.

[a] –, negative.

(b) Experimental systems

(i) Non-human mammals in vivo

Subepithelial inflammation with minimal infiltrate in the submucosa and occasional submucosal intercellular and intracellular oedema were observed in the forestomach of male Fischer 344 rats treated daily with dimethyl hydrogen phosphite (200 mg/kg bw, by gavage) for 6 weeks. No gross changes were observed in the lung or forestomach of rats during necropsy or in the lung during microscopic examination (Nomeir & Uraih, 1988).

In a study conducted by the NTP, male and female F344/N rats were treated by gavage with dimethyl hydrogen phosphite at doses of 0, 50, 100, or 200 mg/kg bw (for males), or 0, 50, and 100 mg/kg bw (for females), 5 days per week for 103 weeks. An increased incidence of chronic inflammation, in the form of chronic interstitial pneumonia, was observed in the treated male rats (both at 100 and 200 mg/kg bw) and in females at the highest dose. No increased incidence of inflammation was observed in either male or female B6C3F$_1$ mice that were treated with dimethyl hydrogen phosphite according to the same protocol as for the male rats (NTP, 1985).

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

In male Fischer 344 rats treated daily by gavage with dimethyl hydrogen phosphite (200 mg/kg bw) for 6 weeks, histological examination of the forestomach identified lesions characterized by epithelial hyperplasia and hyperkeratosis (Nomeir & Uraih, 1988).

Dimethyl hydrogen phosphite was administered via gavage, 5 days per week for 103 weeks, to male (0, 100, or 200 mg/kg bw) and female (0, 50, 100 mg/kg bw) F344/N rats, and to male and female B6C3F$_1$ mice (0, 100, or 200 mg/kg bw). Male rats showed an increased incidence of hyperplasia and squamous metaplasia (highest dose only) in the lung and hyperplasia and hyperkeratosis in the forestomach. Female rats at the highest dose showed an increased incidence of adenomatous hyperplasia and alveolar epithelium hyperplasia in the lung and an increased incidence of forestomach hyperplasia. Dimethyl hydrogen phosphite caused hyperplasia and hyperkeratosis in the forestomach of male rats. No increased incidence of hyperplasia or metaplasia was observed in male or female mice (NTP, 1985).

4.3 Other relevant evidence

Significant increases (> 60% above control values) in levels of nonprotein soluble sulfhydryls were observed in the forestomach of male Fischer 344 rats treated with dimethyl hydrogen phosphite via gavage at a daily dose of 200 mg/kg bw for 6 weeks or with a single intravenous or oral dose of 1000 mg/kg bw, suggesting that dimethyl hydrogen phosphite interferes with sulfhydryl metabolism. [The Working Group noted that the increase in sulfhydryl levels could be a possible contributing factor to the development of lesions in these tissues after long-term exposure to dimethyl hydrogen phosphite.] The activity of soluble carboxylesterase was significantly reduced in the lung and forestomach of rats treated with dimethyl hydrogen phosphite (200 mg/kg bw per day for 6 weeks) (Nomeir & Uraih 1988), which could possibly make these tissues susceptible to further chemical exposures as this enzyme is involved in the hydrolytic detoxification of many toxic chemicals. A significant increase in levels of serum angiotensin-converting enzyme was also observed in rats exposed to dimethyl hydrogen phosphite at 200 mg/kg bw

per day for 4, 5, or 6 weeks, suggesting early lung injury in these animals (Nomeir & Uraih 1988).

4.4 Evaluation of high-throughput in vitro toxicity screening data

Dimethyl hydrogen phosphite was tested in high-throughput toxicity screening assays under the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2019). Chemical samples were procured at high purity, prepared in dimethyl sulfoxide stock solutions at a concentration of about 20 mM, and tested over a period of several years in biochemical and cellular bioassays measuring a wide variety of biological end-points. In addition, chemical analysis of the samples was done in high-throughput fashion at an early and a late stage of the sample testing lifetime for these samples, as described in Tice et al. (2013).

Data on testing results from the concentration–response testing design for all end-points were analysed for significant activity and an active/inactive "hit call" was made for each response, together with a potency value (Filer et al., 2017). For all active calls, individual concentration–response curves were examined to ensure that biologically meaningful activity was detected. Bioassay end-points were mapped, where possible, to the key characteristics of carcinogens using the "kc-hits" software (key characteristics of carcinogens – high-throughput screening discovery tool, available from https://gitlab.com/i1650/kc-hits; Reisfeld et al., 2022) to aid in providing mechanistic insights (Chiu et al., 2018). The detailed results are available in the supplementary material for this volume (Annex 2, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/631) and are briefly summarized below.

The results for dimethyl hydrogen phosphite high-throughput toxicity testing in the CompTox Chemicals Dashboard encompassed 440 assay end-points, of which 191 were mapped to the key characteristics of carcinogens. [The Working Group noted that in the ToxCast database an alternative name of the agent, dimethyl phosphonate, was used.] The cytotoxicity limit was estimated to be > 1 mM (US EPA, 2022b). Dimethyl hydrogen phosphite was inactive in all except seven of the mapped assay end-points, including three with curve-fitting warning flags. All seven of the positive hit calls were mapped to key characteristic 8 (KC8), "modulates receptor-mediated effects", and six of these measured signalling in the estrogen receptor pathway. Four of the six did not have warning flags, and half-maximal activity concentrations (AC_{50}s) ranged from 8.3 to 22.2 μM. However, 11 other assay end-points measuring estrogen-receptor signalling showed negative hit calls. The other assay with a positive hit call for KC8 was for antagonism of the progesterone receptor but with a flag for activity detected only at the highest concentration tested (89 μM). [The Working Group considered this to be very weak evidence of modulation of receptor-mediated effects.]

The results of chemical analysis of the stock solutions for dimethyl hydrogen phosphite (available from the Tox21 Samples database) were inconsistent (NIH, 2022). Two testing samples were analysed: Tox21_201901 and Tox21_302799. Tox21_201901 was reported to have a purity of < 50% on initial analysis and "unknown or inconclusive" on analysis 4 months later. The second sample, Tox21_302799, gave an incorrect molecular weight on initial analysis, but dimethyl hydrogen phosphite was detected with a purity > 90% on the later analysis. [The Working Group considered the testing results for dimethyl hydrogen phosphite to be of low confidence, without the ability to link specific samples to bioactivity testing.]

5. Summary of Data Reported

5.1 Exposure characterization

Dimethyl hydrogen phosphite is a High Production Volume chemical that is used as an intermediate in the manufacture of adhesives, lubricants, organophosphate pesticides (e.g. glyphosate), and pharmaceuticals (α-aminophosphonates). It is also used as a stabilizer in oil and plaster, and as a steel corrosion inhibitor. Dimethyl hydrogen phosphite is used as a reactive flame retardant in textile finishing, cellulosic textiles, acrolein-grafted polyamide fibres and polyethylene. The production and export of dimethyl hydrogen phosphite is stringently controlled under the Wassenaar Arrangement on Export Controls for Conventional Arms and Dual-Use Goods and Technologies because it can be converted by chemical synthesis to nerve gases.

The most relevant occupational exposure route to dimethyl hydrogen phosphite is respiratory. Exposure data were only available for its use in the production of flame retardants. There were no available data on environmental occurrence nor on exposure of the general population.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with dimethyl hydrogen phosphite caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of a single species (rat) in a well-conducted study that complied with Good Laboratory Practice.

Dimethyl hydrogen phosphite was administered orally (by gavage) to male and female F344/N rats in one study that complied with Good Laboratory Practice. In males, dimethyl hydrogen phosphite caused an increase in the incidence of bronchioloalveolar carcinoma of the lung, bronchioloalveolar adenoma or carcinoma (combined) of the lung, squamous cell carcinoma of the lung, forestomach squamous cell carcinoma, and forestomach squamous cell papilloma or carcinoma (combined). In females, dimethyl hydrogen phosphite caused an increase in the incidence of bronchioloalveolar carcinoma of the lung.

5.4 Mechanistic evidence

No data were available on the absorption, distribution, metabolism, and excretion of dimethyl hydrogen phosphite in humans. Dimethyl hydrogen phosphite was absorbed in the gastrointestinal tract of both mice and rats. Distribution was over a wide and similar range of tissues in mice and rats, with tissue concentrations being substantially lower in mice. In both rats and mice, dimethyl hydrogen phosphite was metabolized either to monomethyl hydrogen phosphite and excreted in the urine or to CO_2 and released in expired air. Dimethyl hydrogen phosphite was metabolized to formaldehyde in vitro, but no evidence was available for metabolism in vivo. Dimethyl hydrogen phosphite was excreted in the urine, expired air, and faeces, or as organic volatiles (mice only).

There was no mechanistic evidence available for dimethyl hydrogen phosphite regarding the key characteristics of carcinogens in exposed humans or in human primary cells or tissues.

Overall, the mechanistic evidence regarding the key characteristic of carcinogens "is genotoxic" is suggestive but inconsistent across different experimental systems. Dimethyl hydrogen phosphite induced a mutagenic response in two different experiments in mouse lymphoma cells

only in the presence of metabolic activation. One study using Chinese hamster ovary cells showed that dimethyl hydrogen phosphite induced sister-chromatid exchange and chromosomal aberration in the presence and absence of metabolic activation. Dimethyl hydrogen phosphite was not mutagenic in several *Salmonella typhimurium* strains in the presence and absence of metabolic activation. Dimethyl hydrogen phosphite did not induce sex-linked recessive lethal mutations in two different studies in *Drosophila melanogaster*.

Regarding the key characteristics "alters DNA repair or causes genomic instability", "induces oxidative stress", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply", there was a paucity of available data for each characteristic.

There was one study regarding the key characteristic of carcinogens "alters DNA repair or genomic instability", which showed that dimethyl hydrogen phosphite caused a significant increase in unscheduled DNA synthesis in rodent primary liver cells.

The mechanistic evidence is suggestive for the key characteristic of carcinogens "induces chronic inflammation". Two in vivo studies in Fischer 344 rats were available. The first study observed chronic inflammation in the lungs of male rats and in female rats at a high dose, but not in male or female B6C3F$_1$ mice. The second study showed that dimethyl hydrogen phosphite caused subepithelial inflammation and occasional submucosal and interstitial oedema in the forestomach of male rats.

The mechanistic evidence is suggestive for the key characteristic of carcinogens "alters cell proliferation, cell death, and nutrient supply". There were two in vivo studies in Fischer 344 rats available. The first study showed an increased incidence of hyperplasia and squamous metaplasia (high dose only) in the lung, and of hyperplasia and hyperkeratosis in the forestomach in male rats and an increased incidence of hyperplasia in the lung and forestomach of female rats at a high dose. The second study showed lesions characterized by epithelial hyperplasia and hyperkeratosis epithelial hyperplasia in the forestomach of male rats.

Of note, dimethyl hydrogen phosphite had no effect on the activities of superoxide dismutase or glutathione *S*-transferase in several tissues but caused increased levels of nonprotein sulfhydryls and reduced carboxylesterase activity in the forestomach, as observed in one study in rodents. Carboxylesterase activity was also reduced in the lung.

No data were available for the other key characteristics.

Dimethyl hydrogen phosphite was found to be without effects relevant to the key characteristics of carcinogens in the assay battery of the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, although the dimethyl hydrogen phosphite testing solution was considered problematic for use in the high-throughput assays.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of dimethyl hydrogen phosphite.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of dimethyl hydrogen phosphite.

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

Dimethyl hydrogen phosphite is *possibly carcinogenic to humans (Group 2B).*

6.5 Rationale

The Group 2B evaluation for dimethyl hydrogen phosphite is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in males and females of a single species in one study that complied with Good Laboratory Practice. The mechanistic evidence was *limited*. There is suggestive evidence for several key characteristics (genotoxicity, induction of chronic inflammation, and alteration of cell proliferation in experimental systems). The evidence regarding cancer in humans was *inadequate* because no studies were available.

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LIST OF ABBREVIATIONS

2-AAF	2-acetylaminofluorene
AC_{50}	half-maximal activity concentration
AEGL	acute exposure guideline level
AhR	aryl hydrocarbon receptor
AOAC	Association for Official Analytical Collaboration
AR	androgen receptor
AUC	area under the curve
BALF	bronchoalveolar lavage fluid
B[a]P	benzo[*a*]pyrene
bw	body weight
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
CAT	catalase
CCME	Canadian Council of Ministers of the Environment
CHO	Chinese hamster ovary
CI	confidence interval
CRM	certified reference material
CYP	cytochrome P450
DCFH-DA	2′,7′-dichlorodihydrofluorescein diacetate
DMBA	7,12-dimethylbenz[*a*]anthracene
DMSO	dimethyl sulfoxide
DNEL	derived no-effects level
dUTP	deoxyuridine phosphate
dw	dry weight
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
EGR-1	early growth response protein 1
EI	electron impact ionization
ELISA	enzyme-linked immunosorbent assay
ER	estrogen receptor
ERE	estrogen-responsive element
EROD	ethoxyresorufin-*O*-deethylase
EU	European Union
FLD	fluorescence detection

FSH	follicle-stimulating hormone
fw	fresh weight
GC	gas chromatography
GC-ECD	gas chromatography-electron capture detection
GC-FID	gas chromatography-flame ionization detection
GC-MS	gas chromatography-mass spectrometry
GC-MS/MS	gas chromatography-tandem mass spectrometry
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GJIC	gap junction intercellular communication
GLP	Good Laboratory Practice
GPx	glutathione peroxidase
GR	glucocorticoid receptor
GSH	glutathione
GSR	glutathione reductase
GST	glutathione *S*-transferase
HPBMC	human peripheral blood mononuclear cells
HPLC	high-performance liquid chromatography
HPV18	human papillomavirus type 18
HS-SPME	headspace solid-phase microextraction
HUVEC	human umbilical vein endothelial cell
IL-6	interleukin 6
IQR	interquartile range
KOSHA	Korean Occupational Safety and Health Agency
LC	liquid chromatography
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
LLE-PTV-GC-MS	liquid–liquid extraction and programmed temperature vapourizer-gas chromatography-mass spectrometry
LOD	limit of detection
LOED	lowest observed effective dose
LOQ	limit of quantification
LPO	lipid peroxidation
MAPK	mitogen-activated protein kinase
3-MC	3-methylcholanthrene
MDA	malondialdehyde
MNNG	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
MROD	methoxyresorufin *O*-demethylase
MS	mass spectrometry
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NIOSH	National Institute for Occupational Safety and Health
NOES	National Occupational Exposure Survey
NR	not reported
NRF2	nuclear factor erythroid 2-related factor 2
NTP	National Toxicology Program
ODC	ornithine decarboxylase
OECD	Organisation for Economic Co-operation and Development
OEL	occupational exposure level
OSHA	Occupational Safety and Health Administration

PAH	polycyclic aromatic hydrocarbon
PGE_2	prostaglandin E_2
PM	particulate matter
PPAR	peroxisome proliferator-activated receptor
PTK	protein tyrosine kinase
PUFA	polyunsaturated fatty acids
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	reactive oxygen species
RT-PCR	reverse transcription-polymerase chain reaction
S9	9000 × *g* supernatant
SCE	sister-chromatid exchange
SD	standard deviation
SERCA	sarcoendoplasmic reticulum calcium ATPase
SHE	Syrian hamster embryo
SIC	standard industrial classification
SIM	selected-ion monitoring
SOD	superoxide dismutase
SPME	solid-phase microextraction
TCDD	2,3,7,8-tetrachlorodibenzo-*para*-dioxin
TK	thymidine kinase
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TWA	time-weighted average
UDS	unscheduled DNA synthesis
US	United States
USA	United States of America
US EPA	United States Environmental Protection Agency
UV	ultraviolet
VE-cadherin	vascular endothelial cadherin
v/v	volume per volume
w/w	weight per weight
ZO-1	zona occludens-1

ANNEX 1. SUPPLEMENTARY MATERIAL FOR SECTION 1, EXPOSURE CHARACTERIZATION

These supplementary online-only tables are available from: https://publications.iarc.who.int/631.

Please report any errors to imo@iarc.who.int.

Anthracene

The following table was produced in draft form by the Working Group and was subsequently fact-checked but not edited:

Table S1.12 Exposure assessment review and critique for mechanistic studies in humans exposed to anthracene

2-Bromopropane

The following table was produced in draft form by the Working Group and was subsequently fact-checked but not edited:

Table S1.2 Exposure assessment review and critique for mechanistic studies in humans exposed to 2-bromopropane

Butyl methacrylate

The following table was produced in draft form by the Working Group and was subsequently fact-checked but not edited:

Table S1.3 Exposure assessment review and critique for mechanistic studies in humans exposed to butyl methacrylate

ANNEX 2. SUPPLEMENTARY MATERIAL FOR SECTION 4, EVALUATION OF HIGH-THROUGHPUT IN VITRO TOXICITY SCREENING DATA

These supplementary online-only tables (available from: https://publications.iarc.who.int/631) 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 133.

Please report any errors to imo@iarc.who.int.

1. Anthracene_ToxCastTox21 assay results mapped to the key characteristics of carcinogens
2. 2-Bromopropane_ToxCastTox21 assay results mapped to the key characteristics of carcinogens
3. Butyl methacrylate_ToxCastTox21 assay results mapped to the key characteristics of carcinogens
4. Dimethyl hydrogen phosphite_ToxCastTox21 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 PMID:35561175

SUMMARY OF FINAL EVALUATIONS

Summary of final evaluations for Volume 133

Agent	Evidence stream			Overall evaluation
	Cancer in humans	Cancer in experimental animals	Mechanistic evidence	
Anthracene	*Inadequate*	*Sufficient*	*Limited*	Group 2B
2-Bromopropane	*Inadequate*	*Sufficient*	*Strong*[a]	Group 2A
Butyl methacrylate	*Inadequate*	*Sufficient*	*Inadequate*	Group 2B
Dimethyl hydrogen phosphite	*Inadequate*	*Sufficient*	*Limited*	Group 2B

[a] *Strong* in experimental systems, supported by suggestive evidence of immunosuppression and of modulation of receptor-mediated effects in exposed humans.

IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of four agents: anthracene, 2-bromopropane, butyl methacrylate, and dimethyl hydrogen phosphite.

2-Bromopropane is a solvent used in dry cleaning and in adhesive production and application, and it also occurs as an impurity of 1-bromopropane (used since the 1990s as a substitute for ozone-depleting solvents).

Anthracene, butyl methacrylate, and dimethyl hydrogen phosphite are all chemicals with a high production volume.

Anthracene is a high-production-volume polycyclic aromatic hydrocarbon that is mainly used as an intermediate in the manufacture of dyes and pigments, pyrotechnics, coatings, wood preservatives, pesticides, and organic chemicals. Also formed by tobacco smoke, biomass burning (indoor and outdoor), traffic and industry emissions, and contaminated food, it is ubiquitous in the environment and is a widespread environmental pollutant.

Butyl methacrylate is used in coatings, polyvinyl chloride plastics, polypropylene non-woven materials, glues, caulks, inks and paints, pesticides, and health-care materials.

Dimethyl hydrogen phosphite is used as an intermediate in the manufacture of adhesives, lubricants, pesticides, and pharmaceuticals, and as a stabilizer in oil and plaster, a steel corrosion inhibitor, and a flame retardant.

For all four agents, occupational and environmental exposures may occur.

An *IARC Monographs* Working Group reviewed evidence from cancer bioassays in experimental animals and mechanistic studies to assess the carcinogenic hazard to humans of exposure to these agents and concluded that:

- 2-Bromopropane is *probably carcinogenic to humans (Group 2A)*;
- Anthracene, butyl methacrylate, and dimethyl hydrogen phosphite are *possibly carcinogenic to humans (Group 2B)*.