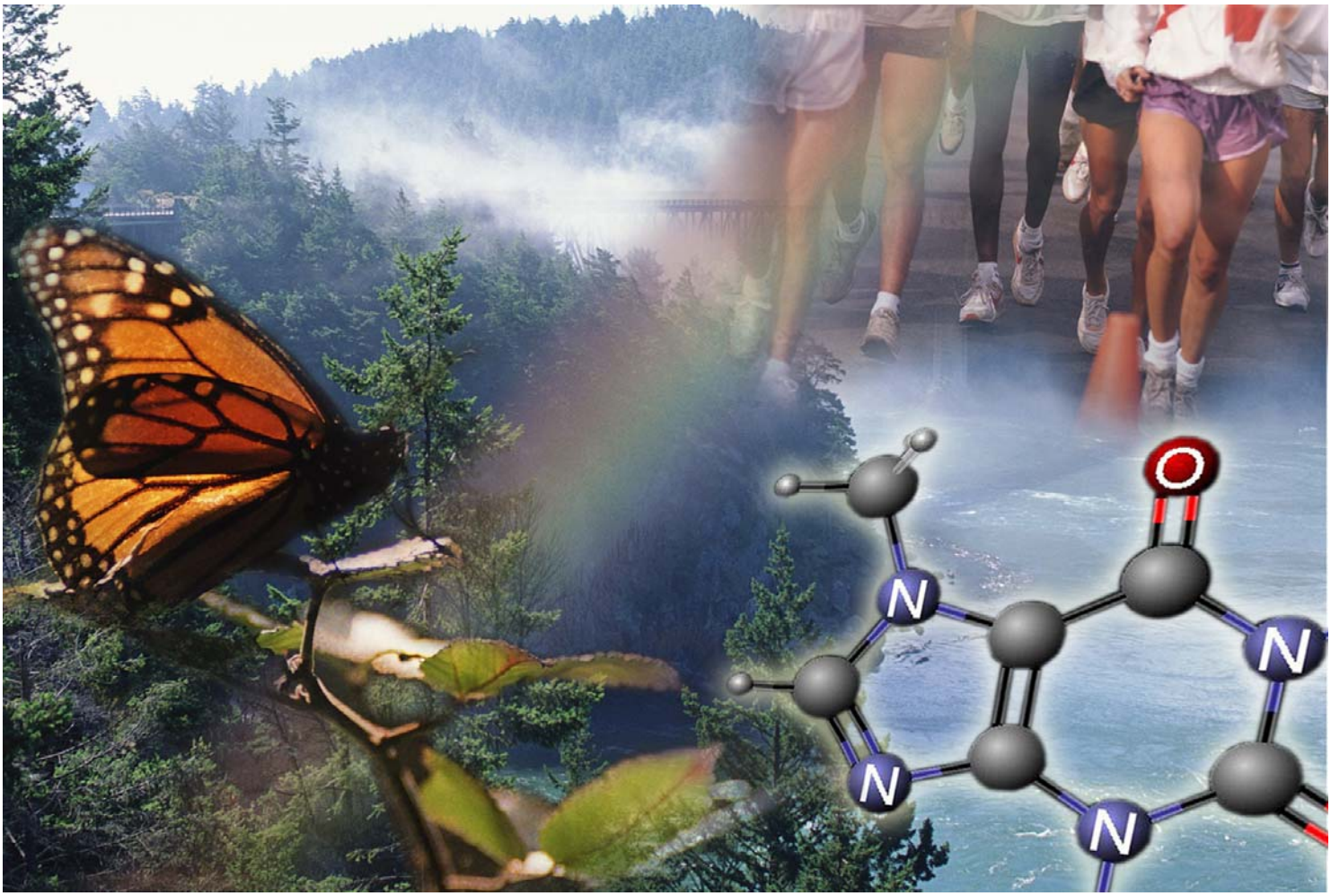
# Guidance on information requirements and chemical safety assessment

## Chapter R.10: Characterisation of dose [concentration]-response for environment




**May 2008**

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# PREFACE

This document describes the information requirements under REACH with regard to substance properties, exposure, use, risk management, and the chemical safety assessment. It is part of a series of guidance documents that are aimed to help all stakeholders with their preparation for fulfilling their obligations under the REACH regulation. These documents cover detailed guidance for a range of essential REACH processes as well as for some specific scientific and/or technical methods that industry or authorities need to make use of under REACH.

The guidance documents were drafted and discussed within the REACH Implementation Projects (RIPs) led by the European Commission services, involving stakeholders from Member States, industry and non-governmental organisations. These guidance documents can be obtained via the website of the European Chemicals Agency (http://echa.europa.eu/reach_en.asp). Further guidance documents will be published on this website when they are finalised or updated.

This document relates to the REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006[1]

1 Corrigendum to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396, 30.12.2006); amended by Council Regulation (EC) No 1354/2007 of 15 November 2007 adapting Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) by reason of the accession of Bulgaria and Romania (OJ L 304, 22.11.2007, p. 1).

**Convention for citing the REACH regulation**

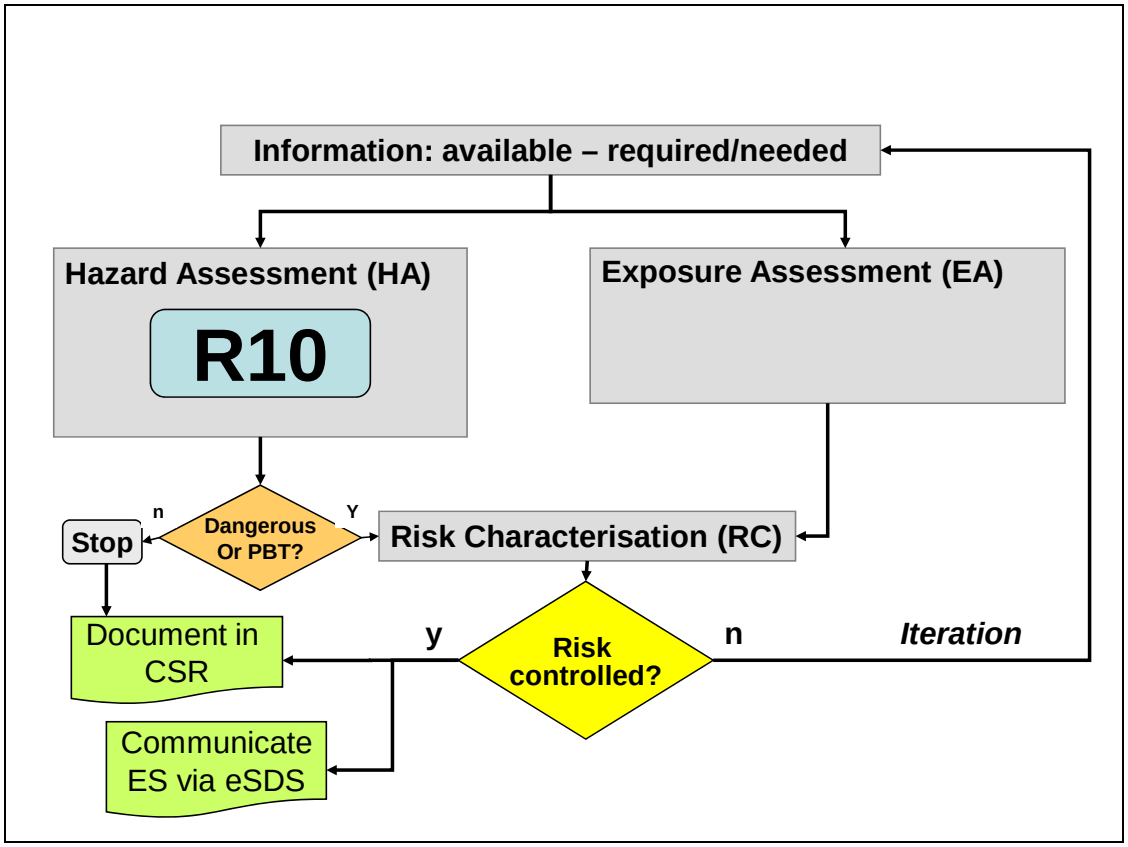
Where the REACH regulation is cited literally, this is indicated by text in italics between quotes.

**Table of Terms and Abbreviations**

See Chapter R.20

**Pathfinder**

The figure below indicates the location of chapter R.10 within the Guidance Document



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# R.10 CHARACTERISATION OF DOSE/CONCENTRATION-RESPONSE FOR ENVIRONMENT

## R.10.1 Aim

This document is guidance on how to characterise the dose (concentration) – response for the different environmental compartments. In other words it is mainly a guidance on how to quantitatively assess the effects of a substance on the environment by determining the concentration of the substance below which adverse effects in the environmental sphere of concern are not expected to occur. This concentration is known as Predicted No-Effect Concentrations (PNECs). If it is not possible to derive the PNEC then this shall be clearly stated and fully justified such as for the air compartment where only a qualitative assessment is normally possible.

## R.10.2 Derivation of PNECs: introduction

### R.10.2.1 Data used for derivation of the PNEC

The derivation of PNECs is required for the chemical safety assessment (CSA) of substances manufactured/imported/used in quantities from 10 t/y onwards. For each tonnage level standard data requirements have been specified in REACH (Annex VII-X, in conjunction with annex XI), but REACH also requires that any other relevant hazard information that is available (i.e. from other available tests and non-test methods) is taken into account. PNEC(s) should be reconsidered if further information becomes available at higher tonnage levels.

For derivation of PNECs, all available hazard information needs to be evaluated (see Chapter R.7 for the individual endpoints).

### R.10.2.2 Evaluation and interpretation of data

For the characterization of the PNEC it is of high importance to evaluate the data with regard to their adequacy and completeness. The evaluation of adequacy shall address the reliability and relevance of data (see Chapter R.4). The evaluation of data is of particular importance for existing substances as tests will often be available with non-standard organisms and/or non-standardized methods. It is suitable to start the effects assessment process with the evaluation of the available ecotoxicological data.
Further guidance on which ecotoxicological information can be used to perform the effects assessment is given in the different endpoint specific sections in Chapter R.7.

In some cases the dose (concentration) - response (effect) relationship is not known, the duration of a test may be different from that of standard tests or the test parameters may not be comparable to those used in standard tests, for example investigations of photosynthesis, of behaviour, investigations on a cellular or a subcellular level. Expert judgement must therefore be used to determine whether such data can be interpreted for use in the assessment.

When there is more than one set of data on the same species, (strain if known), endpoint, duration, life stage and testing condition the greatest weight is attached to the most reliable and relevant one. When there is more than one set of data with the same reliability rating, it might be necessary to look into more detail at the study reports to see whether a specific reason could explain the difference. If no explanation can be found and the results are for the same species and endpoints and are not more than one order of magnitude apart, they can be harmonised by a geometric mean. If

they are more than one order of magnitude apart, this may be questionable. If the endpoint is critical for the outcome of the regulatory decision, a repetition of the study may sometimes be the easiest and most efficient solution, especially for non-vertebrate tests. A decision might also be possible on the basis of additional available data, e.g. from studies of a lower reliability rating or from non-testing methods, if these show a distinct tendency in support of a certain result.

#### R.10.2.2.1 Use of data from laboratory toxicity tests

The data used for derivation of the PNECs are usually results from single species laboratory toxicity tests. The data are typically reported as the concentrations at which x % (e.g. 50%) mortality or inhibition of a function (e.g. growth) was observed and are expressed as the lethal concentration ($LC_x$) or the effect concentration ($EC_x$), e.g. $LC_{50}$ or $EC_{50}$.

$L/EC_{50}$-values are usually obtained from short term tests, while the result of long term tests (e.g. reproductive success of exposed organism) are most frequently reported as $L/EC_x$ (x being very often equal to 10) or as the NOEC (No Observed Effect Concentration) which corresponds to the highest tested concentration for which there are no statistical significant difference of effect when compared to the control group.

The endpoints most frequently used for derivation of PNEC are mortality ($LC_{50}$), growth ($EC_x$ or NOEC) and reproduction ($EC_x$ or NOEC).

Guidance is given in Table R.10-1 with respect to the derivation of L(E)C50 and NOEC values.

Different statistical approaches can be used to analyse data obtained in an ecotoxicity test (see e.g. OECD, 2006b):

Hypothesis-testing methods

Hypothesis testing is a statistical inference technique used to compare the responses among two or more test groups. Hypothesis testing has many uses in ecotoxicology, ranging from detecting whether there is a significant difference in the measured response between the control and a given concentration, to establishing a LOEC and a NOEC.

Several assumptions made when conducting hypothesis tests to determine the NOEC include:

- Concentration-response relationship may or may not be assumed depending on the specific statistical tests used.
- This approach makes only weak assumptions about the mechanisms of the toxicant or the biology of the organism.

Several limitations of using hypothesis testing to determine the NOEC include:

- Since the NOEC (or NOEL) does not estimate a model parameter, a confidence interval cannot be assessed.
- The value of the NOEC is limited to being one of the tested concentrations (i.e. if different values were chosen for the tested concentrations, the value of the NOEC would be different).
- If the statistical power of a test is low (due to high variability in the measured response and/or small sample size), the biologically important differences between the control and treatment groups may not be identified as significantly different. If the power is high, it

may occur that biologically unimportant differences are found to be statistically significantly different.

Concentration-response modelling methods

Regression methods are used to determine the relationship between a set of independent variables and a dependent variable. For designed experiments in ecotoxicology, the main independent variable is the concentration of the test substance and the dependent variable is the measured response (e.g., percent survival, fish length, growth rate). Regression methods fit a concentration-response curve to the data and use this curve to estimate an Effective Concentration (ECx) at a given time point. The mathematical model used may be any convenient function that is able to describe the data; however, some models are more frequently used and accepted within the ecotoxicity testing literature. Several methods are available for model fitting and parameter estimation.

Although statistical power is typically only discussed when hypothesis tests are conducted, both sample size and variation in the response variable within groups affect the inferences of concentration-response models as well. Small sample sizes and high variability in the response within groups will increase the width of the confidence interval of the parameters of interest (e.g. ECx), and the fitted model may not reflect the true concentration-response relationship.

Several assumptions of concentration-response modelling include:

- The fitted curve is close to the true concentration-response relationship.
- This is an empirical model and does not make strong assumptions about the mechanisms of the toxicant or the biology of the organism.

Several limitations of concentration-response modelling include:

- Estimation of ECx values outside the concentration range introduces a great deal of uncertainty (i.e., extrapolation outside the range of the data).
- Once the experiment has been performed, the resulting concentration-response data may not be suitable for the estimation of parameters of a concentration-response model. In particular, when the gaps between consecutive response levels are so large that many different concentration-response models would fit equally well to the observed data, interpolation would not be warranted.

Biology-based methods

Biology-based methods provide models for exploring the effect of the test chemical over time as well as incorporating a toxicokinetic model for the behaviour of the chemical. By modelling concentration and exposure time simultaneously, these methods fit response surfaces to response data to estimate an ECx as a function of exposure time, rather than fitting separate response curves at each time point.

Because of additional assumptions regarding the toxicokinetic behaviour of the chemical and the biological behaviour of the organism in the system, it is sometimes possible to carry out additional extrapolation from the toxicity test. The assumptions are endpoint-specific; therefore, for each type of test, these assumptions need to be defined. The definition of these assumptions usually involves eco-physiological background-research prior to the specification of the test. However, if these additional assumptions can be made, an example of additional outcomes this method can predict is:

chronic responses from acute responses, responses to time-varying concentrations using responses to constant concentrations, and responses by a species using responses to a conspecific or physiologically related species of a different body size for a given test compound .

Several general assumptions made when using biology-based methods include:

- This analysis method incorporates mechanistic models for toxicokinetics and physiology.

Several limitations of biology-based methods include:

- Estimation of parameter values (e.g., ECx) outside the concentration range introduces a great deal of uncertainty (i.e., extrapolation outside the range of the data).
- When the gaps between consecutive response levels are so large that different biology-based models would fit equally well to the observed data parameters estimation would not be warranted, if they differ substantially between the models.
- To date, models have been developed for some of the common aquatic toxicity tests (acute and chronic tests on survival/immobility for daphnids and fish, fish growth test, daphnia reproduction test, and alga growth inhibition test). Nevertheless, such models can be applied to any test species.

**Experimental design implications for the estimation of the NOEC or ECx**

The usual factors (independent variables) studied in ecotoxicity tests are concentration of the tested substance and duration of exposure.

The estimation of an ECx puts different demands on the study design than does the assessment of a NOEC.

To assess a NOEC, an important demand is that the study warrants sufficient statistical power. To that end, the concentration (dose) groups need a sufficient number of replicates (possibly at the expense of the number of dose groups).

To provide an estimate of an ECx, the primary demand on the study design is to have a sufficient number of concentration (dose) groups. This may be at the expense of the number of replicates per group (e.g. keeping the total size of the experiment the same), since the precision of the estimated ECx depends more on the number and spacing of concentrations rather than on the sample size per concentration or dose group.

However, results from ecotoxicological studies may also be reported using other conventions and expressions of effect.

**Table R.10-1 Overview of toxicity test endpoints and guidance on derivation of L(E)C50 and NOEC values**

**Short-term studies:**

If a test report does not indicate the L(E)C50 values but the raw data are presented, the L(E)C50 should be calculated, for example by regression analysis. If only one toxicity value lies between the L(E)C0 and the L(E)C100, the L(E)C50 cannot be calculated e.g. by Probit analysis. Instead, the L(E)C50 may be estimated by, e.g., linear regression.

If results are presented as >L(E)C10 and <L(E)C50, they can be rated as L(E)C50 while results clearly above a L(E)C50 can only be used as an indication of the short-term toxicity of the chemical considered.

**Long-term studies:**

An EC10 for a long-term test which is obtained using an appropriate statistical method (usually regression analysis) will be used preferentially.

The NOEC (no observed effect concentration) is defined as "the highest concentration tested at which the substance is observed to have no statistically significant effect ($p<0.05$) when compared with the control, within a stated exposure period" (OECD 211, 1998b) or the test concentration immediately below the LOEC, which when compared with the control has no statistically significant effect ($p<0.05$) within a stated period (OECD 211, 1998b). There has to be a concentration-effect relationship. In the past, the NOEC was mainly derived on the basis of ANOVA (analysis of variance) and a subordinate test (e.g. Dunett's) or determined directly from the concentration-effect curve by consideration of the deviation of the control (e.g. 10%). The preconditions for the use of ANOVA have to be fulfilled (normal distribution, homogeneous variances). In older investigations, it may be difficult to find out how the NOEC was generated unless test reports or raw data are available. There has been a recommendation within OECD in 1996 to phase out the use of the NOEC, in particular as it can correspond to large and potentially biologically important magnitudes of effect. The advantage of regression method for the estimation of $EC_x$ is that information from the whole concentration-effect relationship is taken into account and that confidence intervals can be calculated. These methods result in an $EC_x$, where x is a low effect percentile (e.g. 5-20%). It makes results from different experiments more comparable than NOECs.

A LOEC (lowest observed effect concentration) stands for the lowest concentration where an effect has been observed. It may therefore not be used as a NOEC. In case only a LOEC is given in the report, it can be used to derive a NOEC with the following procedures:

- LOEC > 10 and < 20% effect: NOEC can be calculated as LOEC/2.

If the effect percentage of the LOEC is unknown no NOEC can be derived.

MATC (maximal acceptable toxicant concentration): In aquatic toxicity the MATC was often calculated. This is the geometric mean of the NOEC and the LOEC. If in the test report only the MATC is presented, the MATC can be divided by $\sqrt{2}$ to derive a NOEC.

It should be noted that in the case of algae studies, which are actually multigeneration studies, it is generally accepted that a 72-hour (or longer) EC50 value may be considered as equivalent to a short-term result and that a 72-hour (or longer) $EC_{10}$ or NOEC value can be considered as a long-term result.

### R.10.2.2.2 (Q)SAR and grouping approaches.

<u>(Q)SAR</u>

Results obtained from valid (Q)SAR may be used instead of testing when the conditions listed in Annex XI are met. Further guidance on the use of QSAR is provided in the endpoint specific Sections (see Chapter R.7) as well as under Section R.6.1.

QSARs may be particularly helpful in assessing long-term aquatic toxicity data from very hydrophobic organic chemicals such as PCBs. Long-term tests with such chemicals are difficult to perform because of their low water solubility and the difficulty of maintaining stable test concentrations. Also, it may take a very long time to reach steady state in the test organisms due to their low elimination rate. By comparing the test result with the "minimum toxicity" obtained from

a QSAR based on the log Kow of the compound, insight can be gained into the validity of the test result (see Section R.6.1).

Available (Q)SAR methods can be summarised using the following categories:

- **Schemes for the prediction of the mode of action/structural class of a compound**

Knowledge about the mode of action of a chemical is a helpful information on the identification of appropriate (Q)SAR models[2]. In general two types of acute modes of action can be distinguished:

  - Baseline toxicity (also referred to as narcosis) with different subtypes

Baseline toxicity describes the minimum toxicity of a chemical due to a narcotic mode of action. Substances acting via narcosis are also described as inert chemicals, narcotics or neutral organics.

  - Excess toxicity (specific acute modes of action or reactive)

Each organic compound can, in principle, act via narcosis. Chemicals that in addition act via a different mode of action (e.g. due to reactivity or specific modes of action such as inhibition of photosynthesis), might show higher toxicity than the predicted baseline toxicity. They can be summarised as substances possibly showing excess toxicity[3].

Information about the acute mode of action of a given chemical can be derived by using schemes as described by Verhaar et al (1992) and Russom et al (1997). A short description of the schemes is given in Table R.10-14 in Appendix R.10-1 Guidance for the characterisation is given in literature cited. As the schemes are based on acute experimental data, they can not be used for the prediction of chronic modes of action.

In addition there are several tools that could support the characterisation according to modes of action and/or chemical classes. Amongst others, ASTER (Russom et al 1991, Russom et al 1997), OASIS/TIMES (Meckenyan et al 2004), MCase, PropertEst and ChemProp (Schüürmann et al 1997) software can be indicated. Other tools such as ECOSAR (U.S. EPA 1994) and TOPKAT (Gombar and Enslein 1995) also characterise chemicals but rather on a chemical class principle than defining a mode of action. An overview of programs for the identification of modes of action of a chemical is provided in Table R.10-15 in Appendix R.10-1

Detailed descriptions of the experimental derivation of different mode of actions and their connection to structural information can be found in the literature (e.g. Verhaar et al 1992 and Lipnick 1991).

- **Qualitative information from structural alerts**

Additional information about the mode of action of a chemical can be obtained from qualitative structure-activity relationships (SARs), e.g. from structural alerts (chemical structures that might indicate an excess toxicity). The alert might be used to indicate which model is to be selected (if available), or simply as an indication that the narcoses models will under predict toxicity.

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[2] In many cases the exact mechanism of action is not known and a response might be due to multiple mechanisms of action. This is acknowledged by using the term “mode of action” instead of “mechanism of action” in the text.

[3] The excess toxicity (Te) can be defined as a ratio between the baseline and the measured $LC_{50}$ value. If the predicted baseline toxicity is lower than the measured toxicity (i.e. when obtaining a measured $LC_{50}$ value which is lower than the predicted $LC_{50}$), excess toxicity is presumed.

Chemical structure and possible modes of action of compounds that might indicate an excess toxicity in fish can be found in the paper of Lipnick (1991). Von der Ohe et al. (2005) identified structural alerts associated with excess toxicity to *Daphnia*. Examples of such structural alerts are given in Table R.10-16 in Appendix R.10-1

- **QSAR predictions from individual models**

Individual QSAR models for aquatic endpoints are mostly experimentally derived by comparing the toxicity of a set of chemicals with one or more chemical descriptors.

- QSARs for narcosis

QSAR models for narcosis are appropriate for chemicals that act via narcosis and do not show additional specific toxicity. In addition they can be used to predict minimum or baseline toxicity (Veith et al 1983). Some QSAR models are based on narcosis subtypes such as polar narcosis, amine narcosis (Newsome et al 1993) and ester narcosis (Jaworska et al 1998)[4]. They are based on the fact that, if log $K_{ow}$ is used as descriptor, polar (or less inert) chemicals, amines and esters tend to show higher toxicity than would be expected by using a non-polar narcosis QSAR model. Examples of regression-based models using log $K_{ow}$ for different types of narcosis to fathead minnow and other aquatic species are given in Table R.10-17 in Appendix R.10-1. For very hydrophobic substances, the toxicity might be overestimated using linear models. In this case models are available with quadratic relationships between toxicity and log $K_{ow}$, developed with training sets that included chemicals with a log $K_{ow}$ above approximately 6 (see Table R.10-17 in Appendix R.10-1).

- QSARs for other modes of action

For substance showing chemical reactivity with biological structures, the effect can not be described via narcosis. Then, different reactivity parameters can be used (e.g. experimentally determined rate constants or quantum-mechanical indices, such as orbital energies, partial charges, and/or superdelocalisability indices). Examples of this approach are provided in Table R.10-18 in Appendix R.10-1. In addition models are available that use descriptors different from log $K_{ow}$ and/or quantum-mechanical indices and are not explicitly based on mechanistic assumptions (although mechanistic interpretation in some degree is possible). Examples are given in Table R.10-19 in Appendix R.10-1.

- QICARs and QCARs for metals and inorganic metal compounds

Development of QSAR methods for metals and inorganic metal compounds have not been as actively pursued as for organic substances. However, for some very data poor inorganic substances with toxicity databases lacking sufficient information with which to include for example speciation modelling, predicting bioactivity from chemical properties may be relevant. Recently in this respect Quantitative Ion Character-Activity Relationships (QICARs) and Quantitative Cationic-Activity Relationships (QCARs) have been developed (Owbny and Newman 2003, Walker et al. 2003.). However, more research efforts are needed in this field to develop and validate appropriate models.

[4] For the ongoing discussion about the possible reasons for the different subtypes of narcosis see e.g. Roberts and Costello (2003), Vaes et al. (1998), and Escher and Hermens (2002).

- **QSAR Predictions from expert systems**

There are a number of expert systems developed that combine multiple QSAR models to predict aquatic toxicological endpoints[5]. Detailed description of formalised expert systems is provided in Table R.10-20 in Appendix R.10-1.

- **Databases of (Q)SAR predictions**

Little reference can be given for such databases as many developments are ongoing. One example is the so called *Danish Database* of QSAR predictions (http://ecbqsar.jrc.it/), which is a compilation of predicted values from a large number of literature and commercial models, including peer-reviewed and non-peer-reviewed models, for a number of endpoints (including acute toxicity to aquatic organisms). The database does not directly contain information relating in a systematic way to the five OECD principles for validation of QSAR models. Nevertheless some overview information concerning model description and validation status is provided in the user manual for the database also available on the above mentioned web site. Furthermore for all predictions made by Multicase models for the various endpoints, the database contains a short *yes/no* statement on whether or not the individual prediction falls within or outside the applicability domain of the model. These statements are made by use of the statistically based features of applicability domain definition of the Multicase platform by use of the most stringent set-up possible.

- **Activity-activity relationships (QAARs) predictions**

In addition to structure activity relationships, information can be derived from quantitative activity-activity relationships. Many models have been developed and described in the literature. They are generally based on the premise that the chemicals might have the same mode of action across the species from different levels, although there might be more or less apparent exceptions (e.g. for aromatic amines, Urrestrazu Ramos et al. 2002). Examples of different QAARs are shown in Table R.10-20 in Appendix R.10-1

Grouping approaches

General guidance for the use of grouping approaches is provided in Section R.6.2. Tools for the identification of possible analogues are also described in Section R.6.2.3.

In order to derive information about the toxicity of chemicals, the comparability search should focus on substances that are comparable with respect to their aquatic toxicity. Additional guidance on this point is provided in Section R.7.8.4.

### R.10.2.3 Environmental compartments

For environmental effects assessment, three main environments are considered: water, soil and air.

The compartmentalisation of the environment is primarily based on the distinction between aquatic and terrestrial ("land") environments. For aquatic risk assessment, fresh water and marine environments are considered separately. In addition to these environments, risk assessment procedures have been developed for special routes of exposure or areas of concern, which are

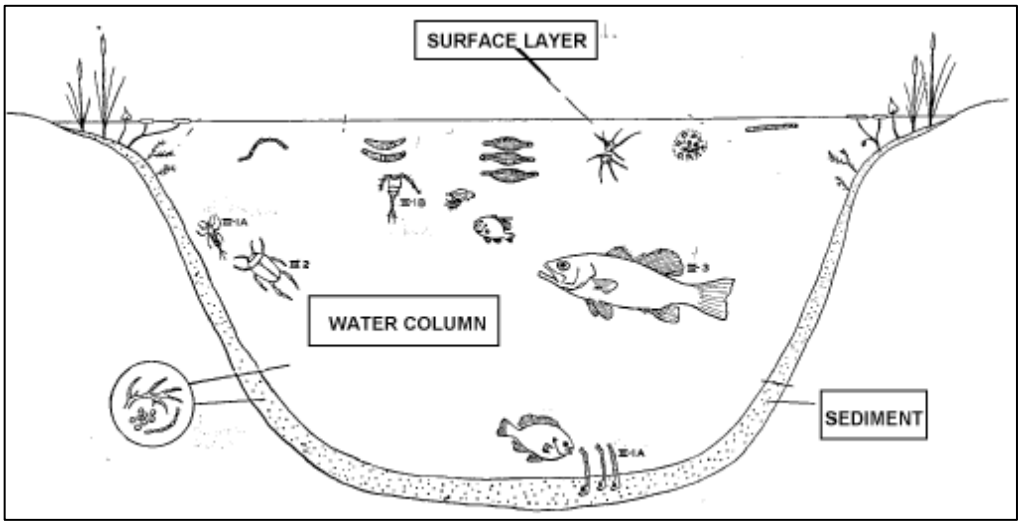
[5] A comprehensive review of such expert systems is available from ECETOC (2003b). Moore et al. (2003) published a comparative analysis on model performance of six software packages that predict acute toxicity to fish.

described as “predators exposed via the food chain” and “micro-organisms in waste water treatment plants”.

Whilst these environments are taken as a starting point for an environmental assessment, in many cases further sub-division is often necessary. Within each environment, two (or three) compartments are defined. In aquatic environments the main compartments are the water column and the sediment. For terrestrial ecosystems, the environment is divided into the soil and the “above soil” compartments. Inland waters that are generally protected against wind (e.g. ponds) may develop a surface layer on top of the water column. This layer forms a special habitat with a special exposure to chemicals; i.e. exposure is mainly via atmospheric deposition and not via the water column.

Compartmentalisation of the environment is illustrated in Figure R.10-1, showing the different possible compartments in fresh water.

**Figure R.10-1: Schematic illustration of environmental compartments of the aquatic environment[6]**




The reason for this compartmentalisation is that conditions differ profoundly between the defined environments and compartments. The presence of different types of particles (organic and clay) in sediment and soil imply that some substances may become strongly attached (sorbed) to these particles. This leads to a large decrease in the availability of these substances to the organisms that live in the compartment (bioavailability). As a result, exposure is reduced as compared to compartments with only few particles such as the water column and air. Furthermore, the types of organisms inhabiting the different environments/compartments are not the same. Therefore, the sensitivity of the organisms and/or populations in various compartments may differ considerably.

An overview of the different compartments is presented in the following tables, which are also indicating the sections in this guidance, where the derivation of the individual parameters are described in detail.

[6] From E.P. Odum (1971): Fundamentals of Ecology, 3rd edition, WB Saunders Company, Philadelphia.

**Table R.10-2. Relationship between different targets of the risk characterisation for different inland compartments**

| Target | Medium of exposure (PEClocal / PECregional) | Section | PNEC | Section |
|---|---|---|---|---|
| Aquatic organisms | Surface water | R.16.5.6.2.<br>R.16.5.6.8 | $PNEC_{water}$ | R.10.3 |
| Benthic organisms | Sediment | R.16.5.6.3<br>R.16.5.6.8 | $PNEC_{sed}$ | R.10.5 |
| Terrestrial Organisms | Agricultural soil | R.16.5.6.6<br>R.16.5.6.8 | $PNEC_{soil}$ | R.10.6 |
| Fish-eating Predators | Fish | R.16.5.7 | PNECoral from $NOAEL_{avian/mammalian}$ | R.10.8 |
| Worm-eating Predators | Earthworms | R.16.5.7 | PNECoral from $NOAEL_{avian/mammalian}$ | R.10.8 |
| Microorganisms | STP aeration tank | R.16.5.5 | $PNEC_{microorganisms}$ | R.10.4 |

**Table R.10-3. Relationship between different targets of the risk characterisation for different marine compartments**

| Target | Medium of exposure (PEClocal / PECregional) | Section | PNEC | Section |
|---|---|---|---|---|
| Aquatic organisms | Seawater | R.16.5.6.4 | $PNEC_{water}$ | R.10.3.2.3 |
| Benthic organisms | Marine sediment | R.16.5.6.5 | $PNEC_{marine\ sed}$ | R.10.5.3 |
| Fish-eating predators | Fish | R.16.5.7 | $PNECoral_{predators}$ | R.10.8 |
| Top predators | Fish-eaters | R.16.5.7 | PNECoral, top predators | R.10.8 |

### R.10.2.4 Calculations - extrapolation methods

Because the conditions of the laboratory test methods differ from natural conditions, it is considered most likely that ecosystems will be more sensitive to the chemicals than individual organisms in the laboratory. Therefore, the results of tests are not used directly for the risk assessment but used as a basis for extrapolation of the PNEC.

Extrapolation methods have been developed for estimating PNEC-values for chemicals in aquatic and terrestrial environments. Two different types of extrapolation methods exist: sensitivity distribution methods and assessment factor methods.

The sensitivity distribution methods

The sensitivity distribution methods are based on statistical calculations and usually require experimentally determined NOEC values for a number of species from different taxonomic groups.

These methods aim at calculating a concentration, which is assumed to protect a certain percentage (e.g. 95%) of the species of the ecosystem against toxic effects. The methods assume that the

species specific NOEC values follow a specific distribution function and that this can be applied for other taxonomic groups of species in the environment. Furthermore, it is assumed that each data point (effect concentration) represents a random sample from the possible data points. The true distribution of the sensitivity is not known but for independent samples it may be described, and average values and standard deviations may be estimated.

The assumptions and requirements for the sensitivity distribution methods are described in detail in Section R.10.3.1.3. When the available data do not fulfil these requirements (which is most often the case), the assessment factor methods are used. Therefore, the assessment factor methods are the most frequently used and mainly these methods are described in this document.

Assessment factor methods

The general principle of these methods is that the result from a laboratory test is divided by an appropriate assessment factor. The sparser the available data, the higher is the assessment factor which is applied. PNECs are estimated by division of the lowest value for the toxicity with the relevant assessment factor. Results of long-term tests (expressed as $EC_{10}$ or NOEC for a sublethal parameter) are preferred to those of short-term tests ($EC/LC_{50}$), because such results give a more realistic picture of effects on the organisms during their entire life cycle.

In establishing the size of these assessment factors, a number of uncertainties have been addressed to extrapolate from single-species laboratory data to a multi-species ecosystem. These areas comprise:

- intra- and inter-laboratory variation of toxicity data;
- intra- and inter-species variations (biological variance);
- short-term to long-term toxicity extrapolation;
- laboratory data to field impact extrapolation.

## R.10.3 Aquatic compartments (freshwater and marine).

### R.10.3.1 Freshwater compartment

#### R.10.3.1.1 Data

The data available depend on the tonnage as the requirements are defined according to Annexes VII-X of the regulation which are based on the tonnage of manufactured and/or imported substance but also on all available information on the substance. The minimum data set available at 10 t/y (Annex VII) includes results of tests with organisms from three trophic levels: Primary producers (plants), represented by algae; plant eating animals, represented by invertebrates (e.g. *Daphnia*) and predators, represented by fish. These groups also represent different taxonomic groups.

#### R.10.3.1.2 Calculation of PNEC for freshwater using assessment factors

The derivation of the PNEC depends on the available data. PNECs are estimated by division of the lowest value for the toxicity with the relevant assessment factor.

The assessment factors recommended for the determination of the PNEC for the (freshwater) aquatic are shown in Table R.10-4.

When only short-term toxicity data are available, an assessment factor of 1000 will be applied on the lowest L(E)C50 of the relevant available toxicity data, irrespective of whether or not the species

tested is a standard test organism (see notes to Table R.10-4). A lower assessment factor will be applied on the lowest EC10 or NOEC derived in long-term tests with a relevant test organism.

For some compounds, a large number of validated short-term L(E)C50 values may be available. Therefore, it is proposed to calculate the geometric mean if more than one L(E)C50 value is available for the same species and end-point. Prior to calculating the geometric mean an analysis of test conditions must be carried out in order to find out why differences in response were present.

The algal growth inhibition test of the base-set is, in principle, a multi-generation test. However, for the purposes of applying the appropriate assessment factors, the EC50 is treated as a short-term toxicity value. The EC10 or NOEC from this test may be used as an additional long term result when other long-term data are available. In general, an algal EC10 or NOEC should not be used unsupported by long-term EC10 or NOECs of species of other trophic levels.

Microorganisms representing a further trophic level may only be used if non-adapted pure cultures were tested. The investigations with bacteria (e.g. growth tests) are regarded as short-term tests. Additionally, blue-green algae should be counted among the primary producers due to their autotrophic nutrition.

The assessment factors presented in Table R.10-4 below should be considered as general factors that under certain circumstances may be changed. In general, justification for changing the assessment factor could include one or more of the following:

- evidence from structurally similar compounds (evidence established by read across from closely related compounds may demonstrate that a higher or lower factor may be appropriate);
- knowledge of the mode of action including endocrine disrupting effects (Some substances, by virtue of their structure, may be known to act in a non-specific manner);
- the availability of test data from a wide selection of species covering additional taxonomic groups other than those represented by the base-set species;
- the availability of test data from a variety of species covering the taxonomic groups of the base-set species across at least three trophic levels. In such a case the assessment factors may only be lowered if these multiple data points are available for the most sensitive taxonomic group.

Specific comments on the use of assessment factors in relation to the available data set are given in the notes below Table R.10-4.

**Table R.10-4 Assessment factors to derive a $PNEC_{aquatic}$**

| Available data | Assessment factor |
|---|---|
| At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae) | 1000 [a)] |
| One long-term EC10 or NOEC (either fish or Daphnia) | 100 [b)] |
| Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae) | 50 [c)] |
| Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels | 10 [d)] |
| Species sensitivity distribution (SSD) method | 5-1<br>(to be fully justified case by case) [e)] |
| Field data or model ecosystems | Reviewed on a case by case basis [f)] |

**Notes to Table R.10-4:**

a) The use of a factor of 1000 on short-term toxicity data is a conservative and protective factor and is designed to ensure that substances with the potential to cause adverse effects are identified in the hazard assessment. It assumes that each of the uncertainties identified above makes a significant contribution to the overall uncertainty. For any given substance there may be evidence that this is not so, or that one particular component of the uncertainty is more important than any other. In these circumstances it may be necessary to vary this factor. This variation may lead to a raised or lowered assessment factor depending on the available evidence. A factor lower than 100 should not be used in deriving a $PNEC_{water}$ from short-term toxicity data except for substances with intermittent release (see Section R.10.3.3.).

Variation from a factor of 1000 should not be regarded as normal and should be fully supported by accompanying evidence.

b) An assessment factor of 100 applies to a single long-term result (e.g. EC10 or NOECs) (fish or Daphnia) if this result was generated for the trophic level showing the lowest L(E)C50 in the short-term tests.

If the only available long-term result (e.g. EC10 or NOECs)is from a species (standard or non-standard organism) which does not have the lowest L(E)C50 from the short-term tests, it cannot be regarded as protective of other more sensitive species using the assessment factors available. Thus the hazard assessment is based on the short-term data with an assessment factor of 1000. However, the resulting PNEC based on short-term data may not be higher than the PNEC based on the long-term result available.

An assessment factor of 100 applies also to the lowest of two long-term results (e.g. EC10 or NOECs) covering two trophic levels when such results have not been generated from that showing the lowest L(E)C50 of the short-term tests. This should, however, not apply in cases where the acutely most sensitive species has an L(E)C50 value lower than the lowest long term result (e.g. EC10 or NOECs) value. In such cases the PNEC might be derived by using an assessment factor of 100 to the lowest L(E)C50 of the short-term tests.

c) An assessment factor of 50 applies to the lowest of two long term results (e.g. EC10 or NOECs) covering two trophic levels when such results have been generated covering that level showing the lowest L(E)C50 in the short-term tests. It also applies to the lowest of three long term results (e.g. EC10 or NOECs) covering three trophic levels when such results have not been generated from that trophic level showing the lowest L(E)C50 in the short-term tests. This should however not apply in cases where the acutely most sensitive species has an L(E)C50 value lower than the lowest long term result (e.g. EC10 or NOECs) value. In such cases the PNEC might be derived by using an assessment factor of 100 to the lowest L(E)C50 of the short-term tests.

d) An assessment factor of 10 will normally only be applied when long-term toxicity results (e.g. EC10 or NOECs) are available from at least three species across three trophic levels (e.g. fish, Daphnia, and algae or a non-standard organism instead of a standard organism).

When examining the results of long-term toxicity studies, the PNECwater should be calculated from the lowest available long term result. Extrapolation to the ecosystem effects can be made with much greater confidence, and thus a reduction of the assessment factor to 10 is possible. This is only sufficient, however, if the species tested can be considered to represent one of the more sensitive groups. This would normally only be possible to determine if data were available on at least three species across three trophic levels.

It may sometimes be possible to determine with high probability that the most sensitive species has been examined, i.e. that a further long-term result (e.g. EC10 or NOECs) from a different taxonomic group would not be lower than the data already available. In those circumstances, a factor of 10 applied to the lowest long term result (e.g. EC10 or NOECs) from only two species would also be appropriate. This is particularly important if the substance does not have a potential to bioaccumulate. If it is not possible to make this judgment, then an assessment factor of 50 should be applied to take into account any interspecies variation in sensitivity. A factor of 10 cannot be decreased on the basis of laboratory studies.

e) Basic considerations and minimum requirements as outlined in Section R.10.3.1.3

f) The assessment factor to be used on mesocosm studies or (semi-) field data will need to be reviewed on a case-by-case basis.

For compounds with a high log *Kow* or substances that exert their effect with relatively slow metabolic (transformation) rates, no short-term toxicity may be found. Also, even in long-term tests this may be the case or steady state (as seen from the incipient LC50) may still not have been reached. In fish tests for non-polar narcotics, the latter can be substantiated by the use of long-term QSARs (see Chapter R.6 on the Use of QSARs and Section R.7.8). Use of a higher assessment factor can be considered in such cases where steady state does not seem to have been reached.

A long-term test has to be carried out for substances showing no toxicity in short-term tests if the log Kow > 3 (or BCF > 100) and if the PEClocal/regional is > 1/100th of the water solubility. The long-term toxicity test should normally be a test on invertebrate (preferred species Daphnia) to avoid unnecessary vertebrate testing. The NOEC from this test can then be used with an assessment factor of 100. If in addition to the required long-term test a NOEC is determined from an algal test of the base-set, an assessment factor of 50 is applied.

#### R.10.3.1.3 Calculation of PNEC for freshwater using statistical extrapolation techniques

The effect assessment performed with assessment factors can be supported by a statistical extrapolation method if the database on species sensitivity distributions (SSDs) is sufficient for its application. If a large data set from long-term tests for different taxonomic groups is available (OECD, 1992), statistical extrapolation methods may be used to derive a PNEC. The main underlying assumptions of the statistical extrapolation methods are as follows (OECD, 1992, Posthuma et al., 2002):

- the distribution of species sensitivities follows a theoretical distribution function;
- the group of species tested in the laboratory is a random sample of this distribution.

In general, the methods work as follows: long-term toxicity data are log transformed and fitted according to the distribution function and a prescribed percentile of that distribution is used as criterion. Several distribution functions have been proposed. The US EPA (1985) assumes a log-triangular function, Kooijman (1987) and Van Straalen and Denneman (1989) a log-logistic function, and Wagner and Løkke (1991) a log-normal function. Aldenberg and Slob (1993) refined the way to estimate the uncertainty of the 95$^{th}$ percentile by introducing confidence levels.

The approach of statistical extrapolation is still under debate and needs further validation. An advantage of these methods is that they use the whole sensitivity distribution of species in an ecosystem to derive a PNEC instead of taking always the lowest long-term NOEC. However, such methods could also be criticised. Among the most common drawbacks, the reasons put forward are: the lack of transparency by using this method compared to the standard approach, the question of representativity of the selected test species, the comparability of different endpoints, the arbitrary choice of a specific percentile and a statistical confidence level etc.

In response to these concerns it is necessary to provide some guidance on when and how to use such methods. What is proposed below has been discussed during an Expert Consultation Workshop on Statistical Extrapolation Techniques for Environmental Effects Assessments, in London on 17-18th January 2001 (EC, 2001). Although the primary objective of this workshop was focused on how statistical extrapolation techniques might be used to derive PNECs in the assessments of metals and their compounds, the general principles outlined here should be also applicable for other substances.

Input data

The methods should be applied on all reliable available NOECs from chronic/long-term studies, preferably on full life-cycle or multi-generation studies. NOECs are derived according to previous considerations (Table R.10-1).

Which taxonomic groups

It is important to include all available information on the mode of action of the chemical, in order to evaluate the need to include possible other (sensitive) taxonomic groups or exclude possible over-representation of certain taxonomic groups, realising that the mode of action may differ between short-term effects and long-term effects and between taxonomic groups. The minimum species requirements when using the Species Sensitivity Distribution method are:

- fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.);
- a second family in the phylum Chordata (fish, amphibian, etc.);
- a crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.);
- an insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.);
- a family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.);
- a family in any order of insect or any phylum not already represented;
- algae;
- higher plants.

It is recognised that for some of the taxa mentioned above, no internationally standardised test guidelines for long-term tests are currently available. The applicability of existing test data and the fulfilment of the above requirements thus need to be assessed on a case-by-case basis. There is a need to evaluate additional information in order to assess how relevant and representative the list of taxonomic groups is to the risk assessment scenario being investigated.

Minimal sample size (number of data)

Confidence can be associated with a PNEC derived by statistical extrapolation if the database contains at least 10 NOECs (preferably more than 15) for different species covering at least 8 taxonomic groups.

Deviations from these recommendations can be made, on a case-by-case basis, through consideration of sensitive endpoints, sensitive species, mode of toxic action and/or knowledge from structure-activity considerations.

How to deal with multiple data for one species?

Where appropriate and possible, a pre-selection of the data should be performed in relation to realistic environmental parameters for Europe (e.g. hardness of water, pH, organic matter and/or temperature). The full database should be carefully evaluated to extract information (e.g., on sensitive endpoints), which may be lost when “averaging” the data to a single value.

The test data applicable to the most sensitive endpoint should be taken as representative for the species. In this context, demographic parameters can be used as endpoints, as can bio-markers if they are toxicologically relevant in terms of population dynamics.

Multiple values for the same endpoint with the same species should be investigated on a case-by-case basis, looking for reasons for differences between the results. For equivalent data on the same end-point and species, the geometric mean should be used as the input value for the calculation. If this is not possible, perhaps because valid results are considered to be too variable, then grouping and combining the values, e.g. by pH ranges, and using reduced numbers of values should be considered. The effects that these different treatments have on the derived value (and on the resulting risk characterisation) should be investigated and discussed.

Where it is considered that the results are limited to certain conditions (e.g. not appropriate for low pH conditions) then these limitations should be explained. The values derived from different treatments of the data may be useful to indicate sensitive regions.

Fit to a distribution

Different distributions like e.g. log-logistic, log-normal or others may be used (Aldenberg and Jaworska, 2000). The log-normal distribution is a pragmatic choice from the possible families of distributions because of the available description of its mathematical properties (methods exist that allow for most in depth analyses of various uncertainties).

The Anderson–Darling goodness of fit test can be used in addition to the Kolmogorov-Smirnov-test, as a criterion for the choice of a parametric distribution for comprehensive data sets, because it gives more weight to the tails of the distribution. A lack of fit may be caused by very different factors. One common factor seems to be the inclusion of several NOECs for species tested in a single laboratory, where the same test concentrations were used for all species. The statistical determination of the NOEC can lead to the same value being obtained for several species, showing up as a vertical row of NOECs in the cumulative distribution plots. Another reason for lack of fit is a possible bimodality of the SSD, due to a specific mode of action of the tested substance towards only some taxonomic groups of species.

Whatever the fit to a distribution, results should be discussed in regards to the graphical representation of the species distribution and the different p values that were obtained with each test. Finally, any choice of a specific distribution function should be clearly explained.

If the data do not fit any distribution, the left tail of the distribution (the lowest effect concentrations) should be analysed more carefully. If a subgroup of species can be identified as particularly sensitive and if the number of data on this subgroup is sufficient, the distribution can be fit to this subgroup. In case of lack of fit, the SSD method should not be used.

Estimated parameter

For pragmatic reasons it has been decided that the concentration corresponding with the point in the SSD profile below which 5% of the species occur should be derived as an intermediate value in the determination of a PNEC. A 50% confidence interval (c.i.) associated with this concentration should also be derived.

Estimation of the PNEC

The PNEC is calculated as:

$$PNEC = \frac{5\%SSD(50\%c.i.)}{AF}$$

**Equation R.10-1**

AF is an appropriate assessment factor between 5 and 1, reflecting the further uncertainties identified. Lowering the AF below 5 on the basis of increased confidence needs to be fully justified. The exact value of the AF must depend on an evaluation of the uncertainties around the derivation of the 5$^{th}$ percentile. As a minimum, the following points have to be considered when determining the size of the assessment factor:

- the overall quality of the database and the endpoints covered, e.g., if all the data are generated from "true" chronic studies (e.g., covering all sensitive life stages);
- the diversity and representativity of the taxonomic groups covered by the database, and the extent to which differences in the life forms, feeding strategies and trophic levels of the organisms are represented;
- knowledge on presumed mode of action of the chemical (covering also long-term exposure). Details on justification could be referenced from structurally similar substances with established mode of action;
- statistical uncertainties around the 5$^{th}$ percentile estimate, e.g., reflected in the goodness of fit or the size of confidence interval around the 5$^{th}$ percentile, and consideration of different levels of confidence (e.g. by a comparison between the 5% of the SSD (50%) with the 5% of the SSD (95%));
- comparisons between field and mesocosm studies, where available, and the 5$^{th}$ percentile and mesocosm/field studies to evaluate the laboratory to field extrapolation.

A full justification should be given for the method used to determine the PNEC.

<u>Further recommendations</u>

NOEC values below the 5% of the SSD need to be discussed in the risk assessment report. For example if all such NOECs are from one trophic level, then this could be an indication that a particular sensitive group exists, implying that some of the underlying assumptions for applying the statistical extrapolation method may not be met;

The deterministic PNEC should be derived by applying the "standard" assessment factor approach on the same database;

If mesocosm studies are available, they should also be evaluated and a PNEC derived following the guidance document according to the standard method (deterministic approach).

The various estimates of PNEC should be compared and discussed and the final choice of a PNEC be based on this comparison.

### R.10.3.2 Marine compartment

#### R.10.3.2.1 Introduction

Marine effects assessment should ideally be based upon data generated using a range of ecologically relevant saltwater species (for example algae, invertebrates and fish). However, such data are rarely available and, therefore, guidance is given on how marine hazard assessment can be based on available data on both freshwater and saltwater organisms.

It is assumed that the greater species diversity in the marine environment, compared to freshwaters, including the presence of a number of taxa that occur only in the marine environment, implies a broader distribution of sensitivities of species and a higher uncertainty in extrapolation. Table R.10-5 describes the assessment factors for marine hazard assessment, which includes a factor of 10,000 for assessments based on data from tests with the three standard fresh water species.

Historically, the patterns of chemical production and usage resulting from urban and industrial development have led to the freshwater environment being considered to be the hydrosphere most at risk from these substances. Consequently, most regulatory schemes for evaluating the hazards and risks posed by new and existing substances have focussed primarily on the protection of freshwater communities. As a result there is a considerable body of data on the ecotoxicity of chemical substances to freshwater organisms (ECETOC, 1994a)[7].

Where there is a need to assess the potential impact of substances entering estuarine and marine waters, any hazard or risk assessment should ideally be based upon data generated using a range of ecologically relevant saltwater species (for example algae, invertebrates and fish). This is particularly important given the greater diversity of species (particularly invertebrates) present in marine waters, relative to freshwaters. There are also circumstances, however, where the special conditions existing in a particular environment such as that existing in the Baltic Sea, give rise to a reduced or limited species diversity and/or specific stresses such as low or variable salinity. In such circumstances of low species diversity, adverse impacts in individual species can have devastating impacts on the specialised ecosystem. Thus, while high species diversity may lead to a wide sensitivity distribution, but also considerable functional overlap, low species diversity may result in a lower sensitivity distribution but increase the ecosystem function dependency on individual keystone species.

In both cases, the effects assessment must use, where possible, data relevant to the environmental compartment that is considered. However, compared to the situation for freshwaters, there are relatively few data on the effects of chemical substances on estuarine and marine organisms. Therefore, in practice there will be situations where saltwater toxicity data are needed for hazard/risk assessments, but may not be available. In these situations it may be necessary to use freshwater data *in lieu* of data for estuarine/marine species (Schobben et al., 1994; Karman et al., 1998). In using data on freshwater species to characterise the risk in the marine waters, a clear understanding of the comparability of effects data generated on both types of species is necessary. Furthermore, there is some evidence, e.g. for some metals, that species living in brackish water are more susceptible because of the salinity (osmotic) stress they have to endure in contrast to those of the

[7] The ECETOC database consists of 2,203 entries on 361 chemicals, covering 121 species. Data on freshwater species accounted for 1862 entries (84.5%) while data for saltwater (estuarine/marine) species accounted for 341 entries (15.5%).

same species living in truly marine conditions. Under these circumstances the applicability of the toxicity data needs to be considered on a case-by-case basis.

#### R.10.3.2.2 Data

It has been recognised for many years that there is a wider diversity of taxonomic groups (particularly invertebrates) in saltwaters compared to freshwaters and that many groups are only found in marine waters (see Russell and Yonge, 1928; Tait, 1978). Moss (1988) stated that 56 phyla were present in marine waters compared to 41 in freshwaters. No phyla are confined to freshwaters only while 15 phyla are found only in marine waters. These differences are partly due to the fact that multicellular animals originated in the seas and they have been well populated since the earliest fossil records.

Nevertheless, an important part of any evaluation of data must involve an assessment of the usefulness of the main body of freshwater ecotoxicity data in predicting effects in the marine environment. Where such data can be used, the focus of further investigation can concentrate on additional factors which specifically characterise the marine conditions. Studies conducted on the comparability of sensitivity of freshwater and marine species have been hampered by the low level of substances for which a comparable dataset has been available. Nevertheless where such data are available, it has tended to show that there is no systematic bias in sensitivity where comparable tests and endpoints are paired. A recent report which collated much of the available data confirmed these findings (ECETOC, 2000). Based on the currently available data, it can be concluded that:

- overall, the data reviewed and current marine risk assessment practice suggest a reasonable correlation between the ecotoxicological responses of freshwater and saltwater biota - at least for the usual aquatic taxa (i.e., fish, crustacea, algae). No marked difference in sensitivity between freshwater and saltwater biota appears that systematically applies across all three trophic levels considered;
- where evaluated, differences between trophic levels within each medium were generally as significant or even more marked than between media. Such variation is implicitly assumed in the use of assessment factors in current risk assessment practice;
- where differences in the apparent sensitivity of freshwater and marine biota were observed for individual compounds, such differences were consistently within a factor of 10 (<1 log unit) and usually somewhat less;
- average differences in sensitivity for such paired species comparisons were typically within a factor of ~2;
- however, within trophic levels differences larger than a factor of 10 were shown for several metals and pesticides indicating that for these substances fresh water and saltwater data should not be pooled for hazard assessment and PNEC derivation.

The use of freshwater acute effects data *in lieu* of or in addition to saltwater effects data for risk assessment purposes is not contra-indicated by the empirical data reviewed. Use of pooled data is therefore recommended. Under such circumstances, PNEC values should be derived from the most sensitive endpoint regardless of the medium.

No comparison of long-term effects data has been made due to the lack of suitable data but again there are no reasons to believe that a systematic bias to freshwater or marine species would exist. Therefore it is proposed that data on freshwater or marine fish, crustacea and algae be used interchangeably for evaluation of the risks to either compartment.

### R.10.3.2.3 Calculation of PNEC for marine water

The greater species diversity in the marine environment compared to freshwater, including the presence of a number of taxa that occur only in that environment, may mean that the distribution of sensitivities of species is broader. It is necessary to consider, therefore, whether the three-taxa model offers sufficient certainty that sensitive species will be covered using the assessment factors developed for the freshwater systems. Since it is not possible to make a clear judgement on the basis of available data, it is considered prudent to assume that this greater diversity of taxa will produce a broader distribution of species sensitivity. Thus, where only data for freshwater or saltwater algae, crustaceans and fish is available, a higher assessment factor than that for the derivation of $PNEC_{water}$ for freshwaters should be applied, to reflect the greater uncertainty in the extrapolation. Where data is available for additional taxonomic groups, for example rotifers, echinoderms or molluscs the uncertainties in the extrapolation are reduced and the magnitude of the assessment factor applied to a dataset can be lowered. Test protocols for these groups are available from organisations such as the American Society for Testing and Materials, the International Council for the Exploration of the Seas and the United States Environmental Protection Agency (OECD, 1998a). The list of standardised tests available for marine species is available in Appendix R.7.8-2. The assessment factors given are based on current scientific understanding on the species comparability of toxicity between freshwater and saltwater species and the issue of differences in diversity in freshwaters and saltwaters. These may need to be revisited as additional information becomes available.

It is recognised that the assumption of a greater species sensitivity distribution covering the additional marine taxa is based on limited data and is precautionary. The generation of additional toxicity data on marine species may allow this assumption to be further refined such that lower or higher assessment factors may be considered following a systematic review of accumulating evidence.

The additional assessment factor is also considered sufficient to cover the situations noted above where low species diversity may result in high ecosystem dependency on individual species.

The assessment factors decrease in magnitude from higher values for short-term acute studies from which L(E)C50 values have been derived to lower values for long-term chronic studies from which EC10 or NOECs have been derived. For long-term studies the magnitude of the assessment factors also decreases as information on a wider range of species becomes available. The assessment factors described in Table R.10-5 are those that would normally be applied to the datasets available. There are some circumstances, however, where expert judgement may be applied to the interpretation of a dataset which may allow a pragmatic approach to the application of the factors and the generation of new data. In each case where expert judgement is so applied, a full justification must be provided.

**Table R.10-5 Assessment factors proposed for deriving PNECwater for saltwater for different data sets**

| Data set | Assessment factor |
|---|---|
| Lowest short-term L(E)C50 from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans and fish) of three trophic levels | 10,000 [a)] |
| Lowest short-term L(E)C50 from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans and fish) of three trophic levels, + two additional marine taxonomic groups (e.g. echinoderms, molluscs) | 1000 [b)] |
| One long-term result (e.g. EC10 or NOEC) (from freshwater or saltwater crustacean reproduction or fish growth studies) | 1000 [b)] |
| Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) | 500 [c)] |
| Lowest long-term results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels | 100 [d)] |
| Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) + one long-term result from an additional marine taxonomic group (e.g. echinoderms, molluscs) | 50 |
| Lowest long-term results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels + two long-term results from additional marine taxonomic groups (e.g. echinoderms, molluscs) | 10 |

**Notes to Table R.10-5:**

Evidence for varying the assessment factor should in general include a consideration of the availability of data from a wider selection of species covering additional feeding strategies/ life forms/ taxonomic groups other than those represented by the algal, crustacean and fish species (such as echinoderms or molluscs). This is especially the case, where data are available for additional taxonomic groups representative of marine species. More specific recommendations as with regard to issues to consider in relation to the data available and the size and variation of the assessment factor are indicated below.

When substantiated evidence exists that the substances may be disrupting the endocrine system of mammals, birds, aquatic or other wildlife species, it should be considered whether the assessment factor would also be sufficient to protect against effects caused by such a mode of action, or whether an increase of the factor would be appropriate.

a)

The use of a factor of 10,000 on short-term toxicity data is a conservative and protective factor and is designed to ensure that substances with the potential to cause adverse effects are identified in the hazard assessment. It assumes that each of the identified uncertainties described above makes a significant contribution to the overall uncertainty.

For any given substance there may be evidence that this is not so, or that one particular component of the uncertainty is more important than any other. In these circumstances it may be necessary to vary this factor. This variation may lead to a raised or lowered assessment factor depending on the evidence available. Except for substances with intermittent release, as defined in Section R.10.3.3., under no circumstances should a factor lower than 1000 be used in deriving a $PNEC_{water}$ for saltwater from short-term toxicity data.

Evidence for varying the assessment factor could include one or more of the following:

- evidence from structurally similar compounds which may demonstrate that a higher or lower factor may be appropriate.
- knowledge of the mode of action as some substances by virtue of their structure may be known to act in a non-specific manner. A lower factor may therefore be considered. Equally a known specific mode of action may lead to a higher factor.
- the availability of data from a variety of species covering the taxonomic groups of species across at least three trophic levels. In such a case the assessment factors may only be lowered if multiple data points are

available for the most sensitive taxonomic group (i.e. the group showing acute toxicity more than 10 times lower than for the other groups).

Variation from an assessment factor of 10000 should be fully reported with accompanying evidence.

b)

An assessment factor of 1000 applies where data from a wider selection of species are available covering additional taxonomic groups (such as echinoderms or molluscs) other than those represented by algal, crustacean and fish species; if at least data are available for two additional taxonomic groups representative of marine species.

An assessment factor of 1000 applies to a single long-term result (e.g. EC10 or NOEC) (freshwater or saltwater crustacean or fish) if this result was generated for the taxonomic group showing the lowest L(E)C50 in the short-term algal, crustacean or fish tests.

If the only available long-term result (e.g. EC10 or NOEC) is from a species which does not have the lowest L(E)C50 in the short-term tests, it cannot be regarded as protective of other more sensitive species using the assessment factors available. Thus, the hazard assessment is based on the short-term data with an assessment factor of 10,000. However, normally the lowest PNEC should prevail.

An assessment factor of 1000 applies also to the lowest of the two long-term results (e.g. EC10 or NOEC) covering two trophic levels (freshwater or saltwater algae and/or crustacean and/or fish) when such results (e.g. EC10 or NOEC) have not been generated for the species showing the lowest L(E)C50 of the short-term tests.

This should not apply in cases where the acutely most sensitive species has an L(E)C50-value lower than the lowest long term value. In such cases the PNEC might be derived by applying an assessment factor of 1000 to the lowest L(E)C50 of the short-term tests.

c)

An assessment factor of 500 applies to the lowest of two long term results (e.g. EC10 or NOEC) covering two trophic levels (freshwater or saltwater algae and/or crustacean and/or fish) when such results have been generated covering those trophic levels showing the lowest L(E)C50 in the short-term tests with these species. Consideration can be given to lowering this factor in the following circumstances:

- It may sometimes be possible to determine with a high probability that the most sensitive species covering fish, crustacea and algae has been examined, that is that a further longer-term result (e.g. EC10 or NOEC) from a third taxonomic group would not be lower than the data already available. In such circumstances an assessment factor of 100 would be justified;
- a reduced assessment factor (to 100 if only one short-term test, to 50 if two short-term tests on marine species are available) applied to the lowest long term result (e.g. EC10 or NOEC) from only two species may be appropriate where:
- short-term tests for additional species representing marine taxonomic groups (for example echinoderms or molluscs) have been carried out and indicate that these are not the most sensitive group, and;
- it has been determined with a high probability that long-term results (e.g. EC10 or NOEC) generated for these marine groups would not be lower than that already obtained. This is particularly important if the substance does not have the potential to bioaccumulate.

An assessment factor of 500 also applies to the lowest of three long term results (e.g. EC10 or NOEC) covering three trophic levels, when such results have not been generated from the taxonomic group showing the lowest L(E)C50 in short-term tests. This should, however, not apply in the case where the acutely most sensitive species has an L(E)C50 value lower than the lowest long term result (e.g. EC10 or NOEC) value. In such cases the PNEC might be derived by applying an assessment factor of 1000 to the lowest L(E)C50 in the short-term tests.

d)

An assessment factor of 100 will be applied when longer-term toxicity results (e.g. EC10 or NOEC) are available from three freshwater or saltwater species (algae, crustaceans and fish) across three trophic levels.

The assessment factor may be reduced to a minimum of 10 in the following situations:

- where short-term tests for additional species representing marine taxonomic groups (for example echinoderms or molluscs) have been carried out and indicate that these are not the most sensitive group, and it has been determined with a high probability that long-term results (e.g. EC10 or NOEC) generated for these species would not be lower than that already obtained;
- where short-term tests for additional taxonomic groups (for example echinoderms or molluscs) have indicated that one of these is the most sensitive group acutely and a long-term test has been carried out for that species. This will only apply when it has been determined with a high probability that additional long

term results (e.g. EC10 or NOEC) generated from other taxa will not be lower than the long term results already available.

A factor of 10 cannot be decreased on the basis of laboratory studies only.

Statistical extrapolation methods for calculation of PNEC for marine organisms could be used when sufficient data are available. More information on these methods and the prerequisites to apply them for risk assessment purposes can be found in Section R.10.3.1.3 in this document.

### R.10.3.3 Calculation of PNEC for water in the case of intermittent releases

The PNEC-values derived for freshwater or marine waters are based on the implicit assumption that the environmental exposure is constant, e.g. arising from a constant or frequent release.

However, in many cases, discharges will be limited in time, e.g. in case of emissions from batch productions (for details regarding the definition of "intermittent releases", see section R.16.2.1.5). In such cases, the environmental exposure will also be limited in time, and it is assumed that when exposure stops rapidly, populations can tolerate higher concentrations than when it is long lasting.

In these cases, short-term $L(E)C_{50}$ values are used to derive a $PNEC_{water,\ intermittent}$. The $PNEC_{water,\ intermittent}$ for such situations is normally derived by application of an assessment factor of 100 to the lowest $L(E)C_{50}$ of at least three short-term tests from three trophic levels. The assessment factor is designed to take account of the uncertainty that exists in extrapolating from the results of short-term laboratory toxicity tests to short-term effects that can be anticipated in the ecosystems.

In undertaking such an extrapolation, due account is taken of the biological variables of intra- and inter-species toxicity, as well as the general uncertainties in predicting ecosystem effects from laboratory data. This extrapolation should be carried out with care. Some substances may be taken up rapidly by aquatic organisms and this can lead to delayed effects even after exposure has ceased. This will generally be taken into account by the assessment factor of 100 but there may be occasions when a higher or lower factor would be appropriate. For substances with a potential to bioaccumulate the lowered assessment factor of 100 may not always be sufficient to provide adequate protection. For substances with a known non-specific mode of action, interspecies variations may be low. In such cases, a lower factor may be appropriate. In no case should a factor lower than 10 be applied to a short-term L(E)C50 value.

## R.10.4 Micro organisms in sewage treatment plants (STP)

### R.10.4.1 Introduction

Since chemicals may cause adverse effects on microbial activity in STPs it is necessary to derive a $PNEC_{microorganisms}$. The $PNEC_{microorganisms}$ will be used for the calculation of the PEC/PNEC ratio concerning microbial activity in STPs.

In general, the aim of the assessment is the protection of the degradation and nitrification functions and process performance and efficiency of domestic and industrial STPs – as also influenced by protozoan populations. The toxicity of a substance to microorganisms in a STP is assessed by comparing the concentration of a substance in STP aeration tank with the microbial effect concentration data for that substance (see also Section R.7.8.16 and R.7.8.17). If the substance under consideration is relevant for industrial and municipal STPs the toxicity assessment should be conducted for both kinds of STPs separately. A $PNEC_{microorganisms}$ should be obtained as a first step in the hazard assessment for microorganisms in both domestic and industrial sewage treatment

plants. The $PNEC_{microorganisms}$ is usually derived from results obtained in the most sensitive test system available.

More information and guidance about information on toxicity to STP micro-organisms is available in Section R.7.8.14 to R.7.8.20.

### R.10.4.2 Calculation of PNEC for micro organisms in STP

Table R.10-6 provides a complete listing of the tests systems mentioned in Section R.7.8.16 and R.7.8.17, effect concentrations that are determined using them as well as the corresponding assessment factors. Some explanations to the table are given below.

An assessment factor (AF) of 10 is to be applied to the NOEC of a sludge respiration test, reflecting the lower sensitivity of this endpoint as compared to nitrification, as well as the short duration of the test. The corresponding AF is 100 when based on the EC50.

The $PNEC_{stp}$ is set equal to a NOEC (AF = 1) for a test performed with specific bacterial populations such as nitrifying bacteria, *P. putida*, ciliated protozoa, the Shk1 Assay. An $EC_{50}$ from this test is divided by an AF of 10 to derive the $PNEC_{stp}$.

If no standard microbial inhibition test data are available, the $PNEC_{stp}$ can also be derived from available ready biodegradation tests. An assessment factor of 10 is applied to the test concentration at which no toxicity to the inoculum was observed. This approach can also be used for inherent biodegradability tests.

From an activated sludge simulation study, a $PNEC_{stp}$ can be derived based on the $PEC_{stp}$ or $PEC_{influent}$, using an AF between 1 and 10 depending on the parameters monitored. The AF of 1 can be used in case there is no impact on nitrification and BOC/COD removal performance (NB: if sludge from an industrial STP was used for the test, the $PNEC_{stp}$ can not be used for the extrapolation to a domestic STP).

No AF is needed to derive a $PNEC_{stp}$ based on good quality field data as this has to be assessed by expert judgement.

**Table R.10-6 Test systems for derivation of $PNEC_{microorganisms}$**

| **Test** | **Available Value** | **Assessment Factor for PNEC Derivation** |
|---|---|---|
| Respiration inhibition tests | NOEC or $EC_{10}$ | 10 |
| EU Annex V of Directive 67/548/EEC C.11; OECD 209 (1984) ISO 8192 (1986)<br>*(Painter 1986)* | $EC_{50}$ | 100 |
| Inhibition control in standardised biodegradation tests<br>- Ready biodegradability tests<br>EU Annex V C.4 A-F; OECD 301A-F (1992)<br>92/69/EEC C4 (1992), OECD 310 (2006)<br>ISO-7827 (1994), -9439 (1999), -10707 (1994), -9408 (1999)<br>- Inherent biodegradability tests<br>EU Annex V C.9; OECD 302 B-C (1981-1992)<br>88/302/EEC (1988)<br>ISO-9888 (1999) | The tested concentration at which toxicity to the inoculum can be ruled out with sufficient reliability (cf. corresponding text section above) can be considered as a NOEC for the toxicity to STP microorganisms | 10 |
| Pilot scale activated sludge simulation tests (CAS)<br>OECD 303A (2001)<br>ISO-11733 (1998) | Based on case-by-case expert judgement, the tested concentration not impairing proper functioning of the CAS [1)] unit can be considered as NOEC for STP microorganisms | Case-by-case: < 5, and down to 1 for a well executed and documented test |
| Inhibition of nitrification | NOEC or EC10 | 1 |
| ISO-9509 (1989) | $EC_{50}$ | 10 |
| Activated sludge growth inhibition tests | NOEC or $EC_{10}$ | 10 |
| ISO-15522 (1999) | $EC_{50}$ | 100 |
| Ciliate growth inhibition tests | NOEC or $EC_{10}$ | 1 |
| (preferably with *Tetrahymena sp.;* OECD 1998) | $EC_{50}$ | 10 |
| Growth inhibition test with *Pseudomonas putida* | NOEC or $EC_{10}$ | 1 |
| ISO-10712 (1996) | $EC_{50}$ | 10 |
| *(Bringmann and Kühn 1980)* | to be used only if no other tests are available | |
| Shk1 Assay<br>*(Kelly et al, 1999)* | $EC_{50}$ | 10 |
| | to be used only if no other tests are available | |
| Pseudomonas fluorescens inhibition test<br>*(Bringmann and Kühn 1960)* | Single species tests with limited relevance for STP as it uses glucose as substrate | |
| Escherichia coli inhibition test<br>*(Bringmann and Kühn 1960)* | Single species tests with limited relevance for STP as it uses glucose as substrate | |
| Vibrio fischeri (MICROTOX® test)<br>ISO 11348-1, -2, -3 (1999) | Single species test based on a marine bacterium, with limited relevance for STP functioning | |

1) CAS: Continuous Activated Sludge test

## R.10.5 Sediments

### R.10.5.1 Introduction

Sediments may act as both a sink for chemicals through sorption of contaminants to particulate matter, and a source of chemicals through resuspension. Sediments integrate the effects of surface water contamination over time and space, and may thus present a hazard to aquatic communities (both pelagic and benthic) which is not directly predictable from concentrations in the water column. Effects on benthic organisms are of concern because they constitute an important link in aquatic food chain and play an important role in the recycling of detritus material. Due to the lack of standardised test methods on, e.g., the role of microorganisms in recycling of detritus material and nutrients, further tests needs to be developed and to be added for guidance in future.

Statistical extrapolation methods for calculation of PNEC for sediment organisms could be used when sufficient data are available (see Section R.10.3.1.3). Further guidance needs to be developed in future.

### R.10.5.2 Freshwater sediment

#### R.10.5.2.1 Calculation of PNEC for freshwater sediment using equilibrium partitioning

In the absence of any ecotoxicological data for sediment-dwelling organisms, the $PNEC_{sed}$ may be provisionally calculated using the equilibrium partitioning method (EPM). This method uses the $PNEC_{water}$ for aquatic organisms and the suspended matter/water partitioning coefficient as inputs (OECD, 1992b; Di Toro et al., 1991).

It has to be considered that the equilibrium partitioning method may result both in an overestimation or underestimation of the toxicity to benthic organisms (Di Toro et al. 2005). Therefore this method can only be used as rough screening to decide whether sediment toxicity tests with benthic organisms are required.

In the partitioning method, it is assumed that the:

- sediment-dwelling organisms and water column organisms are equally sensitive to the chemical;
- concentration of the substance in sediment, interstitial water and benthic organisms are at thermodynamic equilibrium: the concentration in any of these phases can be predicted using the appropriate partition coefficients;
- sediment/water partition coefficients can either be measured or derived on the basis of a generic partition method from separately measurable characteristics of the sediment and the properties of the chemical (for the derivation of the sediment-water partition coefficient and the limits of the calculation methods see Section R.16.4.3.3).

The following formula, which is based on equilibrium partitioning theory, is applied:

$$PNEC_{sed} = \frac{K_{susp-water}}{RHO_{susp}} \cdot PNEC_{water} \cdot 1000$$

**Equation R.10-2**

**Explanation of symbols**

| | | | |
|---|---|---|---|
| $PNEC_{water}$ | Predicted No Effect Concentration in water | $[mg \cdot l^{-1}]$ | |
| $RHO_{susp}$ | bulk density of wet suspended matter | $[kg \cdot m^{-3}]$ | 1150 |
| $K_{susp\ water}$ | partition coefficient suspended matter water | $[m^3 \cdot m^{-3}]$ | Eq. R.16-14 |
| $PNEC_{sed}$ | Predicted No Effect Concentration in sediment | $[mg \cdot kg^{-1}$ of wet sediment] | |

The following qualifying comments apply regardless of whether the $K_{susp\ water}$ is measured or estimated:

- the formula only considers uptake via the water phase. However, uptake may also occur via other exposure pathways like ingestion of sediment and direct contact with sediment. This may become important, especially for adsorbing chemicals, for example those with a log *Kow* greater than 3. For soil invertebrates (earthworms) it was shown that the assumption of EPM holds up to log Kow of 6 (Jager, 2004). For these compounds the total uptake may be underestimated;
- EPM probably overestimates the actual uptake from soil by soil invertebrates (Jager, 2004). However, this relation is complicated and probably depends on the ability to properly calculate the dissolved concentration in the soil (UK Environment Agency, XXX). Therefore it is considered that the possible overestimation of exposure is acceptable when using the equilibrium partitioning method for chemicals with a log *Kow* between 3 and 6;
- for compounds with a log Kow greater than 5 or with a corresponding adsorption or binding behaviour not triggered by the lipophilicity (e.g. log Kow) of the substance but by other mechanisms (e.g. ionisable substances, surface active substances, substances forming covalent bound to sediment, components like e.g. aromatic amines) the equilibrium method is used in a modified way.

In order to take uptake via ingestion of sediment into account, the $PEC_{sed}/PNEC_{sed}$ ratio is increased by a factor of 10. It should be borne in mind that this approach is considered only as a screen for assessing the level of risk to sediment dwelling organisms. If with this method a PEC/PNEC ratio > 1 is derived, the testing strategy developed in Section R.7.8.12 should be applied.

#### R.10.5.2.2 Calculation of PNEC for fresh water sediment using assessment factors

If results from whole-sediment tests with benthic organisms are available the $PNEC_{sed}$ has to be derived from these tests using assessment factors. However, the available sediment tests should be carefully evaluated. Special attention should be given to the pathways through which the test organisms are exposed to the chemical and the test protocol should carefully be checked, whether feeding with unspiked food has possibly reduced exposure via sediment ingestion. For assessing the toxicity of spiked sediment it is necessary to address adequately all possible routes of exposure. Sediment organisms can be exposed via their body surfaces to substances in solution in the overlying water and in the pore water and to bound substances by direct contact or via ingestion of contaminated sediment particles. The route that is most important is strongly influenced by species-specific feeding mechanisms and the behaviour of the organism in, or on, the sediment. Test design parameters can have a bearing on the route of uptake of a substance. Further guidance on the tests to perform is provided in Section R.7.8.

A number of uncertainties have to be addressed (see Section R.10.3.1.2) in establishing the size of the assessment factors. In contrast to the principle adopted for the aquatic compartment, it is not necessary to have 3 acute sediment tests for the assessment factor of 1000 to be applicable. Results from long-term tests with sub-lethal endpoints such as reproduction, growth, emergence, sediment avoidance and burrowing activity are regarded as most relevant due to the generally long-term exposure of benthic organisms to sediment-bound substances. Consequently, if results from short-term tests with sediment-dwelling organisms are only available (at least one) an assessment factor of 1000 is applied to the lowest value. In addition, the $PNEC_{sed}$ should also be calculated from the $PNEC_{water}$ using the equilibrium-partitioning method. A reduction in the size of the assessment factor should only be accepted if results form long-term tests with sediment-dwelling organisms are available.

The $PNEC_{sediment}$ is derived from the lowest available NOEC/EC10 obtained in long-term tests by application of the following assessment factors and is then expressed as mg/kg of dry sediment:

**Table R.10-7 Assessment factors for derivation of $PNEC_{sed}$**

| Available test result | Assessment factor |
|---|---|
| One long-term test (NOEC or EC10) | 100 |
| Two long-term tests (NOEC or EC10) with species representing different living and feeding conditions | 50 |
| Three long-term tests (NOEC or EC10) with species representing different living and feeding conditions | 10 |

### R.10.5.3 Marine sediment

Substances that are highly hydrophobic may be assessed as of low risk for pelagic fauna but can accumulate in sediments to concentrations at which they might exert significant toxic effects (SETAC, 1993). This may be of concern particular in the marine environment, where the sediment may act as a permanent sink for highly hydrophobic substances that can be accumulated to a large extent. Because marine sediment constitutes an important compartment of marine ecosystems it may be important to perform an effects assessment for the marine sediment compartment for those substances.

In principle the same strategy as applied to freshwater sediment is recommended (see Section R.10.5.2) for the effects assessment of marine sediment). Most of the existing whole sediment tests measure acute toxicity; only a few measure long-term, sub-lethal endpoints. Only the latter tests are considered applicable to marine risk assessment because of the long-term exposure of benthic organisms to sediment-bound substances that occur under field conditions.

In Section R.10.3.2 freshwater toxicity data are compared to marine and estuarine data. It is concluded that the use of freshwater acute effects data *in lieu* or together with saltwater effects data is acceptable for risk assessment purposes. Although it is not sure that this also applies to marine and freshwater sediment data, it is nevertheless recommended to use pooled marine and freshwater sediment toxicity data for effect assessment for the sediment compartment. However, when sufficient data for ecologically relevant saltwater species are available lower assessment factors can be applied.

#### R.10.5.3.1 Calculation of PNEC for marine sediment using equilibrium partitioning

In the absence of any ecotoxicological data for sediment-dwelling organisms, but with measured data to predict the $PEC_{marine\ sediment}$, the $PNEC_{marine\ sediment}$ may provisionally be calculated using the equilibrium partitioning method. This method uses the $PNEC_{saltwater}$ for aquatic organisms and the marine suspended matter/water partitioning coefficient. Based on the equilibrium partitioning the following equation is applied:

$$PNEC_{marine-sediment} = \frac{K_{susp-water}}{RHO_{susp}} \cdot PNEC_{saltwater} \cdot 1000 \qquad \textbf{Equation R.10-3}$$

**Explanation of symbols**

| | | | |
|---|---|---|---|
| $PNEC_{saltwater}$ | Predicted No Effect Concentration in saltwater | $[mg \cdot l^{-1}]$ | |
| $RHO_{susp}$ | bulk density of suspended matter | $[kg \cdot m^{-3}]$ | 1150 |
| $K_{susp\ water}$ | partition coefficient suspended matter water | $[m^3 \cdot m^{-3}]$ | Eq. R.16-14 |
| $PNEC_{marine\ sediment}$ | Predicted No Effect Concentration in marine sediment | $[mg \cdot kg^{-1}$ of wet sediment] | |

The equilibrium partitioning method considers uptake via the water phase, while uptake may also occur via other exposure pathways such as ingestion of sediment or direct contact with sediment. This may be important, especially for chemicals that have a tendency to adsorb to sediment organic matter, for example those with a log Kow greater than 3. Direct uptake from marine sediment is also observed in studies with marine benthic organisms and may significantly contribute to the

uptake of organic contaminants such as PAHs (Kaag, 1998). There is also however evidence from studies in soil and in marine sediment that the proportion of the total dose taken up through intake of sediment particles remains low for chemicals with a log Kow up to 5. From other studies it is obvious that feeding mode also influences uptake of substances (via water or ingestion of sediment). Furthermore the absorption of contaminants in the gastrointestinal tract has been found to be increased compared with absorption from the surrounding water (Mayer et al., 1996; Voparil and Mayer, 2000). However, no quantitative conclusions can be drawn from these studies regarding uptake of substances from sediment.

For substances with a log Kow greater than 5 (or with a corresponding $Kp_{sed}$) the equilibrium partitioning method is used in a modified way in order to take account of possible uptake via ingestion of sediment. Thus the resulting PEC/PNEC ratio is increased by a factor of 10 for these compounds. It should be borne in mind that this approach is considered as a screening level assessment of the risk to sediment dwelling organisms. If with this method a PEC/PNEC ratio > 1 is derived then tests, preferably long-term, with benthic organisms using spiked sediment have to be conducted in order for a realistic risk assessment appropriate to the sediment compartment to be carried out.

### R.10.5.3.2 Calculation of PNEC for marine sediment using assessment factors

If results from whole-sediment tests with benthic organisms are available the $PNEC_{marine\ sediment}$ has to be derived using assessment factors. In establishing the size of the assessment factors, a number of uncertainties have to be addressed. Due to the generally long-term exposure of benthic organisms to sediment-bound substances, long-term tests with sub-lethal endpoints like reproduction, growth, emergence, sediment avoidance and burrowing activity are regarded as most relevant.

In contrast to the concept applied to the pelagic marine compartment, it is only necessary to have results from one acute sediment test for the assessment factor of 10000 to apply. Furthermore if only results from short-term tests with freshwater sediment-dwelling organisms are available (at least one) an assessment factor of 10,000 is also applied to the lowest value. The $PNEC_{marine\ sediment}$ should also be calculated from the $PNEC_{saltwater}$ using the equilibrium-partitioning method.

If, in addition to the results of tests with freshwater benthic organisms, a result from an acute toxicity test with a marine benthic organism (preferably representative of the same taxa that is most sensitive in aquatic freshwater or saltwater tests) is available then an assessment factor of 1000 is applicable. Once again a $PNEC_{marine\ sediment}$ should also be calculated from the $PNEC_{saltwater}$ using the equilibrium partitioning method. A reduction of the assessment factor is only permitted if results from long-term tests with sediment-dwelling organisms are available.

A $PNEC_{marine\ sediment}$ is derived by application of the following assessment factors to the lowest LC50 value from acute tests:

**Table R.10-8 Assessment factors for derivation of PNECmarine sediment from short-term sediment toxicity tests**

| Available test results | Assessment factor | $PNEC_{marine\ sediment}$ |
|---|---|---|
| One acute freshwater or marine test | 10,000 | Lowest of LC50 /10,000 and equilibrium-partitioning method |
| Two acute tests including a minimum of one marine test with an organism of a sensitive taxa | 1000 | Lowest of LC50 /1000 and equilibrium-partitioning method |

A $PNEC_{marine\ sediment}$ is derived by application of the following assessment factors to the lowest NOEC/EC10 value from long-term tests:

**Table R.10-9 Assessment factors for derivation of PNECmarine sediment from long-term sediment toxicity tests**

| Available test results | Assessment factor a) |
|---|---|
| One long-term freshwater sediment test | 1000 |
| Two long-term freshwater sediment tests with species representing different living and feeding conditions | 500 |
| One long-term freshwater and one saltwater sediment test representing different living and feeding conditions | 100 |
| Three long-term sediment tests with species representing different living and feeding conditions | 50 |
| Three long-term tests with species representing different living and feeding conditions including a minimum of two tests with marine species | 10 |

a) The general principles of notes (c) and (d) as applied to data on aquatic organisms (Section R.10.3.2) shall also apply to sediment data. Additionally, where there is convincing evidence that the sensitivity of marine organisms is adequately covered by that available from freshwater species, the assessment factors used for freshwater sediment data may be applied. Such evidence may include data from long-term testing of freshwater and marine aquatic organisms, and must include data on specific marine taxa.

If no results from long-term tests with sediment organisms are available and the PEC/PNEC ratio derived from the results of short-term sediment tests or via the equilibrium partitioning method is a cause for concern then the need for long-term testing with sediment organisms should be considered. Further guidance on the testing strategy for sediment is provided in Section R.7.8.7 to R.7.8.12.

## R.10.6 Terrestrial (soil) compartment

Chemicals can reach the soil via several routes: application of sewage sludge in agriculture, direct application of chemicals and deposition from the atmosphere. Consequently the possibility of adverse effects has to be assessed.

Substances discharged into the soil can not only affect the soil organisms but also can influence soil functions. Substances that are hydrophilic and that are readily eluted with the rainwater into the ground water as well as those that geo-accumulate and those that are poorly degradable in soil

should be considered with special care. The terrestrial ecosystem comprises of an above-ground community, a soil community and a groundwater community.

In this section only effects on soil organisms exposed directly via pore water and/or soil are addressed. The scope of the terrestrial effect assessment under the REACH regulation is restricted to non-vertebrate organisms living the majority of their lifetime within the soil and being exposed to substances via the soil pathway. Information requirements and testing strategies for the terrestrial compartment are described in Section R.7.11. It is currently not possible to carry out an effect assessment for the groundwater community because no toxicity data are required. However, ecotoxicity tests with groundwater fauna and microflora have been proposed by Notenboom and Boessenkool (1992) and Van Beelen et al. (1990).

If no hazard information is available for the soil compartment, the equilibrium partitioning method can be applied to aquatic data to identify a PNEC for soil organisms. However, this method cannot replace toxicity data for soil organisms and should only be considered as a screen for identifying substances requiring further testing. In common with the aquatic compartment, the objective of the assessment is to identify substances that present an immediate or delayed danger to the soil communities.

Natural soils used in ecotoxicological tests differ in characteristics such as organic matter and clay content, soil pH, cation exchange capacity (CEC) and soil moisture content. Consequently the OECD terrestrial test guidelines recommend the use of artificial soil, and have specified the organic carbon content depending on the test species. However, natural soil tests can also be used in terrestrial tests, especially in higher tier tests. The results from these natural tests can be converted to a standard soil. The bioavailability of the test compound, and therefore the toxicity observed, is influenced by these soil properties. This means that results from different test soils cannot be compared directly. As far as possible, toxicity tests should be conducted in conditions (as regards the nature of the soil, its organic content and any other parameter that could influence the bioavailability of the substance) where the test substance is bioavailable to the tests organism(s). However, if possible data should be normalized using relationships that describe the bioavailability of chemicals in soils. Results are converted to a standard soil, which is defined as a soil with an organic matter content of 3.4% (see Section R.16.5.4). For standardisation of results for non-ionic organic compounds it is assumed that bioavailability is determined by the organic matter content only. NOECs and L(E)C50s are corrected according to the formula :

$$NOEC\ or\ L(E)C_{50(standard)} = NOEC\ or\ L(E)C_{50(\exp)} \cdot \frac{Fom_{soil(standard)}}{Fom_{soil(\exp)}}$$

**Equation R.10-4**

**Explanation of symbols**

| | | | |
|---|---|---|---|
| NOEC or $L(E)C50_{exp}$ | NOEC or L(E)C50 in experiment | $[mg \cdot kg^{-1}]$ | |
| $Fom_{soil(standard)}$ | fraction organic matter in standard soil | $[kg \cdot kg^{-1}]$ | 0.034 |
| $Fom_{soil(exp)}$ | fraction organic matter in experimental soil | $[kg \cdot kg^{-1}]$ | |
| NOEC or $L(E)C50_{standard}$ | NOEC or L(E)C50 in standard soil | $[mg \cdot kg^{-1}]$ | |

It should be noted that this recommended normalisation is only appropriate when it can be assumed that the binding behaviour of a non-ionic organic substance in question is predominantly driven by its log $K_{ow}$, and that organisms are exposed predominantly *via* pore water.

For standardisation of results in the case of metals correlations between CEC and/or pH and toxicity have been reported (e.g. Jänsch et al (2006), Gorsuch et al. (2006) and Van Gheluwe (2006)). Models may be derived from such sources if scientifically justified.

Three situations can be distinguished for deriving a $PNEC_{soil}$:

- when no toxicity data are available for soil organisms, the equilibrium partitioning method is applied to identify a potential risk to soil organisms. This method is regarded as a "screening approach" (see also Section R.10.5.2).
- when toxicity data are available for a producer, a consumer and/or a decomposer the $PNEC_{soil}$ is calculated using assessment factors (Section R.10.6.2).
- when only one test result with soil dwelling organisms is available the risk assessment is performed both on the basis of this result using assessment factors and on the basis of the equilibrium partition method (EPM). From both $PEC_{soil}/PNEC_{soil}$ ratios the highest one is chosen for the risk characterisation.

### R.10.6.1 Calculations of PNEC for soil using equilibrium partitioning

Equilibrium partitioning method (EPM) is based on the assumption that soil toxicity expressed in terms of the freely-dissolved substance concentration in the pore water is the same as aquatic toxicity. The pore water concentration is correlated with the bioavailable fraction. Although Di Toro *et al.* (1991) based their analysis on sediment partitioning the rationale can also be applied to soils. However the applicability of the equilibrium partitioning method has been evaluated less for soil than for sediment-dwelling organisms. Van Gestel and Ma (1993) have shown the model to be valid for short-term toxicity of several chlorophenols, chlorobenzenes and chloroanilines to earthworms.

The equilibrium partitioning method may not be suitable for lipophilic substances or substances with a specific mode of action nor for organisms that are exposed primarily through food (Van Gestel, 1992).

It should be recognised that substitution of terrestrial toxicity data by aquatic toxicity data should be used with caution. This is because the effects on aquatic species can only be considered as effects on soil organisms that are exposed exclusively to the soil pore water and may only be appropriate for organisms with a water-permeable epidermis. Furthermore, studies have shown that the equilibrium partitioning method can give significant over- or underestimations, due to inaccurate partitioning coefficients or differences in species sensitivities. Therefore, further research is required into the general applicability of the EPM for other organisms. In particular, for Collembola and Oribatid mites, there are indications that direct exposure to soil may be of much greater importance for uptake than is exposure via the food (Løkke and van Gestel, 1998).

Therefore, as illustrated in the integrated testing strategy developed in Section R.7.11.6.3, if the $PEC_{soil}/PNEC_{soil}$ ratio calculated using the Equilibrium Partitioning Method is greater than 1, tests with soil organisms should be considered as an essential requirement for a refined effects assessment.

The $PNEC_{soil}$ is calculated as follows:

$$PNEC_{soil} = \frac{K_{soil-water}}{RHO_{soil}} \cdot PNEC_{water} \cdot 1000$$

**Equation R.10-5**

**Explanation of symbols**

| | | | |
|---|---|---|---|
| $PNEC_{water}$ | Predicted No Effect Concentration in water | $[mg \cdot l^{-1}]$ | |
| $RHO_{soil}$ | bulk density of wet soil | $[kg \cdot m^{-3}]$ | 1150 |
| $K_{soil-water}$ | partition coefficient soil water | $[m^3 \cdot m^{-3}]$ | Eq. R.16-14 |
| $PNEC_{soil}$ | Predicted No Effect Concentration in wet soil | $[mg \cdot kg^{-1}]$ | |

In order to take uptake by soil ingestion into account the same approach is used as for the derivation of the $PNEC_{sediment}$. Thus, the $PEC_{soil}/PNEC_{soil}$ ratio is increased by a factor of 10 for compounds with a log Kow > 5 (or for compounds with a corresponding adsorption or binding behaviour, e.g. ionisable substances).

In principle, toxicity data for aquatic organisms cannot replace data for soil dwelling organisms. This is because the effects on aquatic species can only be considered as effects on soil organisms that are exposed exclusively to the soil pore water of the soil (Samsøe-Petersen and Pedersen, 1994). Therefore, if the $PEC_{soil}/PNEC_{soil}$ ratio that is calculated using the equilibrium partitioning method is greater than 1, tests with soil organisms should be considered as an essential requirement for a refined hazard assessment.

### R.10.6.2 Calculation of PNEC for soil using assessment factors

The same assessment factors used for the aquatic compartment (see Table R.10-4) are applied to the terrestrial compartment (see Table R.10-10). The size of the assessment factor therefore again depends on the type of data that are available i.e. short-term or long-term toxicity test, the number of trophic levels tested and the general uncertainties in predicting ecosystem effects from laboratory data.. A dataset comprising of toxicity data for primary producers, consumers and decomposers is preferred.

In summary, the assessment factors proposed in Table R.10-10 must be regarded as indicative. As more information on the sensitivity of soil organisms becomes available these factors may have to be revised.

**Table R.10-10 Assessment factors for derivation of $PNEC_{soil}$**

| Information available | Assessment factor |
|---|---|
| L(E)C50 short-term toxicity test(s) (e.g. plants, earthworms, or microorganisms) | 1000 |
| NOEC for one long-term toxicity test (e.g. plants) | 100 |
| NOEC for additional long-term toxicity tests of two trophic levels | 50 |
| NOEC for additional long-term toxicity tests for three species of three trophic levels | 10 |
| Species sensitivity distribution (SSD method) | 5 – 1, to be fully justified on a case-by-case basis (cf. main text) |
| Field data/data of model ecosystems | case-by-case |

A $PNEC_{soil}$ is calculated on the basis of the lowest determined effect concentration. If results from short-term tests with a producer, a consumer and/or a decomposer are available, the result is divided by a factor of 1000 to calculate the $PNEC_{soil}$. If only one terrestrial test result is available (earthworms or plants), the risk assessment should be performed both of this test result and on the basis of the outcome of the aquatic toxicity data to provide an indication of the risk. As a matter of precaution, the larger $PEC_{soil}/PNEC_{soil}$ ratio determines which further actions should be taken in the framework of the further testing strategy. If additional soil test results are available the assessment factors given in Table R.10-10 should be applied.

### R.10.6.3 Calculation of PNEC for soil using statistical extrapolation techniques

Calculation of a $PNEC_{soil}$ using statistical extrapolation techniques can be considered when sufficient data are available (see Section R.10.3.1.3 for minimum requirements). For comparable data on the same end-point and species, by default the geometric mean should be used as the input value for the calculation of the species sensitivity distribution. When results are available from tests using different soils and it is likely that the soil characteristics have influence on the results, the effect data should be normalised before further processing. If not possible, the lowest NOEC per end-point and species should be used.

## R.10.7 Air compartment

For the risk assessment of the air compartment biotic and abiotic effects are considered.

### R.10.7.1 Biotic hazard

Methods for the determination of effects of chemicals on species arising from atmospheric contamination have not yet been fully developed, except for inhalation studies with mammals. Therefore, the methodology used for hazard assessment (and therefore the risk characterisation) of chemicals in water and soil cannot be applied yet in the same manner to the atmosphere.

### R.10.7.2 Abiotic hazard

For the evaluation of an atmospheric risk, the following abiotic effects of a chemical on the atmosphere have to be considered:

- global warming;
- ozone depletion in the stratosphere;
- ozone formation in the troposphere;
- acidification.

If for a chemical there are indications that one or several of these effects occur, expert knowledge should be consulted.

## R.10.8 Assessment of secondary poisoning

### R.10.8.1 Introduction

The chemicals of concern with respect to secondary poisoning include lipophilic organic chemicals and some metal compounds.

Secondary poisoning is concerned with toxic effects in the higher members of the food chain, either living in the aquatic or terrestrial environment, which result from ingestion of organisms from lower trophic levels that contain accumulated substances. Previous cases have demonstrated that severe effects can arise after exposure of animals via their food and that bioconcentration, bioaccumulation and biomagnification in food chains need to be considered (see also section R.16.4.3.5 and Section R.7.10).

The risk to the fish-eating predators (mammals and/or birds) is calculated as the ratio between the concentration in their food ($PECoral_{predator}$) and the no-effect-concentration for oral intake ($PNEC_{oral}$).

This section will deal with the derivation of $PNEC_{oral}$. The calculation of $PECoral_{predator}$ is presented in Section R.16.5.8.

### R.10.8.2 Calculation of predicted no-effect concentration in food ($PNEC_{oral}$)

Only toxicity studies reporting on dietary and oral exposure are relevant as the pathway for secondary poisoning is referring exclusively to the uptake through the food chain. Secondary poisoning effects on bird and mammal populations rarely become manifest in short-term studies. Therefore, results from long-term studies are strongly preferred, such as NOECs for mortality, reproduction or growth. If no adequate toxicity data for mammals or birds are available, an assessment of secondary poisoning cannot be made.

REACH (Annex X) indicates that information on long-term or reproductive toxicity to birds should be considered for all substances manufactured or imported in quantities of 1000 t/y or more. However this need for testing should be carefully considered taking into account the dataset on mammalian studies usually available at that tonnage level. Further guidance on avian toxicity and integrated testing strategy for avian toxicity is provided in Section R.7.10.18.

The results of the available mammalian or avian tests may be expressed as a concentration in the food ($mg \cdot kg_{food}^{-1}$) or a dose (NOAEL expressed in $mg \cdot kg$ body $weight \cdot day^{-1}$) causing no effect. For the assessment of secondary poisoning, the results always have to be expressed as the concentration in food in order to be able to compare it to the PEC. In case toxicity data are given as NOAEL only, these NOAELs can be converted to NOECs with the following two formulae:

$$NOEC_{bird} = NOAEL_{bird} \cdot CONV_{bird}$$ **Equation R.10-6**

$$NOEC_{mammal,food_chr} = NOAEL_{mammal,oral_chr} \cdot CONV_{mammal}$$ **Equation R.10-7**

**Explanation of symbols**

| | | | |
|---|---|---|---|
| $NOEC_{bird}$ | NOEC for birds | $(kg \cdot kg_{food}^{-1})$ | |
| $NOEC_{mammal,\ food\ chr}$ | NOEC for mammals | $(kg \cdot kg_{food}^{-1})$ | |
| $NOAEL_{bird}$ | NOAEL for birds | $(kg \cdot kg\ bw \cdot d^{-1})$ | |
| $NOAEL_{mammal,\ oral\ chr}$ | NOAEL for mammals | $(kg \cdot kg\ bw \cdot d^{-1})$ | |
| $CONV_{bird}$ | conversion factor from NOAEL to NOEC | $(kg\ bw \cdot d \cdot kg_{food}^{-1})$ | Table R.10-12 |
| $CONV_{mammal}$ | conversion factor from NOAEL to NOEC | $(kg\ bw \cdot d \cdot kg_{food}^{-1})$ | Table R.10-12 |

Species-specific information on the conversion factor (body weight/daily food intake ratio) should be available in the test report in case of bird testing. For example, a chicken *Gallus domesticus* typically consumes around 1/8th of its body weight per day, and so the conversion factor in this case would be 8 [kg bw.d/kg food][8]. Further considerations for specific test types are provided in EC (2002a), summarised in Table R.10-11. It should be noted that the conversion factor for young birds might differ from adults.

**Table R.10-11 : Food intake considerations for different types of avian test**

| Test | Comment |
|---|---|
| Reproduction | Food consumption: Data are reported on a weekly basis for pairs or groups. Although it is usually higher during egg laying (attributed to the females), the average consumption over the entire exposure period is taken.<br>Body weight: Take average body weight for both sexes over exposure period.<br>Convert each treatment group separately. |
| 5-day dietary test | Food consumption: Usually group consumption rates (expressed as g per bird per day) are given in the report for the 5-day exposure period and the 3-day post-exposure period; the former figure is needed here<br>Body weight: Group means for day 0, 5, and 8 are reported. For the purpose here take the average of day 0 and day 5.<br>The conversion from concentration to daily dose is not appropriate for those treatment groups where a strong food avoidance is obvious (in that case the average dose over 5 days is misleading) as well as for treatment groups with a high mortality (in that case data on the body weight at day 5 and for the food consumption have a poor quality or are missing at all). Further guidance is provided in EC (2002a). |

NOECs derived from NOAELs in this way are assumed to be equivalent to directly measured NOECs. A daily dose approach is considered more appropriate in pesticide risk assessment since birds may avoid food and so the dietary concentration might not truly represent what they are consuming.

[8] Bodyweight/daily food intake ratios have been found to range from 1.1 to 9 for twenty-seven wild bird species (CCME, 1998), indicating that some wildlife species may have a lower bw/dfi ratio than laboratory animals. See EC (2002a) Appendix 1 for more information.

In addition, conversion factors for laboratory animals are presented in Table R.10-12.

**Table R.10-12 Conversion factors from NOAEL to NOEC for several mammalian and one bird species**

| Species | Conversion factor (bw/dfi) |
|---|---|
| *Canis domesticus* | 40 |
| *Macaca sp.* | 20 |
| *Microtus spp.* | 8.3 |
| *Mus musculus* | 8.3 |
| *Oryctolagus cuniculus* | 33.3 |
| *Rattus norvegicus (> 6 weeks)* | 20 |
| *Rattus norvegicus (≤ 6 weeks)* | 10 |
| *Gallus domesticus* | 8 |

* bw = body weight (g); dfi: daily food intake (g/day)

NOECs converted from NOAELs have the same priority as direct NOECs.

The PNECoral is ultimately derived from the toxicity data (food basis) applying an assessment factor.

$$PNEC_{oral} = \frac{TOX_{oral}}{AF_{oral}}$$ **Equation R.10-8**

**Explanation of symbols**

| | | | |
|---|---|---|---|
| PNECoral | PNEC for secondary poisoning of birds and mammals | [in $kg \cdot kg_{food}^{-1}$] | |
| AForal | assessment factor applied in extrapolation of PNEC | [-] | Table R.10-13 |
| TOXoral | either $LC50_{bird}$, $NOEC_{bird}$ or $NOEC_{mammal, food, chr}$ | [in $kg \cdot kg_{food}^{-1}$] | |

If data on avian toxicity is available then the resulting $PNECoral_{bird}$ is derived by applying an assessment factor (AF) to the available toxicity data. The AF is intended to account for interspecies variation and laboratory data to field impact extrapolation as outlined in Section R.10.2.4. Ideally, the $PNECoral_{bird}$ is based on the lowest available reliable chronic NOEC value (for mortality, reproduction or growth), since the assessment is intended to be protective of effects arising from long-term exposures.

Nevertheless, in many cases only acute toxicity data will be available initially. Although there is no link between acute and long-term toxicity (i.e. a substance that is of low acute toxicity will not necessarily be of low long-term or reproductive toxicity), an initial pragmatic approach in the absence of a chronic study is to derive the $PNEC_{bird}$ by applying a high (precautionary) assessment factor to existing 5-d $LC_{50}$ data.

In summary:

$$PNECoral_{bird} = (\text{5-d } LC_{50} \textit{ or } \text{chronic NOEC})$$ **Equation R.10-9**

AF

where AF = 3,000 for acute data, *or* 30 for chronic data.

In case only mammalian oral toxicity data are available the AF to apply are provided in Table R.10-13 below.

The scientific basis for these values is unclear, and there is no evidence to suggest whether they are sufficiently protective or otherwise[9]. In particular, any PNEC based on acute data should be considered tentative. Therefore if both acute and chronic data exist for a substance, preference should be given to the PNEC derived from the chronic data. The slope of the dose-response relationship might be helpful for the interpretation of this value (e.g. a shallow dose-response relationship implies that some individuals may be affected at doses well below the $LC_{50}$). In addition, the ecological significance of the effect might also need to be considered (e.g. as for the PBT assessment – see Chapter R.11).

If a chronic NOEC for *both* birds and mammals is available, the lower of the resulting PNECs is used in the secondary poisoning assessment to represent all predatory organisms.

A $PNECoral_{bird}$ cannot be derived from non-standard avian toxicity test results. However, a *Weight of Evidence* approach may allow conclusions about the relative sensitivities of birds and mammals to be drawn from non-standard or qualitative information. The supportive value of the individual evidence has to be judged carefully, and the arguments must be appropriate and substantiated.

CCME (1998) contains wildlife data on body weight and daily food ingestion rates for 27 bird and 10 mammalian species. In addition, Schudoma et al. (1999) derived the mean body weight and daily food intake for the otter. The currently available set on wildlife bw/dfi ratios ranges from 1.1 to 9 for birds and from 3.9 to 10 for mammalian species. Comparison of these wildlife conversion factors with the values given in Table R.10-12 for laboratory species (8.3 – 40) shows that the wildlife species often have a lower bw/dfi ratio than laboratory animals. The difference can be up to a factor 8 for birds and 10 for mammals. This difference is in theory accounted for in the use of the interspecies variation factor that is part of the standard assessment factor. The interspecies variation, however, should comprise more than just the bw/dfi differences between species, e.g. the differences in intrinsic sensitivity. The protective value of the “normal” interspecies variation factor may therefore be questionable in case of predators. On top of that, many predator species are characterised by typical metabolic stages in their life-cycle that could make them extra sensitive to contaminants in comparison with laboratory animals (e.g. hibernation or migration). Similar to the bw/dfi differences, also this aspect goes beyond the “normal” interspecies variation.

The AForal should compensate for the above-mentioned specific aspects in the hazard assessment of predators. A factor of 30, accounting for both interspecies variation and lab-to-field extrapolation, is considered to be appropriate for this purpose. Additionally, acute/subchronic to chronic extrapolation needs to be taken into account. The resulting assessment factors are given in Table R.10-13.

[9] For example, Sell (undated) analysed a pesticide data set and found that an AF of ~10,000 would have to be applied in order to use the avian dietary test instead of the avian reproduction test. In the 1996 Technical Guidance Document, the AFs were 1,000 and 10 respectively. These were increased by a factor of 3 in the 2003 TGD. The reason given was that the AF should compensate for bw/dfi differences between wildlife species and laboratory animals, as well as metabolic stages (e.g. hibernation or migration) that might make predators more sensitive to contaminants in comparison with laboratory animals. To this might be added the difference in caloric content of the diets of laboratory animals (e.g. grain) versus wildlife (e.g. fish), meaning that wild birds must consume more food to obtain the same amount of energy, leading to a higher body burden of the pollutant (e.g. Everts *et al*., 1993).

**Table R.10-13 Assessment factors for extrapolation of mammalian and bird toxicity data**

| TOXoral | Duration of test | AForal |
|---|---|---|
| $LC50_{bird}$ | 5 days | 3,000 |
| $NOEC_{bird}$ | chronic | 30 |
| $NOEC_{mammal,\ food,chr}$ | 28 days<br>90 days<br>chronic | 300<br>90<br>30 |

If a NOEC for both birds and mammals is given, the lower of the resulting PNECs is used in the risk assessment.

It is highly unlikely that sufficient avian toxicity data will be available for any substance to allow a species sensitivity distribution to be developed (i.e. an insufficient number of species will have been tested in long-term tests), so this is not considered further.

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## APPENDIX R.10- 1: AVAILABLE SOURCES OF (Q)SAR INFORMATION

Examples of available sources of (Q)SAR information.

**Table R.10-14 : Overview of some schemes for the characterisation of modes of action**

| Scheme / Endpoints provided for | Structural classes | Information about the under lying rules |
|---|---|---|
| Verhaar et al. (1992) / Acute fish toxicity | class I – inert chemicals (non-polar narcosis),<br>class II – relatively inert chemicals (polar narcosis),<br>class III – reactive chemicals (different types of reactive chemicals, which in principle are difficult to be modelled together, but the net result of reactivity in all cases is enhanced toxicity),<br>class IV – specifically acting chemicals (e.g. acetylcholinesterase inhibitors or substances that provoke central nervous system effect). | Characterisation as belonging to class I – III is based on structural properties. For a class IV classification additional information about the mode of action is needed. Compounds that cannot be characterised as belonging to class 1, 2 or 3 and that are not known to be compounds acting by specific modes of action can only be characterised as “not possible to classify according to these rules”. |
| Russom et al. (1997) / acute fish toxicity | Narcotics (three distinct groups; narcotics I, II or III))<br>Oxidative phosphorylation uncouplers<br>Respiratory inhibitors<br>Electrophile/proelectrophiles<br>Acetylcholinesterase inhibitors<br>Central nervous system seizure agents | Compounds that do not meet any of the substructural requirements identified, are assumed to exert narcosis I mode. |

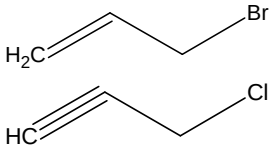
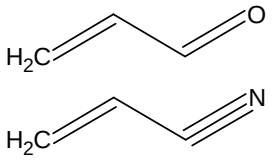
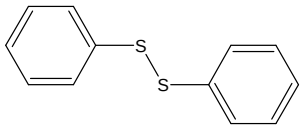
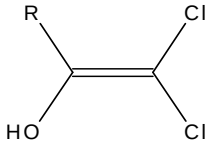
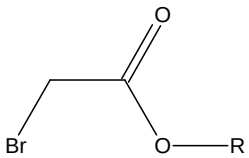
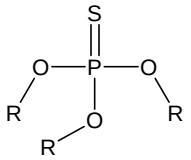
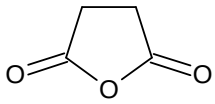
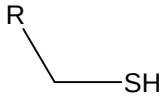
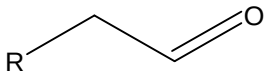
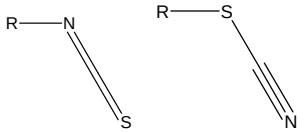
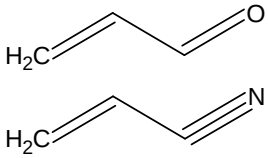
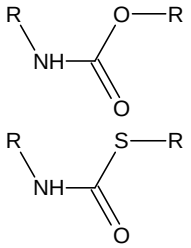
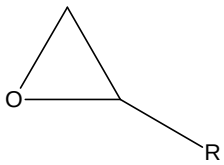
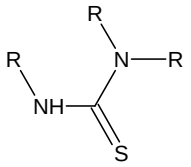
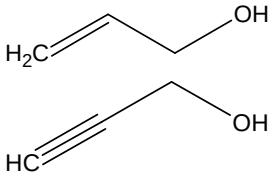
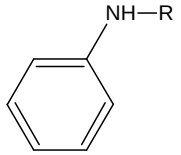
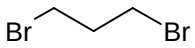
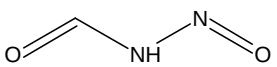
Note that neither of the schemes, which where developed for fish, considers inhibition of photosynthesis

**Table R.10-15 : Overview of programs for the identification of the mode of action of a chemical**

| Program | Availability | Underlying characterisation scheme | Principle of the characterisation | Information about the underlying rules |
|---|---|---|---|---|
| ASTER (Russom et al., 1991, Russom et al. 1997) | ASTER is currently not publicly available (http://www.epa.gov/med/Prods_Pubs/aster.htm) | Russom et al, 1997 | Identification of modes of action (e.g. non-polar, polar, ester narcosis) and chemical classes (e.g. acrylates) | "Unknown" MOA if the substance is not known to the system |
| OASIS/TIMES (Mekenyan et al., 2004) | Commercial product (for information: http://www.oasis-lmc.org/software.php) | New characterisation scheme for acute fish toxicity | Classification into two types of chemicals: non-covalent acting chemicals (comparable to baseline toxicity) and covalent bioreactive chemicals with several subgroups. | "Unexplained" if the substance is not known. For these chemicals minimal toxicity, as defined by the response surface model will be applied. |
| ChemProp (Schüürmann et al., 1997) | On request (contact developer) contact details at http://www.ufz.de, Prof. Dr. Gerrit Schüürmann) | New characterisation scheme for acute toxicity to daphnia and algae (acute fish toxicity under development) | Structural alerts for the identification of substances with higher toxicity than non-polar and polar narcosis and structural rules based on the Verhaar scheme for the identification of narcotic chemicals | Warning if the substance doe not fall within the structural and physico-chemical applicability domain of the system. |
| ECOSAR (U.S. EPA, 1994) | Available for free download at: www.epa.gov/oppt/newchems/21ecosar.htm ECOSAR is also included in EPISuite, which is available for free download at: http://www.epa.gov/oppt/exposure/docs/episuitedl.htm | Fish, daphnids, green algae | Identification of substructures, characterisation on a chemical class principle | If no substructure (chemical class) can be identified the substance is automatically characterised as "neutral organics" |
| PropertEst (Fraunhofer Institute IME) | On request (contact developer) PropertEst will be available in 2006. More details can be found at: http://www.ime.fraunhofer.de/fhg/ime/aoe/chp/propertest.jsp | Fish, daphnids, green algae | according to the Verhaar Scheme | Characterisation as "inert" "less inert" or "classification not possible" |
| Verhaar scheme, plugin for toxTree | Verhaar scheme is available for free download at: http://ambit.acad.b | Fish | according to the Verhaar Scheme | Characterisation as being "not possible according to these rules " if the substance dose |

| **Program** | **Availability** | **Underlying characterisation scheme** | **Principle of the characterisation** | **Information about the underlying rules** |
|---|---|---|---|---|
| (http://ambit.acad.bg/) | g/downloads/verharScheme.jar<br>toxTree is available for free download at: (http://ecb.jrc.it/QSAR/qsar_tools_toxtree.php), | | | not belong to class 1 -3 (non-polar toxicity, polar toxicity, unspecific reactivity) and no specific mode of action is known |
| TOPKAT (Gombar and Enslein, 1995) | Commercial product<br>(for information: http://www.accelrys.com/products/topkat/) | Fish, daphnids | Identification of substructures, characterisation on a chemical class principle (8 classes for fish and 4 classes for *Daphnia*) | The program checks if the substance falls within the optimum prediction space of the model |

**Table R.10-16 : Examples for structural alerts associated with enhanced toxicity in fish (and rat) and for Daphnia**

| **Reproduction from Lipnick, 1991**<br>**For fish (and rat) (Not exhaustive list)** | | **Reproduction from von der Ohe et al., 2005**<br>**For Daphnia (Not exhaustive list)** | |
|---|---|---|---|
| **Structural alert** | **Associated mechanism** | **Structural alert** | **Chemical group** |
|  | Nucleophilic substitution<br>(allylic and propargylic activation) |  | α,β-unsaturated carbonyle and nitrile compounds |
|  | Nucleophilic substitution<br>(benzylic activation) |  | Carbon-carbon double bond activation by two halogens |
|  | Nucleophilic substitution<br>(α-haloactivation) |  | Organophosphorus compounds |
|  | Acid anhydride acylation |  | Aliphatic thioles |
|  | Schiff-base formation |  | Isothiocyanates and thiocyanates |
|  | Michael-type addition |  | Carbamates (simple thiocarbamates) |
|  | Epoxide electrophilicity |  | Thiourea derivatives |
|  | Pro-electrophilicity |  | Primary or secondary anilines without ortho substituents |
|  | Metabolic activation |  | Imid derivatives |

Information about the compliance with OECD principles:

Table R.10-16 provides only examples for different modes of action and chemical groups that might be associated with enhanced toxicity, but is not an exhaustive list of all possible alerts that might be, and were generated. For more comprehensive list of structural alerts and underlying reactivity mechanisms the reader should consider the original papers.

The absence of an alert does not imply the absence of an effect as the set of known alerts is most likely incomplete. In addition, a substructure-based system can become difficult to implement if a structural alert is combined with a physico-chemical property such as hydrophobicity, water solubility, ionization, dissociation, or volatility.

Here, n is number of chemicals, $r^2$ is the squared correlation coefficient, $q^2$ is the squared correlation coefficient in leave-one-out cross validation, s is the standard error of the estimate, and F is the Fisher's criterion. Unless otherwise noted, models were taken from the original references without redevelopment.

**Table R.10-17 : Examples of QSAR models for different types of narcosis**

| Organism/endpoint/ type of narcosis | Equation and statistics | Reference |
|---|---|---|
| **General narcosis (polar and non-polar)** | | |
| *Pimephales promelas* 96-h $LC_{50}$ (mol/L) | Log $LC_{50}$ = -0.81 log $K_{ow}$ – 1.74<br>n = 144, $r^2$ = 0.88, $q^2$ = 0.87, s = 0.45 | Pavan et al., 2005a |
| **Non-polar narcosis** | | |
| *Pimephales promelas* 96-h $LC_{50}$ (mol/L) | log $LC_{50}$ = -0.85 log $K_{ow}$ – 1.39<br>n = 58, $r^2$ = 0.94, $q^2$ = 0.93, s = 0.36* | Verhaar et al., 1995 |
| *Poecilia reticulata* 96-h $LC_{50}$ (mol/L) | Log $LC_{50}$ = -0.84 log $K_{ow}$ – 1.12<br>n = 8, $r^2$ = 0.97, $q^2$ = 0.96, s = 0.24, F = 199** | Roberts and Costello, 2003 |
| *Daphnia magna* 48-h $EC_{50}$ (mol/L) Immobilisation | Log $EC_{50}$ = -0.95 log $K_{ow}$ – 1.32<br>n = 49, $r^2$ = 0.95, $q^2$ = 0.94, s = 0.34 | Verhaar et al., 1995 |
| *Daphnia magna* 48-h $LC_{50}$ (mol/L) | Log $LC_{50}$ = -0.86 log$K_{ow}$ – 1.28<br>n = 36, $r^2$ = 0.90, $q^2$ = 0.94, s = 0.44, F = 311 | Von der Ohe et al., 2005 |
| **Polar narcosis** | | |
| *Pimephales promelas* 96 h $LC_{50}$ (mol/L) | log $LC_{50}$ = -0.73 log $K_{ow}$ – 2.16<br>n = 86, $r^2$=0.90, $q^2$ = 0.90, s = 0.33* | Verhaar et al., 1995 |
| *Poecilia reticulata* 96-h $LC_{50}$ (mol/L) | Log $LC_{50}$ = -0.76 log $K_{ow}$ – 2.00<br>n = 11, $r^2$ = 0.89, $q^2$ = 0.84, s = 0.28, F = 72** | Roberts and Costello, 2003 |
| *Daphnia magna* 48-h $EC_{50}$ (mol/L) Immobilisation | Log $EC_{50}$ = -0.56 log $K_{ow}$ – 2.79<br>n = 37, $r^2$ = 0.77, $q^2$ = 0.73, s = 0.37 | Verhaar et al., 1995 |
| *Daphnia magna* 48-h $LC_{50}$ (mol/L) | Log $LC_{50}$ = -0.80 log $K_{ow}$ – 2.21<br>n = 33, $r^2$ = 0.74, $q^2$ = 0.94, s = 0.45, F = 90<br>(Without anilines) | von der Ohe et al., 2005 |
| *Selenastrum capricornutum* 72-96-h $EC_{50}$ (mol/L) Growth | Log $EC_{50}$ = -1.00 log $K_{ow}$ – 1.23<br>n = 10, $r^2$ = 0.93, $q^2$ = n.d., s = 0.17 | Van Leeuwen et al., 1992 |
| **Amine narcosis** | | |
| *Pimephales promelas* 96 h $LC_{50}$ (mmol/L) | log (1/$LC_{50}$) = 0.67 log $K_{ow}$ – 0.81<br>n = 61, $r^2$ = 0.86, s = 0.53 | Newsome et al., 1993 |
| **Ester narcosis** | | |
| *Pimephales promelas* 96 h $LC_{50}$ (mmol/L) | log (1/$LC_{50}$) = 0.64 log $K_{ow}$ – 0.64<br>n = 14, $r^2$ = 0.95, s = 0.22, F = 207 | Jaworska et al, 1998 |
| **Quadratic functions for very hydrophobic substances** | | |
| Equation of this type was used by Hermens et al. (1984) (*Poecilia reticulata*, 24-days for anilinies and chloroanilines), Veith et al. (1983) (*Pimephales promelas*), Zaroogian et al. (1985) (*Cyprinodon variegates*) | | |

*The models have been re-evaluated by Pavan et al., 2005a. The same models have been evaluated with external test sets and showed high predictivity (89 and 87%, respectively) for chemicals with the same mode of action.
** The models were redeveloped from the data published by Vaes et al., 1998).

Information about the compliance with OECD principles:

The log $K_{ow}$-based regression models for different types of narcoses generally comply well with the OECD principles, if developed for a defined endpoint that has been used for aquatic hazard assessment. The models are transparent if the training set is provided, easy to recalculate and to document/report. If developed on good quality data, and for chemicals with the same mode of action, the goodness-of-fit measured by $r^2$ is typically high (about 0.9 or higher for nonpolar narcosis, and about 0.9 or slightly lower for polar narcosis). The robustness and predictivity depend heavily on the proper use of the model to predict only chemicals that fall in its applicability domain. The narcoses models are typically stable in cross-validation even if developed on a small number of chemicals and have an established mechanistic basis, which has been commented in numerous publications over more than a century. The drawbacks originate from the variability of the descriptor (log $K_{ow}$) and the risk of classifying reactive chemicals as narcotic.

Here $E_{LUMO}$ is the energy of the lowest unoccupied molecular orbital (in eV).

**Table R.10-18 : Examples of QSARs for other modes of action**

| Organism/endpoint/ type of narcosis | Equation and statistics | Reference |
|---|---|---|
| *Pimephales promelas* 96-h $LC_{50}$ (mol/L) aromatic narcotics as well as non-specific (soft) electrophiles | Log LC50 = -0.57 log Kow + 0.45 ELUMO – 2.44, n = 114, r2 = 0.78, q2 = 0.76, s = 0.48* | Pavan et al., 2005b, redeveloped from Veith and Mekenyan, 1993 |
| Similar models can be found in Karcher and Karabunarliev (1996), Karabunarliev et al. (1996a and 1996b), Dimitrov et al. (2004). | | |

Information about the compliance with OECD principles:

The model redeveloped by Pavan et al., 2005b, has been evaluated with an external test set and showed predictivity higher than 80% for chemicals in its domain of applicability.

The endpoint predicted is 96-h acute toxicity to P. promelas (fathead minnow) in mol/L.

**Table R.10-19 : Examples of models that are based on descriptors different from log $K_{ow}$**

| | Hall et al., 1984*<br>Redeveloped by Crookes and Brooke, 2006 | Huuskonen et al., 2003**<br>Redeveloped by Pavan et al., 2005b |
|---|---|---|
| Model | Log $(1/LC_{50}) = 3.275 + \sum (\Delta T_a \times n_a)$ | Log LC50 = $-0.916 - \sum (a_i \times S_i)$ |
| Descriptors and coefficients | Cl $\Delta T_a = 0.557$<br>Br $\Delta T_a = 0.488$<br>$NO_2$ $\Delta T_a = 0.338$<br>$CH_3$ $\Delta T_a = 0.225$<br>$OCH_3$ $\Delta T_a = -0.096$<br>OH $\Delta T_a = 0.004$<br>$NH_2$ $\Delta T_a = -0.082$<br>*o/p*$NO_2$ $\Delta T_a = 1.043$ | $SsCH_3$ -0.194<br>SdsCH -1.707<br>SaaCH -0.171<br>SsssCH -0.406<br>SaasC -0.200<br>SssssC -0.332<br>$SsNH_2$ -0.054<br>StN -0.058<br>SddsN 0.951<br>SsOH -0.080<br>SdO -0.029<br>SsF -0.098<br>SsCl -0.168<br>SsBr -0.236 |
| Statistics | n = 66, $r^2 = 0.90$, $q^2 = 0.90$,<br>s = 0.25, f = 66 | n = 121, $r^2 = 0.84$, $q^2 = 0.68$,<br>s = 0.39, f = 40 |

* The model is based on group-contributions of substituted benzenes. $\Delta T_a$ is the incremental toxicity value of group a, $n_a$ is the number of groups a in the molecule.
** The model uses electrotoplogical (E-state) indices of diverse data. $a_i$ and $S_i$ are the regression coefficients and corresponding structural descriptors. For interpretation of the electrotopological indices see Netzeva (2004).

Information about the compliance with OECD principles:

The training sets are available, and the algorithms allow redevelopment of the models, although this requires some expertise in QSAR techniques. Both models have been evaluated with external test sets which was helpful to identify better their domain of applicability (Crookes and Brooke, 2006, and Pavan et al., 2005b, respectively).

One advantage of the models presented in Table R.10-19 is that the predictions from them do not depend on descriptors variability. The danger of using a general model developed on a diverse dataset without precise definition of its domain of applicability in terms of chemical structure is that the models might not be used properly and the prediction might deviate significantly from the observed values. In addition the following disadvantages appear: The model of Hall et al. (1984) is restricted to substituted benzenes and does not account for the position of the substituents in the molecule and their combination, which in some cases might result in enhanced toxicity due to unlocking of a specific mode of action, or metabolic activation/deactivation. A disadvantage of the Huuskonen model is a potential instability (i.e. higher chance for low accuracy of prediction even for chemicals within its applicability domain), which is evident from the relatively low coefficient of correlation in a leave-one-out cross-validation procedure ($q^2$). Therefore, in a multivariate regression-based model like the one of the Huuskonen et al. (2003), the ratio between the chemicals in the training set of the model and the descriptors in it should be ≥10 (Schultz et al., **2004).**

**Table R.10-20 : Overview of programs for prediction of aquatic toxicity**

| Expert system | Availability | Endpoints available | Principle of prediction | Notes |
|---|---|---|---|---|
| ECOSAR | Freely available from the U.S. EPA (downloadable from http://www.epa.gov/oppt/exposure/docs/episuitedl.htm) | *Acute*<br>Fish (96-h)<br>Daphnid (48-h)<br>Green algae (96-h)<br>*Chronic*<br>Fish (30-d)<br>Daphnid (16-d)<br>Algae | Uses a number of class-specific log $K_{ow}$-based QSARs. | Produces warnings in several occasions (e.g. when the water solubility is very low, or when the prediction is outside the range of log $K_{ow}$ in the training set). |
| TOPKAT | Commercial product of Accelrys Inc. (for information: http://www.accelrys.com/products/topkat/) | Fathead minnow (96-h)<br>*Daphnia* | Uses electro-topological fragments in a range of (Q)SAR models, available for different chemical classes. | Automatically selects the equation from the structural input. Enables the access to experimental test data if available for the query chemicals. Gives information on applicability domain. |
| MCASE | Commercial product of MultiCASE Inc. (for information: http://www.multicase.com/products/products.htm) | Several fish species (blue gill, fathead minnow, rainbow trout, red killifish) | Uses fragment methodology in QSAR models for non-congeneric databases. | Gives information on the domain of validity. |
| OASIS/ TIMES | Commercial product of LMC, Bourgas, Bulgaria (for information: http://www.oasis-lmc.org/software.php) | 17 aquatic species, such as fish, snail, tadpole, hydrozoan, crustacean, insect larvae, and bacteria. | Uses response-surface approach for modelling of acute toxicity for two types of toxico-chemical domains: non-covalent and covalent acting chemicals. | Uses also interspecies models for acute aquatic toxicity. Gives information on applicability domain. |
| Terra QSAR – FHM | Commercial product of TerraBase Inc. (for information: http://www.terrabase-inc.com) | Fathead minnow (96-h) | A stand-alone neural network-based program | |
| PropertEst | On request<br>PropertEst will be available in 2006. (for information: http://www.ime.fraunhofer.de/fhg/ime/aoe/chp/propertest.jsp) | | Currently contains approximately 120 QSAR models on ecotoxicological endpoints (aquatic toxicity, BCF) and physico-chemical endpoints. | Indications are given on how to choose an adequate model for a certain substance. The user can choose a QSAR model and apply expert judgment. |
| ASTER | ASTER is currently not publicly available (http://www.epa.gov/med/Prods_Pubs/aster.htm) | Various | ASTER is an integration of the AQUIRE toxic effects database and the QSAR system. | When empirical data not available mechanistically-based predictive models are used to make estimation. |

Information about the compliance with OECD principles:

The large variety of available expert systems that predict acute aquatic toxicity prevents from a general evaluation for compliance with the OECD principles. While they might have different advantages and disadvantages, it can be recommended that the user applies them after critical evaluation of the results and in combination with results from different models and approaches.

**Table R.10-21 : Examples of QAAR for aquatic toxicity**

| Y | X | Model | Reference |
|---|---|---|---|
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Vibrio fischeri* 5- min EC*50* (mmol/L) | Log (1/Y) = 0.70 log (1/X) + 0.19<br>n = 126, $r^2$ = 0.65, s = 0.79, f = 234<br>(for diverse set)[10] | Cronin et al, 1991 |
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Daphnia magna* 48-h EC*50* (mmol/L) | Log (1/Y) = 0.81 log (1/X) + 0.06<br>n = 46, $r^2$ = 0.75, s = 0.67, f = 136<br>(for diverse set) | Cronin et al, 1991 |
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Tetryhymena pyriformis* 48-h $IGC_{50}$ (mmol/L) | Log (1/Y) = 0.99 log (1/X) + 0.35<br>n = 74, $r^2$ = 0.81, s = 0.44, f = 307<br>(for diverse set) | Cronin et al, 1991 |
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Vibrio fischeri* 5- min EC*50* (mmol/L) | Log (1/Y) = 0.83 log (1/X) + 0.01<br>n = 39, $r^2$ = 0.84, s = 0.66<br>(for non-polar narcosis – alcohols) | Dearden et al., 1995 |
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Tetryhymena pyriformis* 48-h $IGC_{50}$ (mmol/L) | Log (1/Y) = 0.98 log (1/X) + 0.57<br>n = 256, $r^2$ = 0.74, $q^2$ = 0.731, s = 0.63, f = 707 (for diverse set) | Bearden and Schultz, 1998 |
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Tetryhymena pyriformis* 48-h $IGC_{50}$ (mmol/L) | Log (1/Y) = 1.14 log (1/X) + 0.41<br>n = 70, $r^2$ = 0.95, $q^2$ = 0.95<br>(for non-polar narcosis only) | Bearden and Schultz, 1998 |
| *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | *Tetryhymena pyriformis* 48-h $IGC_{50}$ (mmol/L) | Log (1/Y) = 0.95 log (1/X) + 0.42<br>n = 50, $r^2$ = 0.77, $q^2$ = 0.74<br>(for polar narcosis only) | Bearden and Schultz, 1998 |
| *Poecilia reticulata* 96-h $LC_{50}$ (mmol/L) | *Tetryhymena pyriformis* 48-h $IGC_{50}$ (mmol/L) | Log (1/Y) = 1.05 log (1/X) + 0.56<br>n = 124, $r^2$ = 0.85, s = 0.42, f = 682<br>(for diverse set) | Seward et al., 2002 |
| *Oncorhynchus mykiss* 96-h $LC_{50}$ (mmol/L) | *Lepomis macrochirus* 96-h $LC_{50}$ (mmol/L) | Log Y = 0.95 log X – 0.19<br>n = 199, $r^2$ = 0.92, s = 0.44, f = 2168<br>(herbicides, fungicides, insecticides) | Tremolada et al., 2004 |
| *Oncorhynchus mykiss* 96-h $LC_{50}$ (mmol/L) | *Leuciscus idus* 96-h $LC_{50}$ (mmol/L) | Log Y = 0.97 log X – 0.47<br>n = 39, $r^2$ = 0.92, s = 0.48, f = 447<br>(herbicides, fungicides, insecticides) | Tremolada et al., 2004 |
| *Oncorhynchus mykiss* 96-h $LC_{50}$ (mmol/L) | *Ictalurus sp.* 96-h $LC_{50}$ (mmol/L) | Log Y = 0.99 log X – 0.14<br>n = 32, $r^2$ = 0.91, s = 0.44, f = 298<br>(herbicides, fungicides, insecticides) | Tremolada et al., 2004 |
| *Oncorhynchus mykiss* 96-h $LC_{50}$ (mmol/L) | *Pimephales promelas* 96-h $LC_{50}$ (mmol/L) | Log Y = 1.00 log X – 0.22<br>n = 12, $r^2$ = 0.93, s = 0.52, f = 125<br>(herbicides, fungicides, insecticides) | Tremolada et al., 2004 |

[10] Ideally, if the chemicals in the training set of the QAARs act by the same mode of action, the slope of the regression line is expected to be approximately 1. The differences in the intercept might indicate different sensitivity of the species to the chemicals.

Information about the compliance with OECD principles:

The use of QAAR should be applied with some caution and with awareness for possible exceptions. Justification on the choice of QAAR and its applicability to particular chemicals will be required.

A critical analysis of the available data should be performed beforehand. Since the QAARs use experimental activity/toxicity values as independent variables, these values might be subject of variability. With respect to *Vibrio fisheri* the following shortcomings were noted: a) data are of relatively low quality due to differences in the duration, protocols and interlaboratory variability of the test results (Cronin and Schultz, 1997),.b) they capture relatively well the baseline narcosis but due to the short duration do not account completely for toxicity of more reactive chemicals. The relationships between toxicity from similar protocols to similar species (e.g. 96-h fish to fish relationships) offer a meaningful way of using available data but due to the numerous possible combinations require systematic evaluation of such QAARs.

Table R.10-21 does not offer an exhaustive list of QAARs published in the literature but gives some indication for different available models and how they can be interpreted. Usually, the QAARs are one descriptor models that are stable and have mechanistic basis. However, Bearden and Schultz (1998) noted the goodness-of-fit might be excellent for some modes of action (e.g. different narcoses types) and can be poorer for other modes of action (e.g. Schiff-base formation or Michael-type acceptation), to complete lack of correlation for proelectrophilicity, based on the analysis of QAARs in Table R.10-21. It is also possible to have quantitative structure-activity-activity relationships (QSAARs), where measured or theoretical descriptors are use to improve the correlation with measured activity/toxicity. Example for such models can be found in Zhao et al. (1993).

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