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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 1995

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Foreword

OECD Hazard Assessment Activities

The objectives of OECD work on hazard assessment are to promote awareness and improvement and, to the extent possible, harmonization of hazard assessment procedures for chemicals and pesticides, and to encourage mutual use and acceptance of assessments between countries.

Work on hazard assessment is co-ordinated by the Hazard Assessment Advisory Body (HAAB), which provides advice on this programme of work to the Joint Meeting of the Chemicals Group and the Management Committee of the Special Programme on the Control of Chemicals.

Hazard assessment activities in the period 1989-1991 focused on aquatic effects assessment, in particular the application of Quantitative Structure Activity Relationships (QSARs) to estimate aquatic toxicity data; the extrapolation of laboratory data to the real environment; and the effects of chemicals in sediments. The present document integrates the results of these three aquatic effects activities into an OECD scheme for aquatic effects assessment, which can be used for risk assessment of new and existing chemicals.

This document was produced by F. Balk, P.C. Okkerman and J.W. Dogger (BKH Consulting Engineers, Delft, the Netherlands) and financed by the Netherlands Ministry of Housing, Spatial Planning and Environment (VROM). The work was supervised by J. de Bruijn and C.J. van Leeuwen (VROM). The co-operation of J.L.M. Hermens and H.J.M. Verhaar of RITOX (University of Utrecht, the Netherlands) and the members of the HAAB is gratefully acknowledged.

Aquatic Effects Assessment

Now that environmental risk assessment of chemicals is becoming increasingly important as a tool in environmental management, the call for sound risk assessment methods and models is growing. In the past five to ten years, various approaches for environmental risk assessment have been developed up to a level where they can be integrated into a risk assessment procedure that may be used by OECD Member countries. These observations prompted OECD to hold a series of related Workshops, co-ordinated and organized by the HAAB. During 1990 and 1991, three OECD Workshops took place on the effects of chemical substances in the aquatic environment. The topics of these Workshops were: application of QSARs to estimate ecotoxicity data (Utrecht, the Netherlands, 12-14 September 1990); extrapolation of ecotoxicity data to the real environment (Arlington, Virginia, USA, 10-12 December 1990); and effects assessment of chemicals in sediment (Copenhagen, Denmark, 13-15 May 1991).

The overall aim of these activities was to integrate the results into an OECD scheme for aquatic effects assessment suitable for new and existing chemicals, including the OECD work on High Production Volume (HPV) chemicals. The discussions and recommendations

of the three Workshops on aquatic effects assessment have been presented in Environment Monographs No. 58-60 (OECD, 1992a, b, c). These Workshop reports show that several methods and models have been developed, which can be incorporated into one effects assessment procedure that can be used to calculate "low risk" environmental concentrations in order to minimize the hazard or risk for the aquatic environment. The present document gives a first impulse for such a procedure.

In addition to these Workshops on effects assessment, a Workshop on the application of simple methods for environmental exposure assessment was held in Berlin on 11-13 December 1991. The report of this Workshop has been published as Environment Monograph No. 69 (OECD, 1993).

Where possible, the calculation of levels below which it is unlikely that adverse effects on certain species in the ecosystem occur, should be based on (semi-) field tests or field observations. However, the current practice with regard to effects assessment is such that these data are seldom available and are often difficult to interpret. Hence, in most cases acute and chronic laboratory studies are used and extrapolation procedures are applied to translate the results into environmentally relevant protection levels. Estimation procedures may be used to provide data for effects assessment when data on certain physical-chemical properties, as well as data on effects on certain species, are lacking.

The effects assessment procedure presented in this report is set up along these lines. Depending on the quality and quantity of the available data, it guides the reader along a series of methods that can be used to arrive at concentrations where no adverse effects on the aquatic ecosystem are expected. With the exception of hazards related to secondary poisoning, most of these methods have been intensively discussed at the OECD Workshops.

Subsequent to these Workshops, more experience in aquatic effects assessment has been gained through the OECD Existing Chemicals Programme and elsewhere. The European Commission has also developed technical guidance documents on risk assessment of new and existing chemicals. However, this report is intended mainly to integrate the results of the three OECD Workshops on aquatic effects assessment, and therefore does not take into account more recent activities.

The procedure described in this document is not intended to serve as a standard recipe, from which the reader is not allowed to deviate. On the contrary, in the process of effects assessment, information coming from different sources and methods should preferably be combined in order to constitute a sound basis for environmental effects assessment. Notwithstanding the need for transparent and approved methods and models, the effects assessment of chemicals will always remain a combination of information, knowledge and experience.

The Joint Meeting of the Chemicals Group and Management Committee recommended that this document be derestricted. It has been made public under the responsibility of the Secretary-General.

Table of Contents

(continued next page)

Executive Summary

This document results from an OECD Hazard Assessment project on Aquatic Effects Assessment. Three OECD Workshops related to this theme were held in 1990 and 1991 – the Workshop on Quantitative Structure Activity Relationships (QSARs) in Aquatic Effects Assessment, the Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment, and the Workshop on Effects Assessment of Chemicals in Sediment. The discussions and recommendations of these Workshops have been published as separate OECD Environment Monographs (Nos. 58, 59 and 60, respectively). This document integrates the outcome of the three Workshops into an OECD scheme for aquatic effects assessment.

In the scheme developed here, information on the toxicity of a chemical is used to derive a Maximum Tolerable Concentration (MTC) in water, i.e. the maximum concentration of a chemical at which no unacceptable adverse effects on the ecosystem are expected. Various ways of deriving an MTC are described. They depend on the type and extent of the data available.

If a limited toxicity data set is available (e.g. data on fewer than five species), various assessment factors are recommended for adjusting the effects concentration (e.g. $L(E)C_{50}$, No Observed Effect Concentration – NOEC, etc.) and for deriving an MTC. Where laboratory data are missing, Quantitative Structure Activity Relationships (QSARs) may be used to predict toxicity. QSARs quantitatively relate aquatic toxicity to structural characteristics and/or physical-chemical properties (e.g. octanol-water partition coefficient – K_{ow}) of a substance. This document describes two approaches which may be used, one based on the mode of action of the chemical, the other based on chemical classes.

If more data are available, extrapolation methods may be used in the derivation of an MTC. Two of the extrapolation methods described use the variability in the sensitivity among the various test species as a means of calculating a concentration that is expected to be "low risk" for most (e.g. 95 per cent) of the species in aquatic ecosystems, i.e. a concentration (hazardous concentration) is calculated which is hazardous for only a small number of species (5 per cent). For these methods, chronic NOEC values must be available for at least five different species. A third extrapolation method (Final Chronic Value) is described which estimates an effect concentration of a substance corresponding to a cumulative probability of 0.05 in the chronic toxicity values for the genera for which chronic tests have been conducted. This extrapolation method requires chronic NOEC values for at least eight families.

Since chemicals may accumulate in sediments, this document also describes a method (based on equilibrium partitioning theory) for deriving MTCs in aquatic sediments. Effects on benthic organisms are of concern because, in many habitats, the sediment plays an important role in the recycling of detrital material.

Another aspect addressed (although not discussed at the three Workshops mentioned above) is the hazard related to secondary poisoning: i.e. the bioaccumulation of a substance in aquatic organisms may sometimes lead to high exposure of their predators (e.g. fish-eating birds or mammals). A simple method to estimate an MTC using K_{ow} is proposed.

Finally, a series of worked examples are included, illustrating the various approaches described in the document.

Résumé

Le présent document résulte d'un projet de l'OCDE relatif à l'évaluation des effets sur le milieu aquatique. Dans la période 1990-91, trois ateliers de l'OCDE ont été consacrés à cette question, à savoir l'Atelier sur l'utilisation des relations quantitatives structure-activité (RQSA) pour l'évaluation des effets sur le milieu aquatique, l'Atelier sur l'extrapolation au milieu naturel des données de toxicité aquatique obtenues en laboratoire et l'Atelier sur l'évaluation des effets des produits chimiques sur les sédiments. Les comptes rendus des débats et les recommandations de ces réunions ont été publiés dans la série Monographies de l'OCDE sur l'environnement sous les numéros 58, 59 et 60, respectivement. Le présent document incorpore les conclusions des trois ateliers à un schéma OCDE pour l'évaluation des effets sur le milieu aquatique.

Suivant le schéma décrit dans le présent document, les informations relatives à la toxicité d'un produit chimique donné servent à établir une concentration maximale acceptable (CMA) dans l'eau, c'est-à-dire la concentration la plus élevée d'un produit chimique à laquelle l'écosystème ne devrait pas subir d'effets inacceptables. Diverses façons d'établir une CMA sont décrites. Celles-ci varient selon la nature et le nombre des données disponibles.

Si on ne dispose que d'un ensemble limité de données sur la toxicité (pour moins de cinq espèces, par exemple), divers coefficients d'évaluation sont recommandés pour ajuster la concentration entraînant des effets (CL_{50} ou CE_{50}, CSEO – concentration sans effet observé, etc.) et établir une CMA. Lorsqu'on ne dispose pas de données de laboratoire, on peut utiliser des relations quantitatives structure-activité (RQSA) pour prévoir la toxicité. Ces RQSA relient quantitativement la toxicité aquatique aux caractéristiques structurelles ou aux propriétés physico-chimiques d'une substance donnée (le coefficient de partage n-octanol/eau, K_{oe}, par exemple). Le présent document décrit deux méthodes qui peuvent être utilisées, l'une fondée sur le mode d'action du produit chimique, l'autre sur des classes de produits chimiques.

Lorsque davantage de données sont disponibles, on peut procéder à des extrapolations pour établir une CMA. Deux des méthodes d'extrapolation décrites utilisent la variabilité de la sensibilité parmi les différentes espèces d'essai pour calculer une concentration qui ne devrait présenter qu'un «risque faible» pour la plupart (95 pour cent, par exemple) des espèces des écosystèmes aquatiques, en d'autres termes, une concentration qui n'est dangereuse que pour quelques espèces (5 pour cent). Pour appliquer ces méthodes, il faut connaître les CSEO chroniques pour cinq espèces différentes au moins. Enfin, le document décrit une troisième méthode d'extrapolation (Valeur chronique finale), qui consiste à estimer la concentration qui correspond à une probabilité cumulée de 5 pour cent pour les valeurs de toxicité chronique relatives aux genres sur lesquels des essais chroniques ont été effectués. Pour utiliser cette méthode, il faut connaître les CSEO chroniques pour au moins huit familles.

Puisque des produits chimiques sont susceptibles de s'accumuler dans les sédiments, le présent document décrit, en outre, une méthode (fondée sur la répartition à l'équilibre) permettant d'établir les concentrations maximales acceptables dans les sédiments aquatiques.

Les effets sur les organismes benthiques sont préoccupants car, dans de nombreux habitats, les sédiments jouent un rôle important dans le recyclage des matières détritiques.

Un autre aspect évoqué (bien qu'il n'ait pas été abordé lors des trois ateliers mentionnés plus haut), est le danger lié à l'empoisonnement secondaire : la bioaccumulation d'une substance dans des organismes aquatiques peut parfois entraîner une exposition élevée de leurs prédateurs (oiseaux ou mammifères piscivores, pour exemple). Une méthode simple pour évaluer, à partir du K_{oe}, une concentration maximale acceptable est proposée.

Enfin, on trouvera une série d'exemples concrets pour illustrer les différentes méthodes décrites dans le document.

2. Aquatic Effects Assessment Flow Scheme

Various approaches can be taken to estimate the MTC in water or the MTC for a chemical, depending on the type of information available. Aquatic toxicity data will lead to an MTC that protects the aquatic species from direct effects. If data are lacking, the chemical structure of a molecule may provide a means to estimate the toxicity. The chemical structure can also be used to predict the accumulative potential of a substance. The accumulation potential indicates whether the substance may accumulate to hazardous levels in the food chain, or whether benthic organisms are at risk.

Figure 2.1 presents a scheme indicating the pathways that lead to an optimal use of the data available in the database for an assessment of the effects of a chemical on the aquatic system. Section 3 contains a short discussion of the types of information (i.e. the database) used in assessing aquatic effects.

Where possible, the effects of a substance should be tested in a natural system representative of the area to be protected. Studies in more complex systems, ranging from multi-species laboratory systems to microcosms, experimental ponds and field trials, include a variety of environmental conditions and take into account interactions between species and the abiotic environment. However, the results of these tests are hard to evaluate in terms of No Observed Effect Concentrations. In Section 4 a set of criteria is presented for judging whether ecosystem test results can be used to derive MTCs.

When data on the chronic toxicity of a substance for five or more different aquatic species are available, an extrapolation procedure can be applied to estimate an MTC in water as described in Section 5. When data are available on fewer than five species, a set of assessment factors may be applied as described in Section 6.

When no data are available, physical-chemical characteristics may be used to predict the toxic effects and behaviour of the molecule. In some cases it is possible to classify a substance as a neutral chemical (non-reactive, non-polar). In general these chemicals have a narcotic effect, which is considered as a minimum toxicity, i.e baseline toxicity. In this class of chemicals (Class I) the toxicity strongly depends on its lipophilicity. If log K_{ow} is known either as an experimental or a calculated value, reliable Quantitative Structure Activity Relationships (QSARs) are available to estimate both acute and (semi-) chronic toxicity for fish, *Daphnia* and algae from the octanol-water partition coefficient (K_{ow}). If a chemical cannot be classified under Class I with minimum toxicity, it is sometimes possible to place the substance in another well defined class for which QSARs are available. Indeed, another approach which is widely used is based on chemical classes. For example, in the United States, QSARs which are specific to chemical classes have been developed for 35-40 classes. The chemical classification system and the QSAR approaches are explained in Section 7. The QSAR estimates of toxicity may then be included in the database and enable effects assessment according to the approaches described in Sections 5 and 6. In the Mode of Action Approach, QSARs are available for many species for Class I chemicals. For these chemicals the extrapolation method in Section 5 was extended to a general form, which produces an MTC as a function of K_{ow}. The approach is presented in Annex V.

Since data from (semi-) field experiments are seldom available, the data from single-species testing are used to extrapolate to predict effects in the environment. Usually only a limited database is available on the effect of a chemical, consisting mainly of laboratory toxicity tests with some standard test species. Some extrapolation procedure or another, depending on the type and number of data available, can be followed to estimate the effect of the chemical in the real world (OECD, 1989).

If laboratory toxicity data are lacking, QSARs may be used to predict the aquatic toxicity (OECD, 1992a). QSARs quantitatively relate aquatic toxicity to structural characteristics and/or physical-chemical properties of a substance. Several mathematical and statistical techniques are available to develop QSARs for a variety of chemicals. These predicted toxicity data can be used to fill in the lack of experimental toxicity values, to evaluate the data in case few reliable values are available, and finally may be used in extrapolation procedures to estimate the environmental effect of a substance (OECD, 1992b).

Currently, the impact of a substance is mainly assessed against standard test organisms, e.g. algae, *Daphnia* and fish. This approach does not take into account the full structure and complexity of the aquatic ecosystem. It is known that sediments can have an important influence on the fate and effects of chemicals. Many chemicals are sorbed by sediments to give higher concentrations on solids than in the overlaying water. Possible effects on benthic organisms are of concern, because the benthic community is a vital component of aquatic ecosystems and plays a major role in the recycling of detritus and the flow of energy through the ecosystem. The community is highly structured and adapted and constitutes a food web with many complex interactions. Aquatic effects assessment, however, is mostly based on the effects on species that inhabit the water column. An assessment of effects on the benthic community is usually not included and, up to now, no international guidelines for sediments are available. However, for several types of chemicals, MTCs for sediment may be derived from aquatic MTCs by application of the equilibrium partitioning method (OECD, 1992c). It should be noted that there is a limitation on the application of the equilibrium partitioning method (i.e. it is applicable to neutral organic chemicals or solvents, but is not applicable to charged organics, inorganics, organometallics or charged polymers.)

Another important aspect is the hazard connected to the bioaccumulation of chemicals in aquatic organisms (persistent bioaccumulators). Animals that prey on aquatic organisms containing high levels of contaminants may take up a considerable amount of the chemical with their food. Hence, exposure to contaminated food may lead to secondary poisoning of the food chain along the pathway water/fish/fish-catching bird or mammal. A simple methodology has been developed to include the aspect of secondary poisoning in the aquatic effects assessment. Please note that this method has not been discussed by OECD. It is a proposal made by the authors of this document.

In this document a systematic approach for aquatic effects assessment is described. Starting from an evaluated information set on the chemical, the reader is guided along various pathways each leading to a "Maximum Tolerable Concentration" in water, taking into account the quantity and quality of the data, the toxicity, the potential to accumulate, and the results of field tests. The procedure is presented in the form of guidelines on how to interpret and use these toxicity data and physical-chemical characteristics in a systematic way. Depending on the quantity and quality of the data, the procedure will enable the reader to select priority chemicals for further work or derive MTCs for the aquatic ecosystem.

Aquatic effects assessment is a sequential process. It may comprise three stages – initial, refined and comprehensive – where each stage depends on the type and quantity of information that is available. In initial or preliminary effects assessment, the impact of the chemical is generally assessed against only one or two representatives of primary producers (algae), primary consumers (*Daphnia*), and predators (fish), by means of short-term toxicity tests. Intermediate or refined effects assessments are based on chronic or semi-chronic tests, whereas (semi-) field studies provide the basis for comprehensive effects assessments. Hence, the process of effects assessment goes through stages of imprecise to precise estimations of the concentration that will have no adverse effect on the ecosystem under consideration (OECD, 1989).

A Maximum Tolerable Concentration (MTC) in water indicates a maximum concentration of a chemical where no unacceptable adverse effects on the ecosystem are expected. The decision on what is acceptable or not acceptable is not a matter of science, but a political compromise. Anticipating political discussions, a protection level for the ecosystem is assumed as follows: the aquatic ecosystem is supposed to be protected if 95 per cent of the species is protected. This means that in the ecosystem the species No Observed Effect Concentration is not exceeded for 95 per cent of the species (OECD, 1992b). This concept is a theoretical model for general use. For special situations, e.g. where a dominant or otherwise key species would be included in the 5 per cent of the species that is not fully protected, the protection levels may have to be adapted.

As the effects assessment of a chemical goes through the stages from initial to comprehensive when new data are generated, the reliability of the MTC will increase. In the hierarchy of toxicity data, Quantitative Structure Activity Relationships (QSARs) have a lower status than acute tests and chronic tests have a higher status than acute tests. A reliable and representative field test has the highest status. As a consequence, an MTC derived in the confirmatory stage will in general be more suitable for setting environmental quality objectives, whereas sometimes an MTC based on an inititial effects assessment may be used only for setting priorities for further studies. The assessor therefore needs to be aware of the reliability of the MTC in order to judge its significance.

Ideally, the effects of a chemical should be tested in a natural system representative of an area to be protected. In test systems with a higher level of complexity, ranging from multi-species laboratory systems to microcosms, outdoor ponds, enclosures and field trials, effects are studied under variable environmental conditions and including population dynamics and interactions between species and between species and the abiotic environment. However, studies in more complex systems are not only difficult to perform, and expensive in terms of time and money, but are also hard to evaluate, among other things from a statistical point of view (Kooijman, 1987). As it cannot be expected that the effects of exposure to chemicals will be the same in each area, application factors[2] have to be applied, for instance, to account for regional differences between ecosystems.

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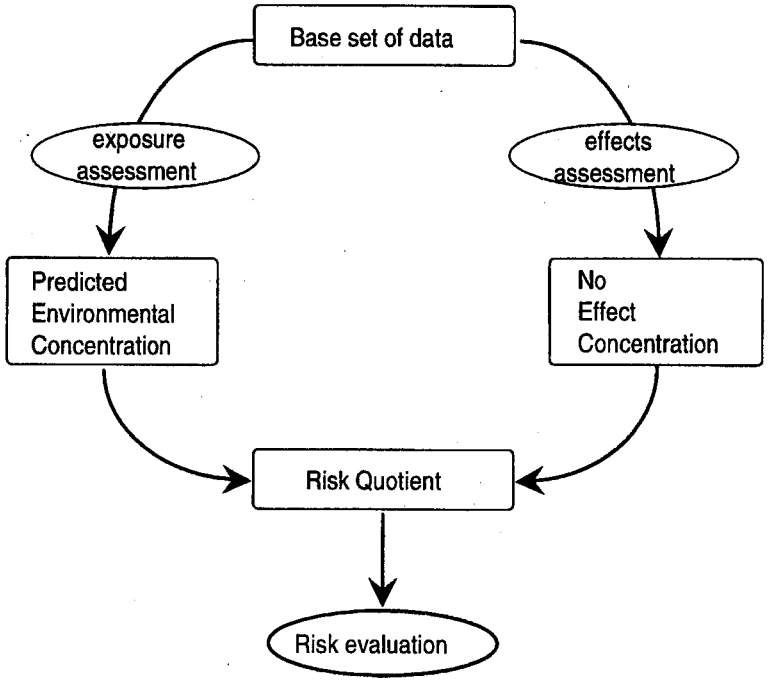
[2] *Application factor* refers to differences in response when comparing acute to chronic toxicity, or single species tests to multispecies tests systems, or when assessing higher order effects, e.g. in ecosystems (Bro-Rasmussen, 1988). *Assessment factor* is often used synonymously.

1. Introduction to Aquatic Effects Assessment

Environmental hazard assessment is defined as the assessment of the potential for a substance to cause adverse effects on environmental species and/or man. Effects assessment can be defined as the identification and quantification of the potential adverse effects of chemicals on individuals, population or ecosystems by means of laboratory testing or field observations (OECD, 1989).[1] In a hazard assessment procedure, a comparison is made of the calculated "low risk" concentration that is considered to minimize the hazard or the risk and the concentrations that are present in the environment, either measured or predicted (**Figure 1.1**). This comparison gives some insights into the risks that the chemical under study poses to human beings or to specific species in any environmental compartment.

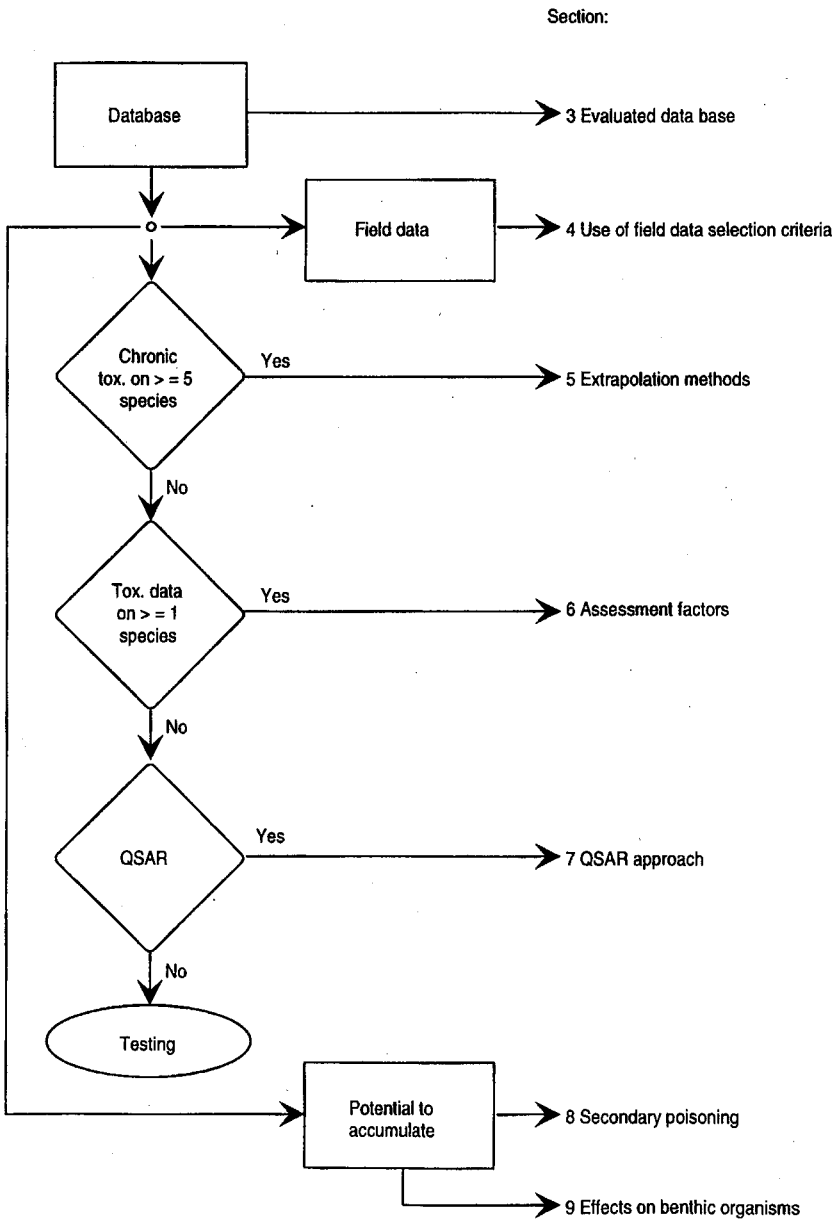
Figure 1.1 Systematic procedure for environmental hazard assessment



[1] Please note that these definitions differ from the terminology used in some countries, such as the United States, where *risk assessment* is used instead of *hazard assessment*, i.e. effects and exposure assessment; and *hazard profile* or *hazard assessment* is used instead of *effects assessment*. In the European Union legislation for risk assessment, different definitions are also used for these terms.

Lipophilic chemicals have a tendency to accumulate in biomass and sorb onto sediments. The octanol-water partition coefficient (K_{ow}) is used as an indication for accumulation potential. Bioaccumulation of a substance in organisms may sometimes lead to high exposure of their predators and may cause "secondary" poisoning. This aspect is elaborated in Section 8. Sorption of a chemical to the sediment may result in higher exposure levels for benthic organisms. The MTC derived from aquatic toxicity data may be converted to an MTC for sediment by methods presented in Section 9. In Section 10 the use of these guidelines is illustrated by some examples.

Figure 2.1 Aquatic effects assessment flow scheme



3. Selection and Evaluation of Data

In order to make a hazard assessment, information on physical-chemical properties and on the toxicity of a chemical substance should be available, either from experiments or from calculations. The quality of the data should be reviewed before they are used for environmental effects assessment.

In the following paragraphs the selection of data for various characteristics is discussed.

3.1 Physical-chemical Properties

- chemical structure of the compound;
- MW: molecular weight;
- MP: melting point (for solids);
- S_w: water solubility;
- K_{ow}: octanol-water partition coefficient (or P_{oct});
- K_{sw}: sediment-water partition coefficient;
- pKa: dissociation constant.

Log K_{ow} may be calculated using the ClogP3 algorithm by Hansch and Leo (1979) from the Pomona-Med Chem program (Leo and Weininger, 1989).

Selections of experimental log K_{ow} values have been taken from the literature and assembled in the THOR/Starlist database as incorporated in the Pomona-MedChem computer program; included are log P* values that are regarded to be the "best" available measured log K_{ow} values (Leo and Weininger, 1989).

Determination of octanol-water partitioning by the shake flask method is not suitable for highly hydrophobic chemicals (log $K_{ow} > 5$). For those chemicals, the K_{ow} determined with the slow stirring method or generator column method can be used (De Bruijn et al., 1989; OECD, 1992a). For some compounds it is not possible to establish a reliable K_{ow} (e.g. anionic surfactants, organometallic compounds). Therefore K_{ow} values should always be reviewed by an expert.

The water solubility is highly correlated with log K_{ow}. For liquid compounds with log K_{ow} between 0 and 5 the following relation may be applied (Müller and Klein, 1992, in Degner et al., 1992):

$$\log S_w = -1.16 \log K_{ow} + 0.79 \qquad r = 0.97, n = 156$$

For solid compounds the relation is extended by the melting point (T_m, in °C). For a "mixed" compound class (not chemical class-specific) the following equation (given by Isnard and Lambert, 1989) can be used when no reliable compound class-specific QSAR is available (Degner et al., 1992):

$$\log S_w = -1.26 \log K_{ow} + 1.0 - 0.0054 (T_m - 25) \qquad r: n.a., n = 300, s = 0.582$$

The sediment-water partition coefficient (K_{sw}) should be normalized to a standard sediment (e.g. content of organic matter, lutum) (OECD, 1992c; Van der Kooij et al., 1991).

3.2 Toxicology

Toxicity studies should be reviewed before using the data in assessments. Data from poor quality tests should not be used. Preference is given to tests carried out according to standardized methods (e.g. OECD Guidelines, EEC, EPA or ASTM methods).

Information may be available on:

- bioconcentration;
- toxicity for aquatic organisms;
- toxicity for fish-eating birds and mammals.

3.2.1 Bioaccumulation

In general, substances with log K_{ow} >3 may bioaccumulate. Substances with:

1) high molecular weight, e.g. > 1000,[3] or

2) calculated least diameter of the molecule > 5.5 Å, or

3) length of the molecule > 5.5 nm

are not expected to bioaccumulate (Kristensen and Tyle, 1990).

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[3] Absorption through biological membranes decreases significantly with MWs > 600. For MW > 1000, absorption is assumed to be negligible.

Moreover, reactive molecules or substances that are easily metabolized are not expected to accumulate to a significant level.[4]

BCF values to be used for the evaluation of secondary poisoning must be expressed on the basis of whole body fresh or wet weights. For conversion, BCFs based on lipid content are multiplied by the fraction of fat of the organism.

With a typical fat fraction for fish of 0.05 (Mackay, 1982), the BCF may be estimated by:

$$BCF = 0.05 * BCF_{fat} \approx 0.05 * K_{ow}.$$

(For fish with a high fat content, a fat fraction of 0.2 may be used.)

If more than one bioconcentration factor is available, the highest factor may be used for a worst case approach. In other cases a geometric mean value might be preferred. Some remarks pertaining to the evaluation of bioaccumulation studies are given in Annex I.

3.2.2 Aquatic toxicity

For effects assessment, results from chronic toxicity tests are preferred. Endpoints which have direct ecological relevance (e.g. survival, growth, reproduction) should be given more weight than other endpoints (e.g. biochemical parameters). General guidance on the interpretation of aquatic toxicity tests may be found in the various test guidelines and also in the OECD Data Interpretation Guide (OECD, 1984).

For the evaluation of acute tests, it should be noted that a 96-hour exposure may not be sufficiently long for some substances (depending on their water solubility) with log $K_{ow} > 5$. An explanation is given in Annex I. This implies that chronic studies are preferred for these substances (Kristensen and Tyle, 1990).

In any case, the water solubility of the test substance must be measured or predicted and the solubility limit must be compared to the (predicted) 96h-LC_{50} value. When the solubility limit is higher than the LC_{50}, the acute toxicity test can be successfully done. However, when the solubility limit is below the LC_{50} value, an acute toxicity test cannot be accepted and chronic testing needs to be invoked. Calculations based on log K_{ow} will not work for surfactants, polymers, inorganics and organometallics (excluding organotin compounds).

If several toxicity data are available for a standard test species, the following rules may be applied:

- If for one test species several toxicity data based on the same toxicological criterion (effect parameter) are available, the geometric mean value is used.

[4] N.B. The metabolism in aquatic organisms may differ strongly, qualitatively as well as quantitatively, from known metabolism in mammals.

- If for one test species several toxicity data are available based on different toxicological criteria (survival, reproduction, growth) from similar tests, only the lowest value is used.

- Data used for the extrapolation methods as described in Section 5 are restricted to NOEC values[5] or geometric mean MATC values.[6] Results of chronic tests reporting only a lowest observed effect concentration (LOEC) might be included when these LOEC values are appropriately converted to estimated NOECs by a formula such as NOEC = LOEC / 2.[7] This LOEC to NOEC conversion can only be used when the LOEC corresponds to an effective concentration (EC) on mortality, growth and/or reproduction of < 20 per cent. If the LOEC corresponds to an EC of > 20 per cent, the chronic toxicity test needs to be redone with lower treatment concentrations. The factor 2 in the formula is taken as an average of the factor between test concentrations, which is usually between 1.8 and 3.2. If the interval between the test concentrations is known, this figure should be taken instead of 2.

3.2.3 Toxicity for birds and mammals

Toxicity tests have seldom been carried out on fish-eating birds and mammals. Therefore data on other bird and mammalian species have to be used. Only toxicity studies on dietary and oral exposure to the chemical are relevant in connection with aquatic effects assessment.

The endpoints of the tests should be expressed as a concentration in food (mg test substance/kg food). Often test results for birds and mammals are expressed in mg/kg body weight/day. These data should be converted to a concentration in food (mg/kg). For the conversion, data on body weight and daily food intake during the tests need to be known. This conversion is only advisable when no other toxicity data for birds or mammals are available. Conversion factors are given in Annex I (Romijn et al., 1993).

Concentrations causing no effect (NOEC) after long-term exposure are preferred. If, in a chronic study, a single dose or the lowest dose of a range causes < 20 per cent effect on mortality or on growth and/or reproduction, a NOEC may be estimated as for aquatic data,

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[5] The No Observed Effect Concentration (NOEC) is the highest test concentration where no significant effect is observed as compared to the control. The Lowest Observed Effect Concentration (LOEC) is by definition the next higher concentration. Therefore a LOEC cannot be derived without a NOEC. However, in some publications LOECs are cited without the NOEC and a conversion may be needed.

[6] MATC is an abbreviation for Maximum Allowable Toxicant Concentration. This is the hypothetical toxic threshold concentration lying between the LOEC and the NOEC. Geometric mean values are often used. Geometric mean MATC-values are calculated using the formula:

$$MATC = \sqrt{NOEC * LOEC}.$$

[7] This conversion was one of the issues not covered in the OECD Workshops.

e.g. from LOEC/2. If the effect is more than 20 per cent, the data cannot be used unless a dose-response curve is available. An application factor of 10 may be applied to NOECs from short-term studies (<1 month) to allow for the extrapolation to long-term exposure (Romijn et al., 1993). If more than one NOEC is available for a single test species, the geometric mean of these values may be used (see Section 3.2.2).

4. (Semi-) Field Tests

Effects assessment is aimed at identifying potential adverse effects of chemicals in the natural environment. Testing under field conditions, with a high degree of realism, would be the most appropriate approach. Some aspects of aquatic ecosystems cannot be predicted from laboratory single-species tests. These include:

- the influence of interspecies interactions on responses to toxicants;
- the response of ecosystem processes to toxicants;
- the recovery rates of ecosystems; and
- the cumulative effects of multiple stresses coupled with varying physical-chemical processes.

According to OECD (1992b), (semi-) field studies provide the basis for comprehensive effects assessment. However, due to the high level of complexity in the field situation, these studies are difficult to perform. Simplified test systems are therefore designed to allow testing at a higher integration level, ranging from multi-species laboratory systems to mesocosms.[8]

Short-term multi-species trials, for instance, are static experiments lasting not more than one month. Most of the time they attempt to measure only acute effects, like mortality of fish or rapid population declines of planktonic species, which may have been predicted in single-species experiments. In comparison with a single-species test, the test system in a multi-species trial is upgraded to a higher integration level; nevertheless there is still a large gap between this type of test and the natural environment and an application factor is therefore needed to arrive at an MTC. Short-term multi-species tests, however, could be a valuable tool during the stage of intermediate or refined effects assessment.

On the other hand, medium to long term mesocosm trials are designed to confirm whether expected fate, predicted chronic effects or bioaccumulation actually occur under reasonably realistic field conditions. They can also reveal secondary effects which may result from species interactions (e.g. algal blooms caused by reduced grazing pressure from susceptible herbivores). These trials may well be used for comprehensive effects assessment.

There are no internationally agreed protocols for ecosystem tests. The approach taken to conducting ecosystem studies is highly dependent upon the objectives of the research. Nonetheless, for the purpose of evaluating the effect of a chemical under field conditions, several criteria may be identified with respect to the design of the experiments, the measurement of biotic responses for relevant species, and the measurement of the test chemical in the system compartments.

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[8] Mesocosms: bounded, and partially enclosed, outdoor experimental units that closely simulate the natural environment.

Criteria for acceptance of the resulting conclusions of an ecosystem study have been suggested (OECD, 1992b; EPPO, 1991; Emans et al., 1993; Van Leeuwen et al., 1994; Okkerman et al., 1991):[9]

- Criteria for the test results

  Desired results include concentrations demonstrated not to cause population or system level impacts (a NOEC for key components or for the ecosystem), and clear concentration-responses.[10] These data can provide evidence for the magnitude of increasing hazard with increasing concentrations. Furthermore, the time it takes for an ecosystem to recover, i.e. the recovery time, is desirable as one indication of the significance of the impact of the chemical. If recovery of ecosystems is dependant on recolonisation, then it has to be simulated in laboratory microcosms and in closed-off mesocosms, e.g. ponds. If recolonisation is not simulated, then the microcosm/mesocosm could over-predict toxicity and impacts.

- The "composition" of the test system

  The test system should include a representative range of taxonomic groups, including fish where possible. Fish can only realistically be included in ponds or large outdoor experimental lakes. If the objective is to represent aquatic ecosystems with some level of realism, the test system must also have components that represent the fundamental properties of such systems, i.e. nutrient cycling, photo-synthesis and trophic structure.

  The duration of the studies should be long enough to enable impacts to be measured, based on knowledge of the nature and fate of the toxicant in aquatic systems and the life history of the organisms expected to be affected. In addition, the scale or size of the test system must be appropriate for the size and important life history characteristics of the organisms included in the study. Typical mesocosm sizes may be 25-50 m^3 and durations may be six to 24 months.

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9 SETAC-Europe has produced a guidance document on testing procedures for pesticides in freshwater mesocosms, indicating the types of information that can be gained from different kinds of mesocosm studies. This document includes sections on: design, composition and characterisation; statistical design and treatment; endpoints and sampling; and data handling (SETAC-Europe, 1992). A European Workshop on Freshwater Field Tests (EWOFFT) was held in Potsdam on 25-26 June 1992. At that workshop the use of freshwater field tests was critically discussed (Hill et al., 1994).

10 Clear concentration-responses are usually toxicologically desirable, but can also be misleading. This issue is discussed in the International Joint Commission for the Great Lakes' 7th Biennial Report on Great Lakes Water Quality (IJC, 1994). The IJC states (page 7) that "conventional scientific concepts of dose-response and acceptable 'risk' can no longer be defined as 'good' scientific and management bases for defining acceptable levels of pollution. They are outmoded and inappropriate ways of thinking about persistent toxics."

- Experimental design

  All studies clearly require a control and at least two or three test concentrations: one should be at a concentration predicted not to cause effects, whereas the other treatments should be selected such that effects are expected and a concentration-response curve can be developed. Finally, each control and treatment should be duplicated although under some circumstances it may be preferable to dispense with replicates in order to extend the concentration range tested. Moreover, replication may not be necessary where responses are predicted to occur within a short time period. The longer the duration of the experiment, the more variable mesocosms are likely to become and the greater the number of replicates needed to determine effects with a given level of confidence (SETAC-Europe, 1992).

- Exposure assessment

  The physical and chemical attributes that affect either exposure and bioavailability or biotic responses must be measured. On several occasions during the experiment the exposure concentrations in water, sediment and biota are measured and a mass balance of the chemical might be attained. The water quality is monitored regularly (e.g. pH, oxygen concentration, hardness, temperature).

- Biological responses

  Because in complex systems not all species can be considered, measurements at the species level should focus on representatives of various trophic levels, functional groups, or otherwise important species. At the individual level, attempts should be made to measure survival, growth, reproduction, and bioaccumulation or other toxicant-specific indicators as appropriate. At the population level, measurements of ecologically or economically important species should include the persistence of the pollution, the age/size structure, production and recovery rate, if any. At the community level, measures must include species composition and relative abundance of important taxa and may include community productivity and respiration.

  The measurements should be made with sufficient frequency to understand the response time, which will frequently differ for each level of biological organization. The process of collecting samples should not be so frequent or extensive as to constitute a stress on the ecosystem.

Another possibility for comprehensive effects assessment is presented when real-world events provide actual case studies. The value of the information that can be obtained depends on the knowledge of baseline ecological conditions and an understanding of exposure conditions, just as in studies under controlled conditions. Ecological baseline information may come either from pre-exposure data, upstream reaches of streams and rivers, or reference streams with similar characteristics.

5. Extrapolation Methods

For the estimation of a "low risk" level where no adverse effects in the environment are expected, an extrapolation factor can be applied to the available toxicity data which accounts for the different sensitivity of other non-tested species in the ecosystem. Various methods have been proposed to extrapolate from single-species toxicity data to a concentration which protects the aquatic ecosystem (OECD, 1992b).[11]

A common feature of these methods is that they use the toxicity data for all tested species to derive a maximum tolerable concentration, as opposed to the method described in Section 6 in which only the lowest test result is used. The variability in the sensitivity of the test species is assumed to be representative for the variability of all species in the aquatic community. Therefore a concentration can be estimated where hazards for most species are expected to be minimized.

In the following chapters these extrapolation methods, e.g. the methods developed by Aldenberg and Slob (1993), Wagner and Løkke (1991) and Stephan et al. (1985), are described. The reader is referred to Section 3.2.2. for the selection and evaluation of the toxicity data used for extrapolation. A computer program which calculates MTCs for the three methods has been developed by Aldenberg (1993).

5.1 Hazardous Concentration (HC_5)

Two extrapolation methods use variability in sensitivity among the various test species as a means to calculate a concentration that is expected to be "low risk" for most (e.g. 95 per cent) of the species in the aquatic ecosystems. In other words, a concentration is calculated that is hazardous for only a small number (5 per cent) of species. Two calculation methods are available, which differ in their basic assumptions regarding the shape of the distribution curve for species sensitivity. One method assumes a log-logistic distribution (Aldenberg and Slob, 1993), whereas the other assumes a log-normal distribution (Wagner and Løkke, 1991). Both methods are based on earlier models of Kooijman (1987) and Van Straalen and Denneman (1989).

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[11] It should be noted that since the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment was held in December 1990, there has been some debate within the scientific community regarding the benefits of statistical extrapolation procedures versus the benefits of using assessment factors (see Section 6). Some state that the statistical procedures show no obvious advantages over the use of assessment factors (Okkerman et al., 1993; Calabrese and Baldwin, 1993; Forbes and Forbes, 1994; Zeeman, in press) although they may recommend them on theoretical grounds (i.e. Okkerman et al., 1993; Calabrese and Baldwin, 1993). Others (Emans et al., 1993) regard statistical extrapolation to be the preferred approach for refined assessments.

The methods may be applied if chronic NOEC (or MATC) values are available for at least five different species. The criterion produced by this method is the Hazardous Concentration for p per cent of the species (HC_p). HC_p is a value such that the probability of selecting a species from the community with a NOEC smaller than HC_p is equal to p (e.g. 5 per cent). The HC_p, e.g. the HC_5, is regarded as a lower boundary for concentrations expected to be harmful for a given community and is considered to be equivalent to the Maximum Tolerable Concentration. The approach is based on the assumption that the NOEC values of the test species as well as of the community species can be conceived of as random trials from a log-logistic or a log-normal distribution, respectively. If the parameters determining the shape and place of the distribution curve were known, it would be possible to find a value for HC_5. However, the real situation is that the parameters determining the shape and place of the distribution curve are not known and have to be estimated. Thus, theoretically, the HC_5 may not provide sufficient protection to 95 per cent of the species. The HC_5 is estimated from the NOEC values for the test species, which are assumed to be independent random trials from the distribution we are looking for. This estimation introduces a second uncertainty which represents the accepted (small) probability that the parameters are not estimated correctly (confidence level). The left confidence limit of HC_5 is used, either with a high level of confidence (95 per cent) for a strict MTC or with a confidence level of 50 per cent for a "most probable" estimation of MTC.

Before the extrapolation method is applied, it has to be confirmed that the data used are a selection from a logistic distribution. This can be checked by means of the so-called Kolmogorov-Smirnov test, which tests for symmetry of the distribution (D'Agostino and Stephens, 1986).

HC_p is estimated from

$$\frac{\overline{NOEC}}{T}$$

$\overline{NOEC}$ is the geometric mean of the NOEC values for m tested species:

$$\overline{NOEC} = \sqrt[m]{NOEC(1)*NOEC(2)*...*NOEC(m)}$$

or

$$\overline{NOEC} = \exp\left\{\frac{lnNOEC(1)+lnNOEC(2)+...+lnNOEC(m)}{m}\right\}$$

and T is an extrapolation factor estimated as:

$$T = \exp^{(s_m * k)}$$

where:

m	:	number of test species
s_m	:	sample standard deviation of lnNOEC values for m species
p	:	percentage of the species in the community that is not protected (e.g. 5 per cent)
k	:	one-side tolerance limit factor for a logistic or normal distribution.

The k value (for a 95 per cent protection level, HC_5) can be read from **Table 5.1** and depends on the selected method, the number of NOEC values used (m), and the confidence level(δ_2) chosen. For the method of Wagner and Løkke (1991), the value of k is found in standard tables for the normal distribution, while for the method of Aldenberg and Slob (1993) k is calculated from computer simulations.

Table 5.1 Extrapolation constants k for the calculation of the extrapolation factor T. The constant k depends on the sample size m (number of species for which toxicity data are available) and is presented for two levels of confidence: 95 per cent and 50 per cent. Protection level: 95 per cent of the species.

	Level of confidence			
	A&S		W&L	
m	95%	50%	95%	50%[a]
2	27.70	2.49	26.26	6.31
3	8.14	2.05	7.66	2.92
4	5.49	1.92	5.14	2.35
[b]				
5	4.47	1.85	4.21	2.13
6	3.93	1.81	3.71	2.02
7	3.59	1.78	3.40	1.94
8	3.37	1.76	3.19	1.90
9	3.19	1.75	3.03	1.86
10	3.06	1.73	2.91	1.83
11	2.96	1.72	2.82	1.81
12	2.87	1.72	2.74	1.80
13	2.80	1.71	2.67	1.78
14	2.74	1.70	2.61	1.77
15	2.68	1.70	2.57	1.76
20	2.49	1.68	2.40	1.73
30	2.28	1.66	2.22	1.70
50	2.10	1.65	2.07	1.68
100	1.95	1.64	1.93	1.66
200	1.85	1.63	1.84	1.65
500	1.76	1.63	1.76	1.65
∞	1.62	1.62	1.65	1.65

A&S Aldenberg and Slob, 1993.
W&L Wagner and Løkke, 1991.
[a] Løkke, pers. comm. 1991.
[b] The extrapolation methods should be used only when $m \geq 5$.

Under the same conditions, the k values for both methods do not differ very much, as can be seen from Table 5.1. Consequently the calculated HC_5 are in the same range, especially when a larger data set is available.

As a general rule, the methods can be applied if data are available for five or more species. However, it should be realized that HC_5 strongly depends on the variability in the sensitivity of the test species (s_m). If the variability is low, five species will give satisfactory results. However, with a high variability in five species, the extrapolation factor will be extremely high, leading to unrealistic low values (Balk and Blok, 1990).

Finally, it is worth noting that both extrapolation methods are based on several assumptions, all of them more or less critical:

- It is assumed that the ecosystem is sufficiently protected if theoretically 95 per cent of the species in the system are fully protected.
- It is assumed that the distribution of the NOECs is symmetrical. As a consequence, high values for unsensitive species lead to low MTCs. This applies especially for those substances that have a specific mode of action for some taxonomic groups.
- It is assumed that the available data are derived from independent random trials of the total distribution. For this reason, elimination of certain test species for some reason or another is not acceptable. In reality, only a limited group of species is used for toxicity tests and those are not a random sample of "all" species. Recent studies regarding the validation of extrapolation methods with multiple-species studies showed that relevant protection levels can be predicted (Emans et al., 1993; Van Leeuwen et al., 1994). Due to the scarcity of reliable field studies, further validation is necessary.

5.2 Final Chronic Value (FCV)

Stephan et al. (1985) presented an adapted EPA method to estimate a Criterion Continuous Concentration, a threshold concentration for unacceptable effects. It aims at protecting 95 per cent of the taxonomic genera and includes the calculation of a Final Chronic Value for animals and a Final Plant Value. The Final Chronic Value (FCV) is an estimate of the effect concentration of a substance corresponding to a cumulative probability of 0.05 in the chronic toxicity values for the genera for which chronic tests have been conducted. The Final Plant Value is obtained from the lowest test result with important plant species. The FCV is calculated from chronic NOEC values for at least eight (animal) families. The following families should be included:

- the family Salmonidae in the class Osteichthyes;
- a second family in the class Osteichthyes (preferably a commercially or recreationally important warm water species);
- a third family in the phylum Chordata;
- a planktonic crustacean;

- a benthic crustacean;
- an insect;
- a family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.);
- a family in any order of insects or any phylum not yet represented.

All chronic values for a species are combined to a species (geometric) mean chronic value. These values are used to estimate mean chronic values for each genus. It is assumed that these values are part of a triangular distribution.

From the cumulative distribution of these genus means, the left fifth percentile is estimated from the lowest four genus means by a non-parametric or graphical method. (If more than 59 genus mean values are available, the four values which have cumulative probabilities closest to 0.05 are selected.) If sufficient chronic data are not available, final chronic values may be estimated from acute values by application of an acute-chronic ratio.

FCV can be calculated as follows:

$$FCV = e^{A}, \qquad \text{where} \qquad A = S * \sqrt{0.05} + L$$

and

$$L = \frac{(\Sigma(\ln GMCV) - S * \Sigma \sqrt{P})}{4}$$

and

$$S = \sqrt{\frac{\Sigma(\ln GMCV)^2 - \frac{(\Sigma \ln GMCV)^2}{4}}{\Sigma P - \frac{(\Sigma \sqrt{P})^2}{4}}}$$

where:

GMCV	:	the genus mean chronic value
S	:	the sample standard deviation of lnGMCV values for the number of genera
N	:	total number of GMCVs available
P	:	cumulative probability of each GMCV, calculated as follows:

The lowest GMCV values are ranked and numbered from R = 1 to 4 (or N). For these GMCV values, P = R/(N+1).

The ability of the method to use only a subsample of the data has particular significance when the distribution of the population from which the data are drawn is not perfectly characterized. Deviations from the assumed distribution that are restricted to the upper part of the distribution will have little impact on the calculation if only the lowest few data in a sample are used. Some errors will arise upon this sort of application, but as long as the distributional assumptions are not grossly violated in the range of the subsample, these errors will generally be minimal. Another advantage of using only the lowest data is that it allows the inclusion of test results with "greater than" values, which must be excluded in other approaches (Erickson and Stephan, 1985).

As a variation on the original method in which only data for specified animal families were used, single-species data (of plants and animals) may be used as input in the formulae for comparison with the other methods. In this case, the calculated FCV is considered to be equivalent to MTC.

Comparison of HC_5 calculated either according to Aldenberg and Slob (1993) or Wagner and Løkke (1991) with (the modified) FCV, showed that the difference is relatively small when the same level of confidence is taken (i.e. 50 per cent; OECD, 1992b).

6. Assessment Factors

When only a limited toxicity data set is available, a factor is used to adjust the effects concentration (laboratory $L(E)C_{50}$, NOEC, etc.) and to estimate environmental concern levels (ECL). The latter is defined as that concentration of a chemical which may cause adverse environmental effects (US EPA, 1984) and is considered to be equivalent to the MTC. The so-called assessment factors are empirically derived; they are not based on any theoretical model, but have developed in line with experience in effects assessment.

Assessment factors may be used to extrapolate from the lowest chronic NOEC to the field situation, from short to long exposure time, and from concentrations with acute effects to no observed effect concentrations (NOEC). For each extrapolation step a factor of 10 is suggested. Consequently, if a data set contains only one LC_{50} for fish, the environmental concern level is estimated from $LC_{50}/(10 * 10 * 10)$ (OECD, 1992b). The suggested assessment factors for an aquatic toxicity data set are presented in **Table 6.1**. The scheme is a modification of a method proposed by US EPA (1984).

Table 6.1 Assessment factors for aquatic toxicity data to derive Environmental Concern Levels (OECD, 1992b)

Available information	Assessment factor applied to the lowest value
NOEC value or QSAR estimate[b] for chronic toxicity derived from a set of data at least consisting of algae, crustaceans and fish	10 [a]
acute $L(E)C_{50}$ or QSAR estimate[b] derived from a set of data at least consisting of algae, crustaceans and fish	100 [a]
acute $L(E)C_{50}$ or QSAR estimate[b] derived from a set of data on one or two aquatic species	1000

[a] If < 3 NOEC values are available, the respective assessment factors are applied and the lowest value of the two is selected.

[b] Evaluated QSARs such as for Chemical Classes I and II as described in Section 7, Tables 7.1 or 7.2.

Assessment factors should be applied with care to *acute* data for substances suspected of having a specific mode of action, with a high log K_{ow}, and which significantly bioaccumulate. The study results must be evaluated to affirm, for example, that the test concentration does not exceed solubility limits and that the duration of the test was sufficiently long in relation to the log K_{ow} or bioconcentration factor. Likewise, if the data set does not contain NOEC data for at least three species, the toxicity tests on which the assessment factor is to be applied should be selected with care: the data need to be evaluated as to their reliability and applicability to the problem (e.g. solubility limits of the substance, test duration in relation to log K_{ow}).

Another set of assessment factors is proposed in the European Union (EU) approach, which focuses on the data for most sensitive species (CEC, 1993).

A similar approach is suggested for the extrapolatation of laboratory toxicity data with vertebrate species (birds, mammals) to a low hazard level for the group of (fish-eating) birds and mammals. These factors are given in **Table 6.2**.

When toxicity data are limited or variable, both statistical methods (Section 5) and assessment factors may be used for the estimation of an MTC.

Table 6.2 Assessment factors to derive a chronic NOEC for fish-eating birds and mammals to assess secondary poisoning (Romijn et al., 1993)

Available information	Assessment factor applied to the lowest value
chronic NOECs for at least three species	10
chronic NOECs for fewer than three species	10 [a)]
acute LC_{50} values[b)] for at least three species	100 [a)]
acute LC_{50} values[b)] for fewer than three species	1000 [a)]

a) If < 3 NOEC values are available, the respective assessment factors are applied and the lowest value is selected.

b) LC_{50} data from standard five-day dietary studies are preferred to LD_{50} values from single oral dose tests.

7. QSAR Approach

7.1 Introduction

The assessment of the aquatic toxicity of chemical substances is often severely hampered by lack of experimental data on even the simplest of parameters, such as the LC_{50}. Therefore much research has been devoted to developing reliable estimation procedures for the toxicity of environmental pollutants. To date, the single most promising technique for estimating toxicities of chemicals, if applied within its recognized limits of applicability, is QSARs, short for Quantitative Structure Activity Relationships. QSARs are formulated in a statistical data analysis procedure, in which the toxicity of various substances is correlated with one or more structural parameters of these substances, normally through uni- or multivariate linear regression or sometimes non-linear regression (Verhaar and Hermens, 1991).

Commonly used structural parameters are: log K_{ow} or aqueous solubilities S_w (hydrophobicity), molar refraction or parachor (dispersion forces), Hammet σ constants, field and resonance effect constants, dipole moment, ionization potentials or assorted quantum chemical parameters, as charge densities, E(homo) and E(lumo) electron energies or superdelocalizabilities (electronic effects) and Taft E_s constants, van der Waals radii, molar volumes, total surface areas or solvent accessible surface areas (steric effects) (Hermens, 1989).

Two approaches to the use of QSARs are introduced in this document. One approach has been developed by US EPA/OPPT and is based on the classification of chemicals by their chemical structure but without any explicit consideration of the chemicals' mode of action (e.g. narcotic effects) in the aquatic environment. In this approach, the reliability of the QSAR is evaluated empirically. One specific equation to estimate one endpoint can be applicable for all chemicals in a class. If the deviation of estimation is very large for a chemical, then that chemical is considered to belong to a separate group of chemicals and another equation will be developed for the chemical.

The other approach focuses on the mode of action of chemicals as the first step. This approach has been suggested by the Netherlands and the US EPA Environmental Research Laboratory-Duluth. Chemicals are classified into four classes depending on their toxicity such as baseline toxicity, etc. on the basis of their chemical structure. The reliability of the QSAR will be evaluated *a priori* class by class. Then specific equations developed for each class are used for estimating endpoints.

7.2 Chemical Classification Approach

QSARs have already been used by US EPA/OPPT for a number of purposes related to aquatic effects assessment, particularly for the regulation of new chemicals under the Toxic Substances Control Act (TSCA). The EPA approach used is simply a regression equation between a chemical's toxicity and one or more of its physical-chemical properties. These equations have been specifically developed for classes of chemicals. Detailed procedures are

mentioned in a compendium of the methods titled *Estimating Toxicity of Industrial Chemicals to Aquatic Organisms using Structure Activity Relationships*, which included 49 QSARs and was published in 1988 (Clements, 1988). Thirty-five to forty classes represented as additional QSARs have since been developed.

It should be noted that SARs (Structure Activity Relationships) developed by US EPA consist not only of 1) QSARs, but also 2) the nearest analog or the two analogs which bracket a chemical to predict toxicity because no physical-chemical property has been found to be correlated with the toxicity for the chemical classes, and 3) generic evaluations of a particular chemical class, e.g. acid dyes.

In the US approach (Nabholz et al., 1993), the standard toxicity profile for all chemicals assessed for potential risk to aquatic ecosystems consists of the following effective concentrations (EC):

- fish acute toxicity (96 h LC_{50});
- daphnid acute toxicity (48 h LC_{50});
- green algal toxicity (96 hr EC_{50});
- fish chronic value (ChV);
- daphnid ChV; and
- algae ChV.

The chronic value (ChV) for fish and aquatic invertebrates is the same as a chronic No Effect Concentration (NEC) and the geometric mean of the Maximum Allowable Toxicant Concentration (MATC,[12] see Section 3.2.2). The ChV for green algae is the 96 h NEC. If the 96 h NEC is not available, then the 96 h NOEC is used; and if the NOEC is not available, then the 96 h EC_{10} is used. In some cases, the algal ChV is predicted by dividing the 96 h EC_{50} by 4.0. The value of four is empirically based. The ratio of the 96-h EC_{50} to the 96-h ChV is about four for neutral organic chemicals and for aliphatic amines, and is about 2.5 for polycationic polymers. Chemicals which act indirectly via chelation of nutrient divalent cations, such as polyanionic polymers and polyanionic monomers which are designed to chelate Ca^{+2} and Mg^{+2}, have larger ratios (of 10 or greater). US EPA/OPPT has been using 4.0 as a last resort algal ChV estimate for about ten years.

Although attempts are made to predict all six endpoints listed above, about half the time only EC values for fish acute, daphnid acute and algal toxicity (i.e. the base set tests) can be predicted. Occasionally only one EC value is available for a chemical.

Validation of all environmental toxicity SARs has been done in US EPA since 1981. As soon as new SARs or QSARs are developed, predictions become hypotheses to be tested. Testing results are integrated into existing SARs, to be used to create new SARs whenever new toxicity information becomes available.

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[12] ChV, NEC and MATC are synonymous.

Validation has been carried out by using validation ratios, i.e. the predicted toxicity value (P-EC) divided by the measured toxicity value (M-EC). The pragmatic goal of US EPA's SAR programme has been that predicted values be within a factor of 10 of the measured values, i.e. ratios of 0.1 to 10.0. If a validation ratio fell outside this range, then an adjustment of the SAR for the chemical class was required. For example, if a measured toxicity value indicated that a chemical was more than ten times more toxic than predicted by current SAR, then the chemical was characterized as exhibiting excess toxicity and a new SAR for that subclass was usually developed. A more specific goal was to have mean validation ratios be slightly less than 1.0. If high accuracy could not be obtained, then to reasonably overpredict the toxicity of new chemicals was preferred for the protection of the environment. A report of the validation study, which shows that prediction by SARs is more accurate than anticipated, is presented as Annex II to this document (Nabholz et al., 1993).[13]

7.3 Mode of Action Approach[14]

In this approach, QSARs are established for compounds with a common mode of toxic action. Recently, six modes of action were distinguished, i.e. non-polar narcosis, polar narcosis, uncoupling of oxidative phosphorylation, respiratory membrane irritation, acetylcholinesterase inhibition and central nervous system seizure (McKim et al., 1987; Bradbury et al., 1989). The mechanism of narcosis is non-specific: the potency of a chemical to induce narcosis is entirely dependent on its hydrophobicity. This implies that, in the absence of all specific mechanisms of toxicity, a chemical will always be as toxic as its hydrophobicity (e.g. log K_{ow}) indicates, or in other words, no chemical will be less toxic than implied by its hydrophobicity (Verhaar and Hermens, 1991). Narcosis type toxicity is therefore also called "baseline" toxicity or minimum toxicity (Könemann, 1981).

Chemicals may be classified on the basis of their chemical structure. According to Hermens (1989) and Verhaar et al. (1992), four broad classes of chemicals can be distinguished:

Class I : inert chemicals (baseline toxicity)

Class II : less inert chemicals

Class III : reactive chemicals

Class IV : specifically acting chemicals

A description of the four Classes is given in Annex III.

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13 An additional independent evaluation (and validation) by the US and the EC of QSARs used by the US EPA/OPPT demonstrated good agreement between predicted and measured toxicity for *Daphnia* and fish (OECD, 1994).

14 The text of Section 7.3 was mainly taken from the report of the OECD Utrecht Workshop on QSARs in aquatic effects assessment (1992a) and other references as indicated.

These general rules provide guidance in the allocation of compounds to appropriate chemical classes for which QSARs can be applied.

Within the classes, various modes of action may be distinguished based on physiological and toxicological responses of organisms. If a chemical is classified as belonging to Class I, effect concentrations can be predicted by QSAR equations given for baseline toxicity. If a chemical is classified as Class II, a range of expected effect concentrations can be calculated. The expected effect concentration is between 0.2 and 0.5 times the baseline toxicity effect concentration.

If a chemical is classified as belonging to Class III or IV, the actual effect concentration is expected to be a factor 10 to 10^3-10^4 lower than the baseline toxicity effect concentration (Verhaar and Hermens, 1991; Verhaar et al., 1992). For classes III and IV, QSARs are available but these have not yet been evaluated by the OECD.

At the time of the OECD Utrecht Workshop (OECD, 1992a), adequate QSAR predictions of aquatic toxicity could only be made for chemicals classified under Class I or II and results have been published recently (Verhaar et al., 1994). The classification for Class I and Class II is presented in the next sections.

7.3.1 Class I chemicals (baseline toxicity)

Narcosis type or baseline toxicity has been observed for a number of classes of inert organic chemicals: aliphatic alcohols, aliphatic ketones, aliphatic ethers and alkoxyethers, aliphatic halogenated hydrocarbons, saturated alkanes and halogenated benzenes. Structural requirements for these chemicals with narcosis type toxicity are currently restricted to organic compounds that consist of carbon, hydrogen, nitrogen, oxygen and/or halogens (excluding Iodine). Furthermore, in this subsection narcosis refers only to the type I or non-polar narcosis.

Chemicals can be classified as Class I according to the flow chart presented in **Figure 7.1**. A more detailed description of the classification scheme in this figure is given in Annex III. It should be noted, however, that some chemicals that are known to be more toxic than baseline toxicity do classify as Class I (e.g. γ-HCH). It is always advisable to check whether a specific compound is listed under Class IV – specifically acting chemicals. This list is included in Annex III.

If a chemical can be classified as a Class I chemical, it is assumed to act by (non-polar) narcosis and QSAR equations may be used to predict the aquatic toxicity. QSAR equations were evaluated for the following endpoints: acute and chronic toxicity to fish, acute and chronic toxicity to *Daphnia magna*, and chronic toxicity to algae. The applicable equations are presented in **Table 7.1** (OECD 1992a). Equations for more species are summarized in Annex IV.

It should be noted that these relations are only valid for liquids at room temperature. When predicting the toxicity of solids at room temperature, either a measured water solubility or a predicted water solubility (using K_{ow} and melting point, see Section 3.1) must be known. If the predicted toxicity value is higher than the solubility limit, the effect cannot be obtained within the time period of the test and a longer study has to be done.

Figure 7.1 Flow scheme for the classification of Class I chemicals according to Verhaar and Hermens (1991, 1992)

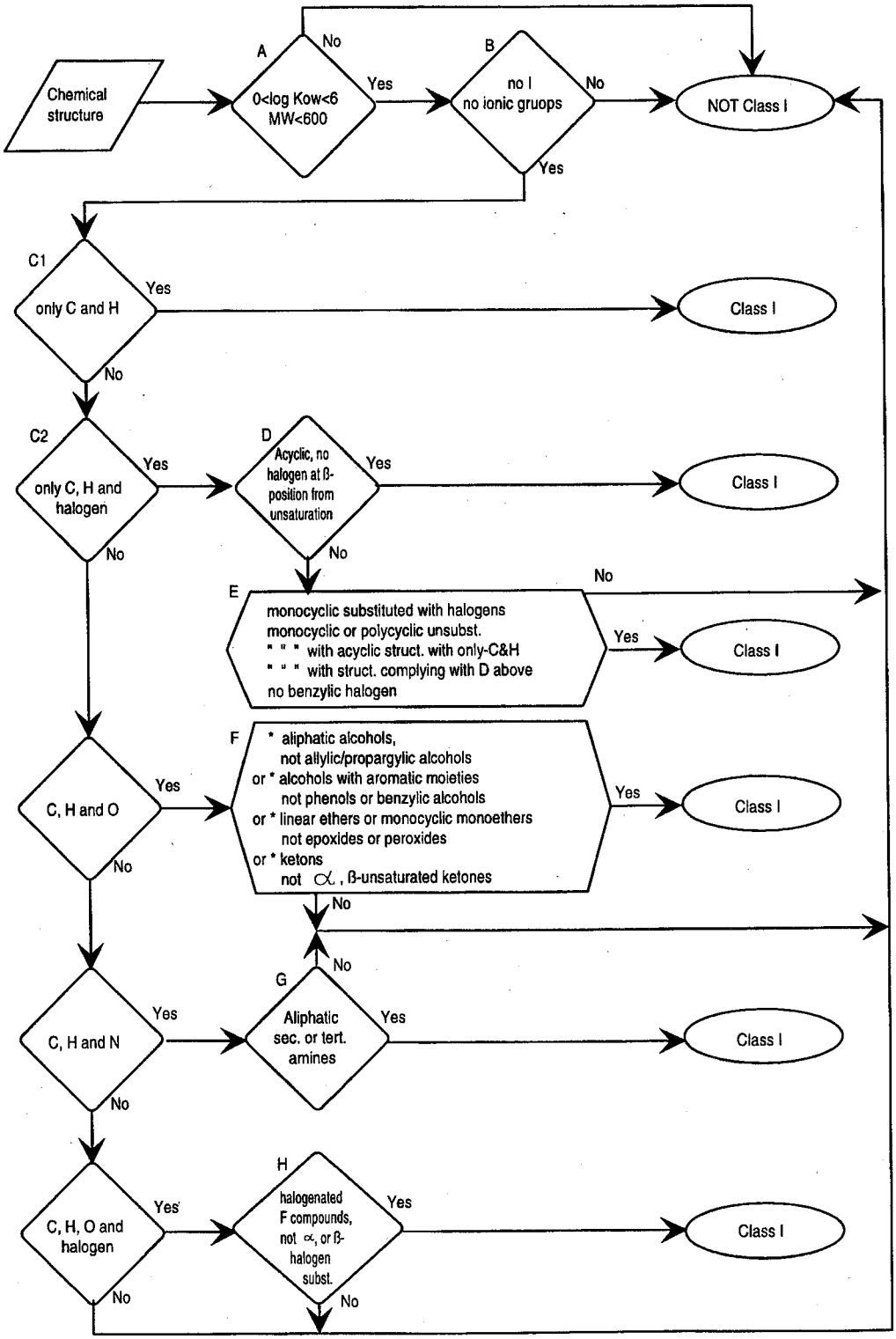


Table 7.1 Mode of action and applicable QSARs* for Class I chemicals as presented at the OECD Utrecht Workshop (OECD, 1992a)

1. Acute toxicity to fish				
(I) log LC_{50} (mmol/l) = -0.94 log K_{ow} + 0.94 log (0.000068 K_{ow} + 1) + 1.75			**n = 65**	**r^2 = 0.98**
Species: fathead minnow (*Pimephales promelas*), 30 days, 0.12 g Endpoint: 96-h LC_{50} Log K_{ow} range: -1.17 to 5.00 Ref.: Veith et al., 1983 Remark: Bilinear curve. Decreased toxicity for high K_{ow} (steady state is not reached within 96 hours)				
(ii) log LC_{50} (mmol/l) = -0.87 log K_{ow} + 1.87	**n = 50**	**r^2 = 0.976**	**s = 0.24 [a)]**	
Species: Guppy (*Poecilia reticulata*), 2-3 months Endpoint: 7-day and 14-day LC_{50} Log K_{ow} range: -1.35 to 5.69 Ref.: Könemann, 1981				
2. Chronic toxicity to fish				
log NOEC (mmol/l) = -0.90 log K_{ow} + 0.8	**n = 30**	**r = 0.914**	**s = 0.33 [a)]**	
Species: Zebrafish (*Brachydanio rerio*) and fathead minnow (*Pimephales promelas*) Endpoint: 28-day NOEC, early life stage (growth, hatchability) Log K_{ow} range: 0.54 to 5.69 Ref.: Van Leeuwen et al., 1990; Call et al., 1985				
3. Acute toxicity to *Daphnia magna*				
log EC_{50} (mmol/l) = -0.91 log K_{ow} + 1.72	**n = 19**	**r^2 = 0.98**	**s = 0.24 [a)]**	
Endpoint: 48-h immobilisation Log K_{ow} range: -1.35 to 5.69 Ref.: Hermens et al., 1984				
4. Chronic toxicity to *Daphnia magna*				
(i) log NOEC (mmol/l) = -1.04 log K_{ow} + 1.30	**n = 17**	**r^2 = 0.98**	**s = 0.25**	
Endpoint: 18- to 21-day NOEC on reproduction Log K_{ow} range: -0.24 to 5.18 Ref.: De Wolf et al., 1988; Kühn et al., 1989				
(ii) log NOEC (mmol/l) = -1.07 log K_{ow} + 1.25	**n = 10**	**r^2 = 0.97**	**s = 0.40**	
Endpoint: 18- to 21-day NOEC on growth Log K_{ow} range: -0.24 to 5.18 Ref.: De Wolf et al., 1988				
5. Chronic toxicity to algae				
log NOEC (mmol/l) = -1.00 log K_{ow} + 1.77	**n = 10**	**r^2 = 0.93**	**s = 0.17**	
Species: *Selenastrum capricornutum* Endpoint: 72- and 96-h EC_{50} for population growth Log K_{ow} range: 2.19 to 4.14 Ref.: Calamari et al., 1983; Galassi et al., 1988				

* Some equations in this table marked [a)] differ slightly from the equations in Annex IV. The reason for the small difference is that, for the preparation of the list in Annex IV, the relationships were recalculated using more recent values for K_{ow} (De Bruijn et al., 1989; Van Leeuwen et al., 1992).

7.3.2 Class II chemicals

Class II chemicals are not reactive under normal physiological conditions and do not interact with specific receptors in an organism, but are slightly more toxic than baseline toxicity.

The following groups of chemicals can be classified as Class II chemicals:

- non- or weakly acidic phenols;
- aromatic amines and anilines;
- aliphatic primary amines;
- weakly basic pyridines.

Two QSAR equations were selected to predict the acute fish toxicity of phenols and primary aromatic amines that can be identified as polar narcotics or uncouplers (OECD, 1992a; Bradbury, Hermens, pers. comm.). As already stated in Section 7.2, these relations are generally only applicable for liquids at room temperature or low melting solids.

The phenolic or primary aromatic amine compound under investigation has to be classified with respect to its probable mode of action to ensure that the most appropriate QSAR is selected. For the selection of the most appropriate QSAR, the decision scheme in **Figure 7.2** (OECD, 1992a) should be followed. Guidance for the subdivision in Figure 7.2 is given below. Examples of Class II chemicals are presented in Annex III.

The QSARs referred to in the figure and some background information are presented in **Table 7.2.**

Guidance for the decision scheme in Figure 7.2:

The basic procedure consists of several steps to identify those phenols and primary aromatic amines for which no QSAR predictions can be recommended, then to identify those for which uncoupler QSARs are recommended, and lastly to identify those phenols and primary aromatic amines which can be assumed to be polar narcotics. Examples of Class II chemicals are given in Annex III.

The decision scheme is to be applied as follows:

1) The first step is to identify all outliers such as electrophiles, alkylating structures, reactive structures, dihydroxy compounds, diamino compounds, nitrophenols, phenolic aldehydes, hydroxyanilines and nitroanilines. For these compounds, no appropriate QSARs are available and prediction cannot be recommended as the toxicity may be much higher than estimated using uncoupler or polar narcotic QSARs. Consequently further testing of the compound is necessary.

2) If the compound is a phenol or primary aromatic amine that does not meet the criteria summarised under 1) above for being an outlier, the user is to proceed to the next step, which is to identify likely uncouplers such as dinitrophenols,

phenols with pKa ≤ 6.3 and phenols with more than three halogen substituents, dinitroanilines and primary aromatic amines with more than three halogen substitutes. If the compound is an uncoupler, QSAR (ii) in Table 7.2 may be applied.

3) If the compound is a phenol or primary aromatic amine that does not meet the criteria summarised under 1) and 2) above for being an outlier or an uncoupler, the user is to proceed to the next step, which is to identify some further criteria. If the phenol or primary aromatic amine under investigation contains any of the following features, it can be assumed to be a polar narcotic: fewer than nine carbon atoms in alkyl chains, fewer than four halogen substituents, 0-alkyl substituent, 0-phenyl substituent. In the case of phenols, if pKa > 6, the same assumption can be made. If according to the above listed criteria the phenol or primary aromatic amine under investigation can be assumed to be a polar narcotic, QSAR (i) can be applied with a sufficient level of confidence.

4) Before the appropriate QSAR is applied, the user is to check if the log K_{ow} and/or pKa for the phenol or primary aromatic amine under investigation are within the range covered by the QSAR. If not, further testing is necessary.

7.4 Application of QSARs

If no toxicity data are available and the chemical is classified as a Class I chemical, QSARs can be used to estimate the toxicity for fish, *Daphnia* and algae. For a Class II chemical, QSARs may be used to estimate the acute toxicity for fish. Assessment factors (10, 100 or 1000) may be applied to derive a maximum tolerable concentration.

If few experimental data are available and the chemical is classified as a Class I chemical, QSARs may be used to verify if the experimental toxicity of a chemical agrees with baseline toxicity. If so, evaluated QSAR equations may be used to extend the database with data on fish, *Daphnia* and algae. An MTC may be derived by the application of assessment factors. As an alternative, the MTC can be estimated directly from log K_{ow} of the substance.

Figure 7.2 Decision scheme for application of QSARs for phenols and primary aromatic amines according to OECD (1992a) [for QSARs (i) to (iii), see Table 7.2]

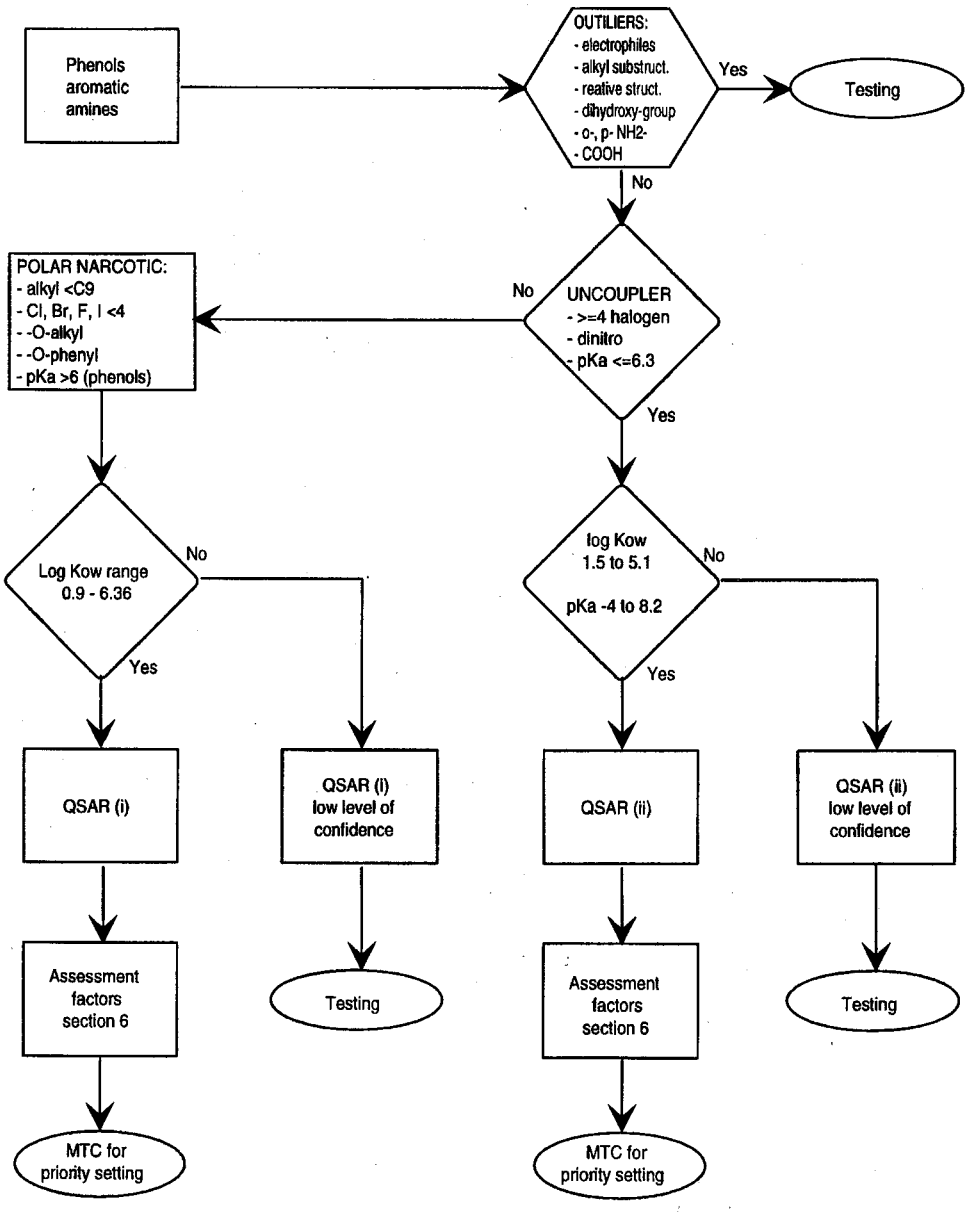


Table 7.2 Mode of action and applicable QSARs for Class II chemicals as presented at the OECD Utrecht Workshop (OECD, 1992a)

1. Polar narcosis type chemicals (phenols, anilines)

These chemicals are not reactive under normal physiological conditions and do not interact with specific receptors in an organism, but are slightly more toxic than baseline toxicity. In general, effect concentrations of these chemicals are between two and five times lower than predicted, using baseline toxicity QSAR equations.
In LC_{50} tests with binary mixtures, all chemicals are concentration-additive with phenol.
The QSAR developed by Veith and Broderius (1987) is recommended:

(i) $\log LC_{50}$ (mmol/l) = -0.65 $\log K_{ow}$ + 0.7 **n = 40 r = 0.949**

Species: fathead minnow (*Pimephales promelas*)
Endpoint: 96 h LC_{50} to fish
Log K_{ow} range: 0.90 to 6.36.
This equation is derived from data for both phenols and aniline derivatives.

The structural requirements related to this model are: **phenols and aromatic amines with various substituents, except derivatives with four or more rings, substituted halogens and two or more nitro groups.**

2. Uncouplers

Some chemicals are more toxic than predicted by the equation for non-polar narcosis. These chemicals probably act as uncouplers of oxidative phosphorylation. This is supported by mode of action studies (McKim et al., 1987; Bradbury et al., 1989). Within this mode of action two QSARs are recommended:

(ii) $\log LC_{50}$ (mmol/l) = -0.59 $\log K_{ow}$ - 0.2 **n = 6 r = 0.978**

Species: fathead minnow (*Pimephales promelas*)
Endpoint: 96 h LC_{50} to fish
Log K_{ow} range: 0.96 to 5.04.

The structural requirements related to QSAR(ii) are: **substituted phenols with alkyl (including unsaturated), chlorine, bromine, nitro, methoxy, amino, phenoxy, N-acetyl and combinations.**

Reference: Schultz at al., 1986.

(iii) $\log LC_{50}$ (mmol/l) = -0.67 $\log K_{ow}$ + 0.05 **n = 11 r = 0.906**

Species: fathead minnow (*Pimephales promelas*).
Endpoint: 96 h LC_{50} to fish.
Log K_{ow} range: 1.54 to 5.12.
pKa range: -4.0 to 8.2.

The structural requirements related to this model are: **substituted phenols and anilines (primary aromatic amines) with four or more ring substituted halogens and two or more nitro groups.**

Reference: US EPA ERL-Duluth (Bradbury, unpublished).

7.4.1 QSAR approach when few experimental toxicity data are available. for Class I chemicals

When for at least one species a NOEC or L(E)C_{50} value is available, an MTC may be derived in various ways. Assessment factors may be applied to the experimental data, or QSAR estimates may be used to extend the database before an MTC is derived. The approach is schematically presented in **Figure 7.3.**

Guidance on the decision scheme in Figure 7.3:

- Assessment factors

  When some experimental data are available, assessment factors of 10, 100 or 1000 (see Section 6) may be applied to the lowest value to derive a maximum tolerable concentration which can be used for priority setting.

- Chemical structure Class I

  On the basis of the chemical structure, a number of chemicals can be identified that act only by (non-polar) narcosis (Section 7.2). In general, these chemicals are relatively non-reactive and do not ionize strongly (are non-polar). If a chemical is classified as a Class I chemical, the next step is to calculate the baseline toxicity for this chemical with QSARs for non-polar narcosis. If a chemical is not classified as a Class I chemical, further testing may be necessary.

- Apply QSAR.I

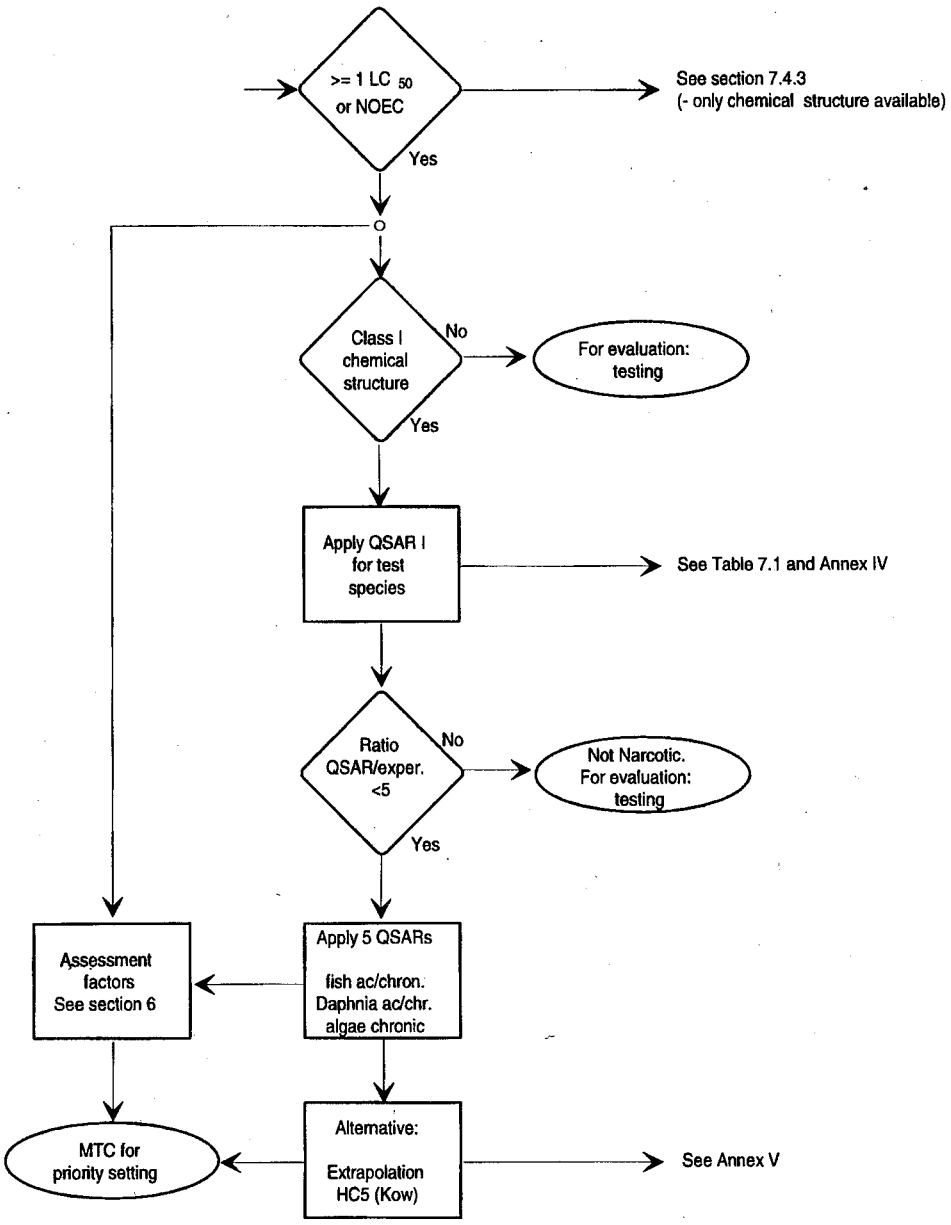
  The QSARs designed for Class I chemicals (further called QSAR.I) are based on the chemical descriptor K_{ow} (octanol-water partition coefficient). The QSARs that may be used are listed in Table 7.1 and in Annex IV. The choice of the QSAR.I(s) to be applied depends on:

  1) The phylum of the test species for which the toxicity data are available (e.g. *Daphnia magna* belongs to the phylum arthropods). If no QSAR is available for the particular test species, all relevant QSARs (depending on endpoint and duration of the available tests, see sub 2) for the same phylum could be applied. The lowest QSAR could be taken as a starting point.

  2) The endpoint (NOEC, EC_{50}, NOLC or LC_{50}) and duration of the toxicity tests.

  After selection of the appropriate QSAR(s), the baseline toxicity for the chemical can be calculated using a reliable K_{ow} value.

Figure 7.3 Application of QSARs when few experimental data are available



- ratio $\frac{Calculated.effect.conc.(QSAR.I)}{observed.effect.concentration} < 10$ for a particular endpoint

  In the above ratio, the available experimental data are compared to the results of a QSAR estimate to verify whether these substances really fit into the QSARs for narcotic chemicals. A substance is not considered to be a narcotic chemical in case the baseline toxicity calculated with QSAR.I exceeds its experimentally derived $L(E)C_{50}$ or NOEC by more than a factor of 5. If the calculated baseline toxicity exceeds one or more of the experimental results, further testing of the chemical is necessary for hazard assessment. In that case, an MTC can be derived by application of the assessment factors to the experimental data as described in Section 6. The MTC can be used for priority setting.

- Apply five QSARs

  Once it is established that a chemical may be considered to act solely as a narcotic chemical, the toxicity for fish (acute and chronic), *Daphnia* (acute and chronic) and algae can be estimated from the recommended relations given in Table 7.1. It should be noted, however, that valid experimental data have preference over QSAR estimates for the same test species. Assessment factors applied to the QSAR estimates (100 or 10, see Table 6.1) result in an MTC which may be used for priority setting.

- Alternative

  As an alternative to the previous step [Apply five QSARs], an MTC may be derived from the extrapolation procedures described in Section 5. When the chronic toxicity can be estimated from QSAR.I for a number of test species, these estimates can be used as input in the extrapolation methods. This procedure was carried out for some 20 species and more than 100 Class I chemicals. For each substance a hazardous concentration (HC_5, i.e. 95 per cent protection level) can be derived, and in this way a connection is made (via the QSAR toxicity estimate) between log K_{ow} and the hazardous concentration for Class I chemicals (Van Leeuwen et al., 1992). The procedure is presented in Annex V. The HC_5 (K_{ow}) may be used as an MTC for priority setting.

7.4.2 QSAR approach when few experimental toxicity data are available for Class II chemicals

When few experimental data are available for a Class II chemical (phenols, aromatic amines), the database can be extended with a QSAR estimate of the acute toxicity for fish. For the selection of the most appropriate QSAR from Table 7.2, the decision scheme in Figure 7.2 should be followed. It should be noted that QSAR estimates should not be used when data on acute fish toxicity are available from reliable experiments.

A maximum tolerable concentration can be derived by application of assessment factors (see Section 6) to the combined dataset of experimental data and QSAR estimates. The MTC may be used for priority setting.

7.4.3 QSAR approach when only chemical structure is available

Even when no experimental toxicity data are available, it might be possible to derive an MTC that may be used to set priorities (or Environmental Concern Levels). If the chemical structure complies with the structural requirements for Class I or Class II, the QSARs as presented in Tables 7.1 and 7.2 may be used. These QSARs were selected as the most reliable ones by experts during the OECD QSAR Workshop (OECD, 1992a). The measured or calculated log K_{ow} (see Section 3.1) is the input variable for the QSAR equations.

For Class I chemicals, the acute toxicity for fish and *Daphnia* and the chronic toxicity for fish, *Daphnia* and algae may be estimated. Assessment factors of 100 and 10, respectively, may be applied to derive an MTC. As an alternative, the $HC_5(K_{ow})$ extrapolation method referred to in Section 7.4.1 may be applied.

For Class II chemicals, the acute toxicity for fish may be estimated and an assessment factor of 1000 should be applied to derive an MTC.

For chemicals that are not classified as Class I or II, no method evaluated by the OECD (OECD, 1992a)[15] is available for deriving levels where no adverse effects are expected. Even for setting priorities, further testing of the chemical is necessary.

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[15] However, Clements (1988) presents a large number of QSARs for specific chemical classes. See also Section 7.1.

8. Secondary Poisoning

8.1 Introduction

Chemicals in surface water distribute between water, sediment and biota (such as fish). Fish can accumulate chemicals to levels far beyond the concentration in the water phase. When fish are eaten by predatory species such as birds and mammals, the predators are exposed to chemicals present in their prey. The uptake of chemicals through ingestion of contaminated fish may lead to indirect effects on fish-eating animals, i.e. secondary poisoning. The presented method arises from discussions in the Netherlands and has not been discussed within the OECD.

When secondary poisoning is to be avoided, the concentration of chemicals in the food should be below the No Observed Effect Level in dietary toxicity test with animals representative of fish-eating birds or mammals. The No Observed Effect Level is considered as a maximum concentration in food which will not lead to adverse effects. When the bioconcentration factor (BCF) of a substance is known, the maximum concentration in food (fish) can be converted to a maximum concentration in water that will not cause adverse effects on fish-eating predators (Maximum Tolerable Concentration, MTC).

The same considerations can also be made when assessing the ecological risks to piscivorous fish. Models have been developed to predict the concentrations of extremely hydrophobic chemicals in predatory fish (Barber et al., 1988, 1991; Gobas et al., 1988; Norstrom et al., 1976; Thomann and Connolly, 1984), but in those cases the BCF does not exceed a factor of 10. Therefore no further attention is paid to this exposure route.

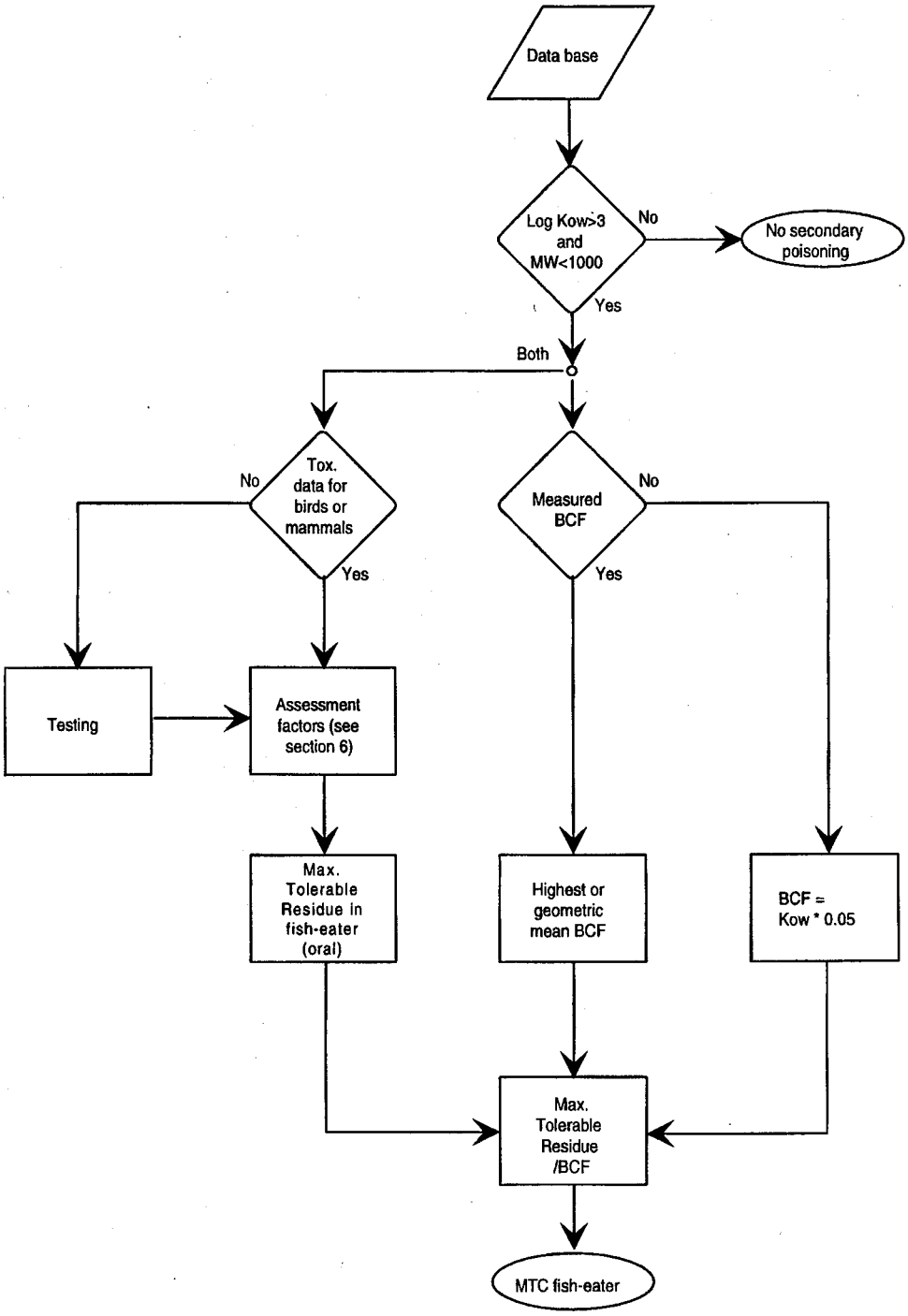
8.2 Proposed Approach

For the assessment of effects of secondary poisoning, the following procedure is proposed:

- It is decided whether the compound has a potential for bioaccumulation (based on the K_{ow} and molecular weight);
- The level of bioaccumulation in fish (BCF) is derived from experimental or calculated data;
- The available toxicity data are used for estimating a maximum concentration in food that is expected to be "low risk" for fish-eaters;
- The maximum concentration in food is divided by the BCF in fish to give a maximum level for fish-eating birds or mammals expressed as a concentration in surface water; in other words, an MTC.

The flow scheme in **Figure 8.1** is designed to provide guidance for deriving MTC levels for fish-eaters.

Figure 8.1 Procedure to derive Maximum Tolerable Concentrations to avoid secondary poisoning of fish-eating birds and mammals



Guidance for the flow scheme in Figure 8.1:

- $K_{ow} > 3$ or MW < 1000

  In the past, a number of reports have shown a linear correlation between the n-octanol/water partition coefficient (K_{ow}) and the bioconcentration (BCF) in fish in the log K_{ow} range between 2 and 6.

  If log $K_{ow} < 3$ ($K_{ow} < 1000$), the compound is not expected to bioaccumulate significantly and therefore secondary poisoning of fish-eaters is not considered as a critical pathway. This is also the case when the compound has a molecular weight greater than 700 (Kristensen and Tyle, 1990) or 1000 (Auer et al., 1990).

- Measured BCF values available?

  Although in general the BCF for neutral chemicals can be adequately predicted from log K_{ow}, preference is given to the use of experimentally derived BCFs. The reason for this is that other factors than lipophilicity could influence bioconcentration (e.g. metabolization of the compound by the fish). These factors are included in experimental results. The measured BCF values used here must be based on "whole body" weight of the fish. BCF values based on the lipid fraction of the fish can be converted to "whole body" weight BCFs if the lipid content of the fish is known.

  Sometimes more than one measured bioconcentration factor is available. As the effects assessment is carried out to determine whether secondary poisoning is a critical pathway, it is suggested to use the highest factor to account for a worst case. In other cases a geometric mean value might be preferred.

  If measured BCF values are not available, the BCF for fish can be predicted from the relationship published by Mackay (1982, see also Section 3.2.1) between K_{ow} and BCF for fish (on wet weight basis): $BCF = K_{ow} * 0.05$. In this relation the fish contains 5 per cent fat. The relation applies to lipophilic substances with log K_{ow} between 2 and 6. Other relations have been published in the past (Davies and Dobbs, 1984; Hawker and Connel, 1986; Van Gestel at al., 1985), but the relation given above is simple and is satisfactory for most (non-fat) fish species.[16] For a "worst case" analysis, a fish containing 20 per cent fat could be taken: $BCF = K_{ow} * 0.20$. Various methods are available to calculate K_{ow}. Often a large variation is found in the K_{ow} values of a chemical by using different methods. Therefore the K_{ow} value must have been evaluated by an expert (see Section 3).

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[16] One of the recent non-linear regression relationships (Bintein et al., 1993) is as follows:

$\log BCF = 0.910 \log P - 1.975 \log (6.8 \times 10^{-7} p + 1) - 0.786$

where n = 154, r = 0.950, s = 0.347 and f = 463.51

- Toxicity data for birds and mammals available

  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. The results of these tests may be expressed as a concentration in the food (mg/kg) or a dose (mg/kg body weight/day) causing no effect. For the assessment of secondary poisoning, the results are converted to the concentration in food (mg/kg food). Conversion factors are given in Annex I.

  Effects on birds and mammal populations are seldom caused by mortality after short-term exposure. Therefore results from long-term studies are preferred, such as chronic NOECs for mortality, reproduction or growth (Romijn et al., 1993).

  If toxicity data for mammals or birds are not available, the examination for secondary poisoning cannot be made.

- Assessment factor

  The toxicity data are extrapolated to a Tolerable Residue Level (TRL) in food for fish-eaters with assessment factors as described in Section 6, Table 6.2. This TRL is expressed as a concentration in food (fish).

- MTC

  The Tolerable Residue Level in fish (mg/kg fish) is divided by the BCF for fish to convert the figure to a tolerable level in surface water or, in other words, to an MTC:

$$\text{MTC fish-eater} = \frac{\text{Tolerable Residue Level in fish for fish-eaters}}{\text{BCF}}$$

Comparison of the MTC (fish-eater) to maximum tolerable concentrations for aquatic organisms shows whether secondary poisoning could be a critical pathway. This is the case when MTC values for fish-eating birds and mammals are lower than MTC values derived for aquatic organisms.

The water – fish – fish-eating bird or mammal food chain is one example of a secondary poisoning pathway. Tolerable levels for fish-eating animals do not exclude that other birds or mammals feeding on other aquatic organisms (e.g. mussels, worms) are at risk. Therefore the MTC values for fish-eating birds and mammals must be considered indicative of secondary poisoning as a critical pathway.

9. Maximum Tolerable Concentrations in Sediment

9.1 Introduction

Sediments may act as a sink for, and source of, chemicals through sorption of chemical contaminants to particulate matter. 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 in many habitats the sediment plays an important role in the recycling of detrital material. Therefore sediment quality objectives should be derived that may serve as a basis for developing standards to protect benthic organisms and the aquatic system from the effects of sediment contamination (OECD, 1992c).

At the 1991 OECD Workshop on effects assessment of chemicals in sediments, three methods were recommended for the development of sediment quality objectives: equilibrium partitioning, interstitial water quality, and spiked sediment testing (OECD, 1992c).

A relatively simple approach to derive sediment quality criteria is the so-called Equilibrium Partitioning (EP) method (Shea, 1988; Di Torro, 1991). The method assumes a sorption equilibrium between the concentrations in water and in the sediment. The method is applicable to chemicals for which a partitioning model, describing the sediment partitioning as a function of the properties of the chemical and the characteristics of the sediment, is available. In general, this is the case for neutral organic chemicals which are liquid at room temperature. As the melting point increases, the EP method will become less and less predictive. The EP approach is not suitable for highly polar and charged organic chemicals for which adsorption cannot be modelled in a simple manner, or for chemicals which are rapidly degradable. The approach for heavy metals is comparable, but factors like speciation make it more difficult to apply (Van der Kooij et al., 1991).

The interstitial water quality method is similar to the equilibrium partitioning method, but measured interstitial water concentrations are used instead of predicted concentrations. The method is used for chemicals for which no adequate partitioning model is available. In the spiked sediment/toxicity testing, dose-response relations are established for individual chemicals and mixtures. The method is applicable only to chemicals for which analytical techniques are available to determine interstitial water concentrations. Both methods rely on the availability of measured data, which is usually a limitation. Moreover, sediment toxicity testing is still developing and toxicity data on sediment-dwelling organisms are scarce. The Equilibrium Partitioning approach is therefore explained here in more detail.

With the Equilibrium Partition method, maximum tolerable concentrations for sediment can be derived from maximum tolerable concentrations in water that were established for aquatic organisms or for the secondary poisoning pathway. The method is based upon the observation that the toxicity of the chemicals in sediments to benthic organisms is related more closely to the concentrations in interstitial water than to the bulk sediment concentrations (Ziegenfuss et al., 1986).

The EP method relates – by assuming steady state – the concentration in sediment to a concentration in (interstitial) water. Consequently an MTC for (interstitial) water can be converted to an MTC for sediment assuming that:

- the concentrations in sediments, interstitial water and benthic organisms are at equilibrium;
- the concentration of a chemical substance in any of these phases can be predicted using appropriate partition coefficients and concentrations in one phase;
- concentrations in sediments causing effects, expressed on the basis of the organic carbon content, can be predicted using partitioning predictions and effect concentrations in water;
- the water quality objective is an appropriate effects concentration for deriving sediment quality standards, i.e. pelagic test organisms and sediment-dwelling organisms are equally sensitive.

9.2 Proposed Approach

By definition, concentrations in water and solids are related through a partition coefficient:

$$K_{sw} = \frac{C_s}{C_w} \quad \text{or} \quad C_s = K_{sw} * C_w \qquad (1)$$

where:

K_{sw} : solids-water partition coefficient (l/kg)
C_s : concentration in the solid phase (mg/kg)
C_w : concentration in the water phase (mg/l)

If K_{sw} is known for a chemical, the maximum tolerable concentration in sediment (MTC_{sed}) can be calculated from the MTC for surface water using:

$$MTC_{sed} = MTC * K_{sw} \qquad (2)$$

For non-ionic organic chemicals, values for K_{sw} can be derived from the K_{ow} of the compound and the organic carbon content of sediment, using the referencing procedure formulated by Karickhoff et al. (1979):

$$K_{sw} = K_{oc} * f_{oc} \qquad (3)$$

with:

K_{oc} : organic carbon-referenced partition coefficient (l/kg_{oc})
f_{oc} : fraction of organic carbon (dimensionless)

and K_{oc} is approximately equal to K_{ow} (OECD, 1992c).

The equation relating K_{oc} and K_{ow}, as developed by Karickhoff et al. (1979), is based on a small set of chemicals such as polycyclic aromatic hydrocarbons, some insecticides and a few other compounds. Application of this relationship is therefore restricted to relatively unreactive, non-polar, organic chemicals. Most of the compounds that act by narcosis follow these restrictions.

Combining (1) and (3) gives the concentration in sediment (C_s):

$$C_{sed} = C_w * K_{ow} * f_{oc} \qquad (4)$$

A tolerable concentration in sediment is derived from the Maximum Tolerable Concentration in the aqueous phase according to the EP method:

$$MTC_{sed} = MTC_{aq} * K_{ow} * f_{oc} \qquad (5)$$

Since the derived MTC_{sed} strongly depends on the octanol/water partition coefficient, it is extremely important that correct K_{ow} values are used.

In a standard sediment the organic carbon fraction may be set at 0.05. It is known, however, that in the real world large (geographical) variations of f_{oc} occur.

The EP approach is mainly applicable for non-ionic hydrophobic organic chemicals and some metals. For acidic ionizing organic chemicals such as phenols, a correction to the method has to be applied because dissociation of the compounds may occur and the ions are far less hydrophobic than their uncharged equivalents (Jafvert, 1990; Jafvert et al., 1990; Jafvert and Heath, 1991).

The fraction of the non-dissociated (f_{ni}) molecules is calculated from the dissociation constant pK_a and the pH. The following model accounting for the degree of ionization is used:

$$K_{sw} = f_{oc} * K_{ow} * f_{ni} \qquad (6)$$

where:

$$f_{ni} = 1/(1 + 10^{pH-pKa}) \qquad (7)$$

The pH may be fixed at 8 for a standard sediment or adapted to local conditions.

Analogous to the method for neutral organic chemicals, the MTC_{sed} can be calculated using:

$$MTC_{sed} = MTC * K_{ow} * f_{ni} * 0.05 \qquad (8)$$

The approach for metals is essentially the same, but in this case values of K_{sw} are to be derived from the results of routine surveys of the water quality. It should be noted that K_{sw} values for metals show a great variability and depend on many physical-chemical factors, e.g. salinity, pH, dissolved oxygen concentration and sulphide concentrations (Acid Volatile Sulphide, AVS) in the sediment (Di Torro et al., 1990, Van der Kooij et al., 1991).

10. Examples of Effects Assessment

The methods described in these guidelines have been applied as an illustration of the approach. As the data are based on a limited data search, the examples are not to be considered as a formal effects assessment of the specific substances.

10.1 Example I – 4-chloro-2-nitroaniline

Mol. form.	$C_6H_5ClN_2O_2$
Mol. weight	172.6
log K_{ow}	2.23
K_{oc}	140
K_{sw}	7
pKa	–
Henry's const.	$1.3 * 10^{-7}$ atm. m^3/mol
BCF	–
BCF_{calc}	29

Table 10.1 Toxicity data for 4-chloro-2-nitroaniline (Balk et al., 1991)

Test species	Expo time	Parameter	Results (mg/l)
Chinook salmon	24 h	NOEC	1
	20 h	LC	5
Coho Salmon	24 h	NOEC	1
	4-6 h	LC	5
Lepomis macrochirus	0.5 h	EC sickness	5
	3 h	LC	5
Northern squawfish	24 h	NOEC	1
	1 h	EC	5
	22 h	LC	5
Poecilia reticulata	3 h	LC	5
Salmo gairdneri	0.5 h	EC sickness	5
	24 h	LC	5
Birds	no data		
Mammals			
rat		LD_{50} (oral)	6430*
mouse		LD_{50} (oral)	1250*

* mg/kg body weight

Aquatic effects assessment

For 4-chloro-2-nitroaniline, no chronic data are available. Therefore extrapolation methods (Section 5) cannot be applied. Because several acute toxicity data are available for fish (see **Table 10.1**), assessment factors as described in Section 6 may be applied to derive an MTC for priority setting. It has to be noted, however, that these acute data are not expressed as $L(E)C_{50}$, but as "no effect" concentration, effect concentration and lethal effect concentration (no percentage). The assessment factor (Table 6.1) is 1000, which is applied to the lowest concentration with reported mortality: 5 mg/l, leading to an MTC of 5 µg/l.

Possibly additional toxicity data can be derived from QSARs. In that case, the chemical structure of the substance is used to classify the chemical as explained in Section 7. From this section it is derived that 4-chloro-2-nitroaniline is a Class II substance and identified as likely to be a polar narcotic. As the log K_{ow} of 2.23 is in the range of 0.9-6.36, QSAR (i) (in Table 7.2) can be applied with a high level of confidence to a 96 h LC_{50} for *Pimephales promelas* of 31 mg/l, which is higher than the experimental data shown in Table 10.1.

Another estimation of toxicity data and MTC based on the US EPA/OPPT SAR method (see Section 7.2) is shown in **Table 10.2.**

Table 10.2 Estimated data by QSAR for 4-chloro-2-nitroaniline (Clements et al., 1994)

Hazard Profile		
log K_{ow} = 2.6 (CLOGP)		
Predicted toxicity values are:		
fish 96-h LC_{50} =	19.0	mg/l
fish 14-d LC_{50} =	4.9	mg/l
daphnid 48-h LC_{50} =	0.810	mg/l
green algal 96-h EC_{50} =	7.9	mg/l
fish Chronic Value (ChV) =	0.110	mg/l
daphnid ChV =	0.020	mg/l
algal ChV =	2.0	mg/l
based on SARs for anilines, pH = 7, hardness < 180.0 mg/l as $CaCO_3$, and TOC < 2.0 mg/l;		
high concern;		
assessment factor =	10.0	
concern concentration (= MTC) =	0.002	mg/l (ppm) or
=	2.0	µg/l (ppb)

By using the MTC of 5 µg/l, which is derived from the experimental data, the MTC_{sed} of 35 µg/kg is calculated according to the formulas described in Section 9.

Secondary poisoning

Secondary poisoning is not likely to occur, as the K_{ow} is lower than 3. Therefore no $MTC_{fish\text{-}eater}$ is estimated.

10.2 Example II – 1,4-dichlorobenzene

Mol. form.	$C_6H_5Cl_2$
Mol. weight	147.01
log K_{ow}	3.4
Henry's constant	0.160 kPa.m^3/mol
log K_{oc}	2.74

Table 10.3 Chronic toxicity data for 1,4-dichlorobenzene (Balk et al., 1991)

Species	Expo time	Parameter	Results (mg/l) or (mg/kg)	Standard soil (mg/kg)
Aquatic test organisms				
Algae				
Selenastrum Capricornutum	4 d	NOEC growth	0.57	
Crustaceans				
Daphnia magna	21 d	NOEC repr., mort.	0.5	
Fish				
Branchydanio rerio	28 d	ELS NOEC growth survival hatching	0.65	
Brachydanio rerio	28 d	NOEC hatch mort. growth morph.	≥ 5.6 3.2 1.0 3.2	
Pimephales promelas	32 d	ELS NOEC	0.57	
Pimephales promelas	32 d	ELS LOEC	1.0	
Terrestrial test organisms				
Plants				
Lactuca sativa	14 d	NOEC growth	10	50
Mammals				
rat	90 d	NEL(oral) teratogen.	19**	

** mg/kg body weight/day

Aquatic effects assessment

For 1,4-dichlorobenzene, chronic NOECs are available for four species (**Table 10.3**). These data are used as input in the method of Aldenberg and Slob, 1991 (A&S) and Wagner and Løkke, 1991 (W&L) as described in Section 6. Although for the correct use of these extrapolation methods, data for at least five species should be available, these methods are applied here as an example. The Kolmogorov-Smirnov test revealed that the data could be derived from a logistic distribution. The methods lead to MTC_{95} of 180 and 190µg/l and MTC_{50} values of 390 and 400 µg/l, for A&S and W&L, respectively.

For sediment, an MTC_{sed} is estimated with the formulas presented in Section 9. The MTC_{95} is approximately 5 mg/kg and the MTC_{50} is 11 mg/kg in standard sediment.

Additional toxicity data may be derived from QSARs. Therefore the substance has to be classified on the basis of its chemical structure as proposed in Section 7. From this section it is concluded that 1,4-dichlorobenzene is a Class I substance with narcosis or baseline toxicity. Therefore the alternative mentioned in Figure 7.3 may be applied: the HC_5 (Hazardous Concentration, 95 per cent protection level) is estimated directly from log K_{ow} (see Annex V). For water, the MTC_{95} is 70 µg/l and the MTC_{50} is 290 µg/l. For sediment, the MTC_{95} is 5 mg/kg and the MTC_{50} is 22 mg/kg.

Table 10.4 Bioconcentration factors for 1,4-dichlorobenzene (Balk et al., 1991)

Substance Species	Uptake period	Depuration period	k_1 ($days^{-1}$)	k_2 ($days^{-1}$)	BCF (wet weight)	BCF (lipid weight)
Fish						
Jordanella floridae ± 498	28d	14d	291 ± 26	0.98 ± 0.04	296 ± 29	3596
Leuciscus idus melanotus		3d			50	
Pimephales promelas					110	
Salmo gairdneri (alevin)	7d	24h			40	
Salmo gairdneri (alevin)	7d	24h			85	
Salmo gairdneri (egg-alevin)	60d	24h			100-1400 [3]	
Salmo gairdneri (alevin)	7d	24h			112	
Salmo gairdneri (250 g [4])	7-119d				720 ± 130 [1]	8640 [2]

1) average lipid content: 8.8 per cent
2) converted BCF: "wet-weight" BCF*12
3) BCK=1400 at hatching, BCF=100 at the end of the test
4) initial weight.

Secondary poisoning

There are indications that secondary poisoning may occur, as 1,4-dichlorobenzene has a log K_{ow} of 3.4. and a MW of 147.01. Therefore, if toxicity data are available for mammals or birds and also BCF values for aquatic organisms, then an MTC fish-eater can be calculated from the equations described in Section 8.

For 1,4-dichlorobenzene one oral NOEC is available for the rat (19 mg/kg bodyweight/day), corresponding to 380 mg/kg food (see Annex I, Table I.1). Applying an assessment factor of 10, as described in Section 6, a NOEL fish-eater of 38 mg/kg food can be calculated.

Several BCFs are available for fish. For the evaluation of the experiments, the considerations in Annex I are used. If BCF is derived from the ratio C_f/C_w, the duration of the experiment should be sufficiently long to allow a steady state to be approached. Moreover the test concentrations should be below the toxic level. The depuration rate constant k_2 can be calculated from an empirical relationship between log K_{ow} and k_2 (see Annex I): $k_2 = 0.05$ (day^{-1}). The test duration should be 58 days. In **Table 10.4** an experimental k_2 is given: $k_2 = 0.98$, and it follows that the minimum test duration is 3.1 days. (The higher depuration rate may be explained by metabolism of the test substance.) Therefore the 3-d test with *L. idus* is not included. Although in the test with *P. promelas* the test concentration was as high as the NOEC, the BCF is not outside the range of BCFs in the other tests. Four tests with *S. gairdneri* alevins by the same author gave BCF values between 40 and 112, geometric species mean 79. Other BCF values are 296, 110 and 720, giving a geometric mean BCF of 207. The highest BCF was 1400. From this, an MTC fish-eater is estimated varying from 27 to 184 µg/l.

10.3 Example III – chromium

Mol. weight	52
K_{sw}	290 (l/g)[17]
BCF fish and worms	125-200 wet weight

Aquatic effects assessment

For chromium, a large number of NOECs are available for several taxonomic groups, mostly tested with K_2CrO_7 or Na_2CrO_7 (see **Table 10.5**). It should be kept in mind that the toxicity of chromium depends strongly on the pH of the test medium. The NOEC values follow a distribution as presented in **Figure 10.1**. The Kolmogorov-Smirnov test revealed that the data could be derived from a logistic distribution. These data were used as input into the methods of Stephan et al., 1985 (Steph), A&S and W&L leading to an MTC of 0.59 µg/l for the Stephan method, MTC_{95} values of 2.9 and 3.3 µg/l, and MTC_{50} values of 8.5 and 8.0 µg/l, for the A&S and W&L method, respectively.

Using the range of calculated MTC values, the MTC_{sed} varies from 115 to 1640 mg/kg.

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[17] K_{sw} is determined as the ratio of the concentration in suspended solids/concentration in water. The concentration in suspended solids is a factor 1.5 above the concentration in the sediments. Therefore a correction factor of 1.5 is applied to K_{sw} (Van der Kooij et al., 1991).

Secondary poisoning

Only a restricted number of accumulation data are available for aquatic organisms. In accumulation studies with invertebrates (mussels and worms) BCFs vary between 125 and 200, for exposure to Cr(III) as well as to Cr(VI). Concentrations were found to be higher in invertebrates than in fish, indicating that higher species can perhaps regulate chromium.

Results from oral chronic tests with mammals are rather variable (Slooff et al., 1989): rats were exposed in a chronic test (90 days or life long), to maximal 5 per cent Cr(III) oxide-pigment (Cr_2O_3, insoluble) in food, resulting in a No Effect Level of 1210 mg/kg bodyweight. This may be converted to 24.2 g/kg food (see Annex I, Table I.1). The absorbed fraction was estimated at 0.5 per cent, resulting in an absorbed dose of 121 mg/kg food. In another test rats received chromium salts in their drinking water during one year and no effects were observed. The doses are equivalent to 2.5 mg Cr(III) or Cr(VI)/kg bodyweight/day [N.B. No effect dose ≥ 2.5 mg/kg (day)]. This is converted to a level of ≥ 50 mg/kg food.

In this example, the No Effect Level for the rat is taken to be 100 mg/kg food. With an assessment factor of 10, the $MTC_{fish\text{-}eater}$ is 10 mg/kg food. With BCF = 125 to 200, this level of accumulation in the food may be reached (by invertebrates) when the concentration in water is 0.05 mg Cr/l. This concentration is the tolerable level in water for the fish/invertebrate eater.

Figure 10.1 Frequency distribution of NOEC values for chromium (De Bruijn, 1991, pers. comm.)

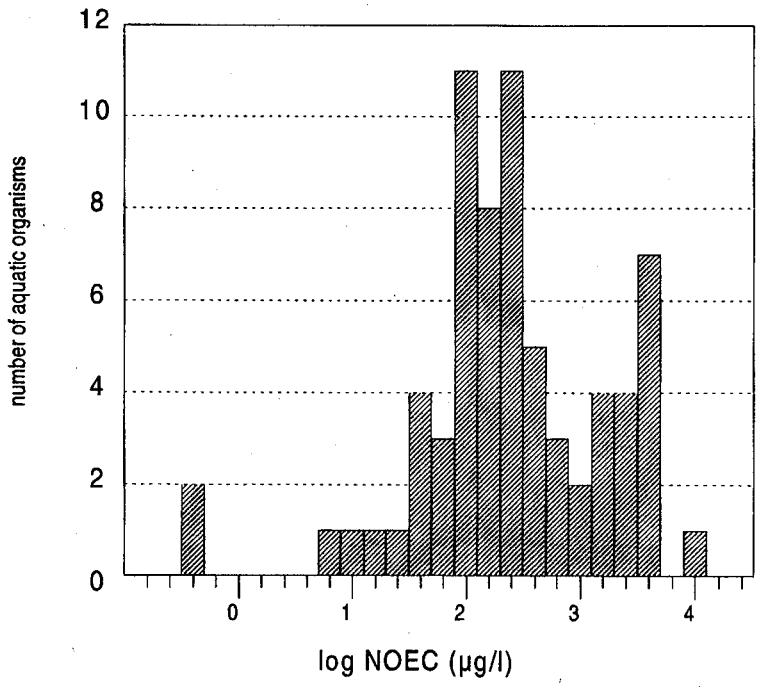

Table 10.5 Chronic toxicity data on chromium (Slooff et al., 1989)

Organism	Chemical structure	Time of exposure	Criterion	Result (µg/l)	Reference	Bay. stat. (µg/l)	Aldenberg & Slob (µg/l)	Bay. stat. with tax. groups (µg/l)
Protozoa								
Euglena gracilis	$K_2Cr_2O_7$	4 d	NOEC[g]	35	Adema et al., 1981	35	35	35
Vorticella microstoma	$K_2Cr_2O_7$	2 d	NOEC[g]	100	Sudo & Aiba, 1973	100	100	
Colpidium campylum	$K_2Cr_2O_7$	2 d	NOEC[g]	3200	Sudo & Aiba, 1973	3200	3200	
Opercularia sp.	$K_2Cr_2O_7$	2 d	NOEC[g]	6400	Sudo & Aiba, 1983	6400	6400	
Coelenterata								
Hydra oligactis	$K_2Cr_2O_7$	3 w	NOEC[g,l]	1120	Slooff & Canton, 1983	1120	1120	1120
Molluscs								
Lymnaea stagnalis	$K_2Cr_2O_7$	6 w	NOEC[r,l]	112	Slooff & Canton, 1983	112	112	112
Lymnaea stagnalis	$K_2Cr_2O_7$	1 w	NOEC[r]	350	Slooff & Canton, 1983	350		
Crustaceans								
Daphnia magna	$K_2Cr_2O_7$	3 w	NOEC[r,l]	35	Adema et al., 1981	35*	39	9
Daphnia magna	$K_2Cr_2O_7$	3 w	NOEC[r,l]	350	Van Leeuwen et al., 1987	350*		
Daphnia magna	Na_2CrO_4	4 w	EC[r,l]	10	Trabalka & Gehrs, 1977	5[1]*		
Daphnia magna	$CrCl_3$	3 w	NOEC[r,g,c,l]	<330	Biesinger & Christensen, 1972	165		
Atlanto-astacus pallipes p.	$--Cr_2O_7$	20 w	NOEC[r,g,l]	9	Chaisemartin, 1978	9	9	
Insects								
Culex pipiens	$K_2Cr_2O_7$	4 w	NOEC[d,l]	1120	Slooff et al., 1983	1120	1120	1120

Table 10.5, cont'd Chronic toxicity data of chromium

Organism	Chemical structure	Time of exposure	Criterion	Result (µg/l)	Reference	Bay. stat. (µg/l)	Aldenberg & Slob (µg/l)	Bay. stat. with tax. groups (µg/l)
Fish								
Brachydanio rerio	$K_2Cr_2O_7$	>4 w	NOEC[r,g,l]	3500	Canton et al., 1984	3500	3500	
Catostomus commersoni	$Na_2Cr_2O_7$	10 w	NOEC[r,g,l]	290	Sauter et al., 1976	290	290	
Channa punctatus	$K_2Cr_2O_7$	17 w	NOLC	2600	Sastry & Sunita, 1983	2600	2600	
Ictalurus punctatus	$Na_2Cr_2O_7$	10 w	NOEC[r,g,l]	150	Sauter et al., 1976	150	150	
Jordanella floridae	$K_2Cr_2O_7$	4 w	NOEC[g,l]	1120	Adema et al., 1981	1120	1120	
Jordanella floridae	$K_2Cr_2O_7$	6 w	NOEC[g,r,l]	1120	Adema et al., 1981	1120		
Lepomis macrochirus	$Na_2Cr_2O_7$	9 w	NOEC[r,g,l]	522	Sauter et al., 1976	522	522	
Oryzias latipes	$K_2Cr_2O_7$	4 w	NOEC[g,l]	3500	Adema et al., 1981	3500	3500	
Oryzias latipes	$K_2Cr_2O_7$	6 w	NOEC[r,g,l]	3500	Adema et al., 1981	3500		
Pimephales promelas	$K_2Cr_2O_7$	16 m	NOEC[r,g,l]	1000	Pickering, 1980	1000		
Pimephales promelas	$Na_2Cr_2O_7$	4 w	NOLC	3000	Broderius & Smith, 1979	3000	400	
Pimephales promelas	$Na_2Cr_2O_7$	4 w	NOEC[g]	400	Broderius & Smith, 1979	400		
Poecilia reticulata	$K_2Cr_2O_7$	4 w	NOEC[g,l]	3500	Adema et al., 1981	3500	3500	
Rutilus rutilus	$K_2Cr_2O_7$	4 w	NOEC[c,b,l]	1000	Strik et al., 1975	1000	1000	
Salmo gairdnenii	$Na_2Cr_2O_7$	13 w	NOEC[r,g,l]	51	Sauter et al., 1976	51		
Salmo gairdnerii	Na_2CrO_4	32 w	NOEC[r,g,l]	200	Van der Putte, 1981	200*		
Salmo gairdnerii	Na_2CrO_4	32 w	NOEC[r,g,l]	20	Van der Putte, 1981	20*		
Salmo gairdnerii	$Cr(NO_3)_3$		NOEC[r,g,l]	48	Stevens & Chapman, 1984	48		
Salmo gairdnerii	$Cr(NO_3)_3$		NOEC[r,g,l]	157	Stevens & Chapman, 1984	157		

Table 10.5, cont'd Chronic toxicity data of chromium

Organism	Chemical structure	Time of exposure	Criterion	Result (µg/l)	Reference	Bay. stat. (µg/l)	Aldenberg & Slob (µg/l)	Bay. stat. with tax. groups (µg/l)
Fish (continued)								
Salmo gairdnerii	Na_2CrO_4	32 w	NOEC[g,l]	200	Van der Putte, 1981	200	63[2]	63
Salmo gairdnerii	Na_2CrO_4	32 w	NOEC[g,l]	200	Van der Putte, 1981	200		
Salmo gairdnerii	Na_2CrO_4	12 w	NOEC[c,g,h,l]	2000	Van der Putte, 1981	2000		
Salmo gairdnerii	Na_2CrO_4	12 w	NOEC[c,g,h,l]	200	Van der Putte, 1981	200		
Salmo gairdnerii	$Na_2Cr_2O_7$	8 m	NOEC[g]	100	Benoit, 1976	100		
Salmo gairdnerii	$K_2Cr_2O_7$	6 m	NOEC[c]	<200	Arillo et al., 1982	100		
Salvelinus fontinalis	$Na_2Cr_2O_7$	8 m	NOEC[g]	100	Benoit, 1976	100	100*	
Salvelinus fontinalis	$Na_2Cr_2O_7$	22 m	NOEC[g,l]	<350	Benoit, 1976	175		
Salvelinus namaycush	$Na_2Cr_2O_7$	16 w	NOEC[r,g,l]	105	Sauter et al., 1976	105	105*	
Amphibia								
Xenopus laevis	$K_2Cr_2O_7$	14 w	NOEC[d,g,l]	350	Slooff & Canton, 1983	350	350	350
Algae (green)								
Chlorella pyrenoidosa	$K_2Cr_2O_7$	4 d	NOEC[g]	63	Adema et al., 1981	63	63*	
Chlorella pyrenoidosa 211-8b	$K_2Cr_2O_7$	4 d	NOEC[g]	2000	Meisch & Schmitt-Beckman, 1979	2000*	447*	
Chlorella pyrenoidosa 211-8b	$K_2Cr_2O_7$	4 d	NOEC[g]	100	Meisch & Schmitt-Beckman, 1979	100*		
Chlorella pyrenoidosa 211-8b	$K_2Cr_2O_7$	4 d	NOEC[g]	100	Meisch & Schmitt-Beckman, 1979	100*		
Chlorella pyrenoidosa 211-8b	Cr-glycine	4 d	NOEC[g]	2000	Meisch & Schmitt-Beckman, 1979	2000*		

Table 10.5, cont'd Chronic toxicity data of chromium

Organism	Chemical structure	Time of exposure	Criterion	Result (µg/l)	Reference	Bay. stat. (µg/l)	Aldenberg & Slob (µg/l)	Bay. stat. with tax. groups (µg/l)
Algae green (continued)								
Chlorella WR 1	$K_2Cr_2O_7$	4 d	NOEC[g]	100	Meisch & Schmitt-Beckman, 1979	100*	266*	112
Chlorella WR 1	$K_2Cr_2O_7$	4 d	NOEC[g]	100	Meisch & Schmitt-Beckman, 1979	100*		
Chlorella WR 1	Cr-glycine	4 d	NOEC[g]	<500	Meisch & Schmitt-Beckman, 1979	250*		
Chlorella WR 1	Cr-glycine	4 d	NOEC[g]	≥2000	Meisch & Schmitt-Beckman, 1979	2000*		
Scenedesmus pannonicus	$K_2Cr_2O_7$	4 d	NOEC[g]	112	Adema et al., 1981	112*	112	
Scenedesmus pannonicus	$K_2Cr_2O_7$	14 d	NOEC[g]	112	Adema et al., 1981	112*		
Selenastrum capricornutum	$K_2Cr_2O_7$	4 d	NOEC[g]	196	Adema et al., 1981	196	196	
Algae (blue)								
Gomphonema parvulum	$K_2Cr_2O_7$	4/7 d	NOEC[g]	35	Hanstveit et al.,1985	35	35	35
Microcystis aeruginosa	$K_2Cr_2O_7$	4 d	NOEC[g]	196	Adema et al., 1981	196*	197	
Microcystis aeruginosa	$K_2Cr_2O_7$	8 d	NOEC[g]	196	Adema et al., 1981	196*		
Microcystis aeruginosa	$K_2Cr_2O_7$	4 d	NOEC[g]	112	Hanstveit et al., 1985	112*		
Microcystis aeruginosa	$K_2Cr_2O_7$	4 d	NOEC[g]	350	Sloof & Canton, 1983	350*		
Oscilltoria agardhii	$K_2Cr_2O_7$	4/7 d	NOEC[g]	35	Hanstveit et al., 1985	35	35	
Diatoms								
Stephanodiscus hantzschii	$K_2Cr_2O_7$	4 d	NOEC[g]	0.35	Adema et al., 1981	0.35*	0.35	0.35
Stephanodiscus hantzschii	$K_2Cr_2O_7$	7 d	NOEC[g]	0.35	Canton & Mathijsen-Spiekman, 1984	0.35*		

Table 10.5, cont'd Chronic toxicity data of chromium

Organism	Chemical structure	Time of exposure	Criterion	Result (µg/l)	Reference	Bay. stat. (µg/l)	Aldenberg & Slob (µg/l)	Bay. stat. with tax. groups (µg/l)
Macrophytes								
Lemna gibba	Na_2CrO_4	1 w	NOEC[9]	100	Staves & Knaus, 1985	100	100*	100
Lemna minor "M19"	$K_2Cr_2O_7$	1 w	NOEC[9]	11	Adema & De Zwart, 1984	11*	35*	
Lemna minor "M19"	$K_2Cr_2O_7$	1 w	NOEC[9]	112	Slooff & Canton, 1983	112*		
Lemna paucicostata "6746"	$K_2Cr_2O_7$	1 w	NOEC[9]	500	Nasu & Kugimoto, 1981	500*	354*	
Lemna paucicostata "6746"	$K_2Cr_2O_7$	1 w	NOEC[9]	<500	Nasu & Kugimoto, 1981	250*		
Spirodela punctata	Na_2CrO_4	1 w	NOEC[9]	100	Staves & Knaus, 1985	100	100	
Spirodela polyrhiza	Na_2CrO_4	1 w	NOEC[9]	100	Staves & Knaus, 1985	100	100	

[1] A factor of 2 is used to convert this value into a NOEC;
[2] This value is incorrect. The geometric mean of 51, 200, 20, 48 and 157 should be calculated (= 69), because the criterion is the same: NOEC[r,g,l].

10.4 Example IV - lindane

Mol. weight	291
K_{ow}	3.85
BCF	100-1000

Aquatic effects assessment

For lindane, several chronic NOECs are available for several taxonomic groups (**Table 10.6**). The Kolmogorov-Smirnov test revealed that the data could be derived from a logistic distribution. These data are used in the methods of Stephan et al. (1985), Aldenberg and Slob (1993) and Wagner and Løkke, leading to an MTC of 1.5 µg/l for the Stephan method; MTC_{95} values of 0.041 and 0.062 µg/l and MTC_{50} values of 0.75 and 0.60 µg/l, for the Aldenberg and Slob and Wagner and Løkke method, respectively.

As lindane is listed under Class IV chemicals acting by a specific mechanism, the alternative approach presented in Annex V, using the direct relation between K_{ow} and MTC, cannot be applied. (If calculation is performed, the MTCs differ by a factor of 260, showing that lindane is in fact much more toxic than expected for a narcotic chemical.)

Using the range of calculated MTC values, the MTC_{sed} varies from 15 to 530 µg/kg.

Secondary poisoning

There are indications that secondary poisoning may occur, as lindane has a log K_{ow} of 3.85 and a MW of 291. The toxicological No Effect Level for a short-term test with rats is 2 mg/kg food. The BCFs for water organisms vary between 100 and 1000. From this the $MTC_{fish\text{-}eater}$ is estimated at between 0.2 and 2 µg/l.

Table 10.6 Chronic toxicity data for lindane (Slooff and Matthijsen, 1987)

Organism	Life stage	Time of exposure	Criterion	Result (µg/l)	Reference	Bay. stat (µg/l)	Aldenberg & Slob (µg/l)	Bay. stat with tax. groups (µg/l)
Molluscs								
Lymnaea stagnalis	4 m	10 m	36% fecundity decrease	1000	Bluzat & Seugé, 1979a	500[1]	500	500
Crustaceans								
Daphnia magna		64 d	NOEC[r]	11	Macek et al., 1976	11	11	4.3
Gammarus fasciatus		120 d	NOEC	4.3	Macek et al., 1976	4.3	4.3	
Insects								
Chironomus tentans	eggs		NOEC[d,l]	2.2	Macek et al., 1976	2.2	2.2	2.2
Fish								
Lepomis macrochirus		18 m	NOEC[r]	9.1	Macek et al., 1976	9.1	9.1	8.8
Pimephales promelas		43 w	NOEC[g,r]	9.1	Macek et al., 1976	9.1	9.1	
Salvelinus fontinales		261 d	NOEC[g,r]	8.8	Macek et al., 1976	8.8	8.8	
Amphibia								
Xenopus laevis		12 w	NOEC[r]	<500	Marchal-Segault & Ramade, 1981	250	250	250
Algae (green)								
Scenedesmus quadricauda		8 d	inhibition of cell multiplication	300	Bringmann & Kühn, 1978	150[1]	150	150
Algae (blue)								
Microcystis aeruginosa		8 d	inhibition of cell multiplication	1900	Bringmann & Kühn, 1978	950[1]	950	950

[1] The result is divided by a factor of 2, because the criterion is not NOEC.

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Annex I

Evaluation of the Data

1. Bioaccumulation and toxicity

Bioconcentration factors may be determined experimentally in two ways:

1. The bioconcentration factor is calculated as the concentration in whole fish (C_f) (or parts thereof) at "near steady-state" divided by the mean concentration of the substance in water during the exposure period (C_w), and

2. The bioconcentration is calculated as the ratio between the rate constants of uptake (k_1) and depuration (k_2), assuming first order kinetics.

In many cases, bioconcentration factors reported on lipophilic substances may have been underestimated when based on C_f/C_w ratios from experiments with high contents of particulate or dissolved organic matter (C_w may be overestimated, the bioavailability of the substances in water is reduced). BCF may also be underestimated if the mean concentration of the chemicals during the exposure period is greater than the water solubility limit of the chemical. Bioconcentration factors based on k_1/k_2 ratios are expected to be less affected by the degree of bioavailability, except for experiments where the organic matter content has been fluctuating greatly during the exposure period (Kristensen and Tyle, 1990). When bioconcentration factors are derived from C_f/C_w ratios, the duration of the experiment should have been sufficiently long to allow a steady state to be reached or approached. The time needed to approach a steady state (e.g. 95 per cent of the steady state) can be estimated from the following equations:

(Theoretical:) $t_{95} = 3.0/k_2$

and

(Empirical:) $\log k_2 = -0.414 * \log K_{ow} + 0.122$

Note that a 96-hour exposure period for LC_{50} tests may not be sufficiently long for substances with $\log K_{ow} > 5$. For relatively unreactive non-polar chemicals that act by narcosis, it is assumed that the internal concentration is constant at a particular effect (Van Hoogen and Opperhuizen, 1988). The relation between the internal lethal concentration and the exposure (external) concentration in the water is reflected in the bioconcentration process. For acute LC_{50} values this has the following implications: if a chemical needs a long time to reach a "steady state" in fish, a "steady state" concentration will not be reached in an acute test of 96 hours. This implies that the exposure concentration needs to be very high in order to reach a lethal internal concentration. Therefore, the LC_{50} value will also be high. When the exposure period is sufficiently long to allow a steady state to be reached, lethal internal concentrations will be reached at lower exposure concentrations and, as a consequence, the LC_{50} will be lower.

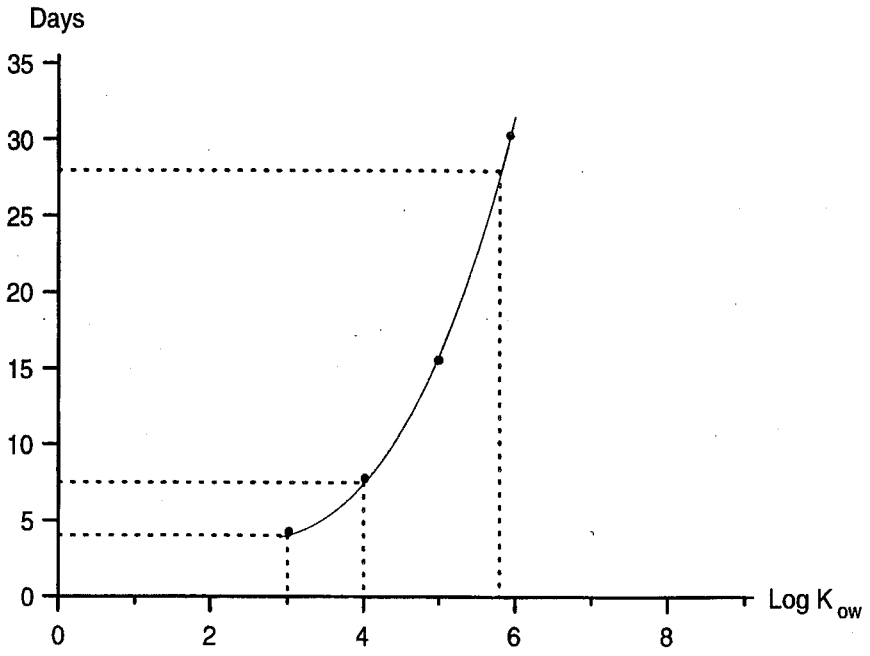
For neutral organic chemicals which are liquids at room temperature, 96-h LC_{50} values can be obtained up to $\log K_{ow} = 5.0$. Anilines and phenols are acutely toxic up to log

K_{ow} = 7.0 and aliphatic amines are acutely toxic up to log K_{ow} = 22.0. For neutral organics which are solids at room temperature, the higher the melting point the greater the probability that an LC_{50} cannot be obtained within 96 hours. In cases of solids, water solubility must be measured or predicted and the aqueous solubility limit must be compared to the predicted LC_{50} value. When the aqueous solubility limit is higher than the predicted 96-h LC_{50}, then the acute toxicity test can be successfully done. However, when the aqueous solubility limit is lower than the predicted 96-h LC_{50}, then the acute toxicity test cannot be successfully done at or below the aqueous solubility limit and chronic toxicity tests need to be invoked.

For substances with log K_{ow} > 6, the water solubility is usually very low. Therefore it may be possible that a 96-h LC_{50} cannot be determined because the exposure concentration should exceed the water solubility in order to reach a lethal concentration.

Log K_{ow} may be used to estimate the appropriate duration of an acute toxicity test. The above mentioned equations are combined, and the time needed to reach 95 per cent of the steady state concentration of the substance in fish can be read from **Figure I.1**.

Figure I.1 Relation between log K_{ow} and the minimum test duration



2. Toxicity for fish-eating birds and mammals

The data presented in literature can be converted into a value expressed in mg/kg food. Data on body weight, daily food intake and daily water intake that can be used for the transformation are given in **Table I.1**. For transformation of toxicity data expressed on the basis of bodyweight or water intake to food intake, the toxicity data should be multiplied by the conversion factor (BW/DFI or DWI/DFI).

Table I.1 Conversion factors for toxicity data (Sax, 1989; Romijn et al., 1993)

	BW (g)	DFI (g)	DWI (l)	BW/DFI	DWI/DFI
Canis domesticus	10.000	250		40	
Macaca spec.	5.000	250		20	
Microtus spec.	25	3		8.3	
Mus m.	25	3		8.3	
Oryctolagus c.	2.000	60		33.3	
Rattus n.	200	10		20	
Rattus n. (age > 6 weeks	200	10		20	
Rattus n. (age < 6 weeks)	200	10		10	
Gallus domesticus		64.3	128.5		2

BW : bodyweight (g)
DFI : daily food intake, (mg/kg body weight/day)
DWI : daily water intake (mg/l)
BW/DFI : conversion factor to mg/kg food
DWI/DFI : conversion factor to mg/kg food

Annex II

Validation of Structure Activity Relationships Used by the US EPA's Office of Pollution Prevention and Toxics for the Environmental Hazard Assessment of Industrial Chemicals

Reference: *Nabholz, J.V.,[1] Clements, R.G.,[1] Zeeman, M.G.,[1] Osborn, K.C.,[2] and Wedge, R.,[2]* ***"Validation of Structure Activity Relationships Used by the US EPA's Office of Pollution Prevention and Toxics for the Environmental Hazard Assessment of Industrial Chemicals,"*** *Environmental Toxicology and Risk Assessment: 2nd Volume, ASTM STP 1216, Joseph W. Gorsuch, F. James Dwyer, Christopher G. Ingersoll, and Thomas W. La Point, Eds., American Society for Testing and Materials, Philadelphia, 1993, pp. 571-590.*

Abstract: *The Toxic Substances Control Act (TSCA) allows for the regulation of new industrial chemicals if a chemical may present an unreasonable risk towards the environment or if a chemical has significant exposure towards the environment. As part of this regulatory process, an environmental hazard assessment is used to identify the effects of a chemical towards organisms in the environment, and their populations, communities, and ecosystems. In the United States Environmental Protection Agency's (US EPA) Office of Pollution Prevention and Toxics (OPPT), the aquatic toxicity of chemicals has been predicted using structure activity relationships (SAR) because of a lack of measured toxicity data. A SAR is the relationship between a chemical's toxicity and its chemical structure. Quantitative and qualitative SARs have been developed for dozens of chemical classes and their validation has been an ongoing process in OPPT since 1981.*

The object of SAR validation is to test a SAR's accuracy and consists of comparing the predicted toxicity values of chemicals with the measured toxicity values. When predicted and measured values are similar, the SAR is assumed to be accurate. When the predicted and measured values are significantly different, either the SAR is reformulated or a new SAR is identified for another chemical class. The goal of SAR validation is to increase the accuracy of SARs. Newly measured toxicity data are either integrated into existing SARs or used to develop new SARs.

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A summary and discussion of this validation process for several chemical classes will be presented: neutral organic chemicals, organic chemicals which show excess toxicity relative to neutral organic chemicals with similar structure, anionic surfactants, cationic surfactants, polycationic polymers, cationic dyes, acid dyes, polyanionic monomers which are strong chelators of nutrient elements, and compounds which undergo hydrolysis (e.g. acid chlorides and alkyloxysilanes).

Keywords: *environmental hazard assessment, structure activity relationships, validation, aquatic toxicity, acute toxicity, chronic toxicity, toxicity assessment, Toxic Substances Control Act*

Introduction

The Toxic Substances Control Act (TSCA) allows for the regulation of new industrial chemicals under Sec. 5 if a chemical may present an unreasonable risk towards the environment or if a chemical has significant exposure to the environment. As part of this regulatory process, an environmental hazard (or toxicity) assessment is developed to identify as many of the effects of a chemical as possible towards organisms in the environment, including their populations, communities, and ecosystems. The standard toxicity profile for all chemicals assessed for potential risk to aquatic ecosystems consists of the following effective concentrations (EC):

fish acute toxicity (96 h LC_{50});
daphnid acute toxicity (48 h LC_{50});
green algal toxicity (96 h EC_{50});
fish chronic value (ChV);
daphnid ChV; and
algal ChV.

The chronic value (ChV) for fish and aquatic invertebrates is the same as a chronic no-effect concentration (NEC) and the geometric mean of the maximum allowable toxicant concentration (MATC). The MATC is the range of concentrations between the lowest-observed-effect concentration (LOEC) and the no-observed-effect concentration (NOEC). The ChV for green algae is the 96 h NEC. If the 96 h NEC is not available, then the 96 h NOEC is used, and if the NOEC is not available, then the 96 h EC_{10} is used. In some cases, the algal ChV is predicted by dividing the 96 h EC_{50} by 4.0.

A toxicity profile is predicted for every chemical assessed by OPPT using its chemical structure, its physical/chemical properties, and SAR methods. The goal of OPPT is to predict all six ECs for all chemicals; however, about half the time only the environmental base set of ECs can be predicted, i.e. the fish acute value, the daphnid acute value, and the algal toxicity value. Occasionally, only one EC is available for a chemical. For a more detailed discussion of OPPT's environmental SAR methods, see Nabholz et al. (1993).

OPPT has used structure activity relationships (SAR, Auer et al. 1990; Nabholz et al. 1993; Clements 1988, 1993a, 1993b) since 1979 to predict the toxicity of 95 per cent of new chemicals reported under Sec. 5 of TSCA. To date, over 20,621 chemicals described in

Sec. 5 premanufacturing notices (PMN) have been assessed by OPPT for environmental risk. Most of these assessments have been based on toxicity predictions derived from the chemical structure and physical/chemical properties reported in the PMN. Only about 4.8 per cent of these PMNs have contained test studies for environmental toxicity. Of course, if measured toxicity data are available for a chemical, then those data are used for risk assessment provided that those data are valid.

OPPT began to use structure activity relationships (SAR) in 1979 and quantitative structure activity relationships (QSAR) in 1981. Use of QSARs has become more and more frequent. A QSAR is simply a regression equation between a chemical's toxicity and one or more of its physical/chemical properties. QSARs have been developed from as little as one datum, i.e. one fish acute value, to dozens of data points for a chemical class.

The science of SAR consists not only of (1) QSARs, but also, (2) nearest analog analysis which uses the closest structural analog or the two analogs which bracket a chemical to predict toxicity because no physical/chemical property has been found to be correlated with the toxicity for the chemical class, and (3) generic evaluations of a particular chemical class, e.g. acid dyes. For a more detailed discussion of SAR as it is used by OPPT, see Auer et al. 1990; Nabholz 1991; Nabholz et al. 1993; Zeeman et al. this volume.

SAR validation within OPPT is done by measuring how accurately the toxicity of a chemical was predicted. This method of validating SARs is common and was used early in the development of environmental SARs (Könemann 1981). When OPPT requests that the chemical industry test a chemical for toxicity, OPPT determines how close the measured toxicity values are to the predicted values. One requirement is that the measured value be determined under similar test conditions as were used to test the chemicals included in the SAR. For example, OPPT predicts the toxicity of aliphatic amines from an SAR in which all the toxicity data were measured at about Ph 7 or completely ionized. Therefore, when an amine is tested, OPPT requests that the stock solution be neutralized prior to testing.

Validation of all environmental toxicity SARs has been an ongoing process in OPPT since 1981. As soon as new SARs or QSARs are developed, predictions become hypotheses to be tested. Testing results are integrated into existing SARs or used to create new SARs whenever new toxicity information becomes available.

Validation in this study was conceptualized as a validation ratio, i.e. the predicted toxicity value (P-EC) divided by the measured toxicity value (M-EC). If this ratio was 1.0, perfect accuracy would be demonstrated. Ratios of less than 1.0 would indicate that toxicity had been over-predicted, and ratios greater than 1.0 would indicate that toxicity had been under-predicted. Perfect accuracy is the goal of OPPT with regards to SAR analysis, but we all know that the real world is more variable than not. For example, differences in species sensitivity is generally 10 times or greater and the results of interlaboratory tests (or round robin or ring tests) frequently show differences of 10 times or more (Zeeman et al. this volume).

The pragmatic goal of OPPT's SAR program has been that predicted values be within a factor of 10 of the measured values, i.e. ratios of 0.1 to 10.0. If a validation ratio fell outside this range, then an adjustment of the SAR for the chemical class was required. For example, if a measured toxicity value indicated that a chemical was more than 10 times more toxic than predicted by our current SAR, then the chemical was characterized as exhibiting excess toxicity (Auer et al. 1990; Lipnick 1991) and a new SAR for that subclass was usually

developed. A more specific goal was to have mean validation ratios be slightly less than 1.0. If perfect accuracy could not be obtained, then OPPT should reasonably over-predict the toxicity of new chemicals because the U.S. Environmental Protection Agency is a regulatory agency whose goal is to protect the environment. Auer et al. (1990), Nabholz (1991) and Nabholz et al. (1993) have more detailed discussions of the role of SAR in OPPT's risk assessment process, and Suter (1993) discussed SAR in the broader context of ecological risk assessment.

We also realized that the mean validation ratios were more important than the range of ratios. The range of ratios from 1979 to present would represent a historical range. A historical range would contain all mistakes and would not reflect the adjustments which had been made in the SAR Program over the years. For example, toxicity predictions for a chemical assessed in Fiscal Year (FY) 1985 were made using the current SAR methods available for the chemical class in FY85. If the measured toxicity information for the chemical indicated that the toxicity was significantly over- or under-predicted, then the SAR was adjusted using the measured toxicity data for the chemical class so that future predictions for the chemical class would be more accurate. The original predictions were not changed, thus, the ranges observed in this validation study reflect all of the inaccuracies inherent in the SAR Program since 1979.

Methods

Test study summary sheets were analyzed for predicted and measured toxicity values and ratios were calculated. Ratios were then sorted by chemical class and toxicity endpoint. As an ongoing activity, OPPT summarizes the results of each environmental toxicity test submitted under TSCA. For each toxicity test, a test study summary sheet is prepared which summarizes the pertinent data from the toxicity test regarding the chemical tested, species tested, testing conditions, and effective concentrations, such as EC_{50}, LC_{50}, LC_{100}, EC_{100}, LOEC, NOEC, NEC, EC_{90}, and EC_{10}. The toxicity summary sheets are contained in a database called the OPPT PMN ECOTOX DATABASE and the physical/chemical information is contained in the OPPT PMN PHYCHEM DATABASE.

A contractor (ICF Inc., Clement International Corp, Fairfax, Virginia) was hired to analyze these summary sheets. The following guidelines were used during this evaluation: (1) only aquatic toxicity studies which contained data identical to the six ECs which comprise OPPT's standard toxicity profile were to be used; however, if a daphnid 24-h LC_{50} value, a fish 72-h LC_{50} value, and a green algal 72-h EC_{50} value were the only ECs available, then they were used, (2) studies were limited to those which had been validated by Nabholz in order to ensure consistency in data evaluation, (3) the only toxicity predictions used were those made with OPPT environmental SAR methods and by Nabholz, again for consistency, (4) toxicity values were not used if they were significantly higher than either the aqueous solubility limit of non-surfactant chemicals or the dispersibility limit of self-dispersing surfactants and polymers, (5) studies in which the chemical identity of the tested substance was not sufficiently known to allow for predictions were not used, (6) studies not used included those in which the new chemical was tested as a minor component, e.g. 5 per cent active ingredients (AI), and was tested with other components which were known to be toxic to aquatic organisms, and (7) studies done in dilution water which was known to contain material which would interfere significantly with the intrinsic toxicity of the chemical were also rejected.

For example, OPPT predicts the toxicity of polycationic polymers assuming dilution water will contain less than 2.0 mg/L (ppm) of total organic carbon (TOC). Therefore, when a polycationic polymer was tested in dilution water containing 12.0 mg TOC/L, the study was not used.

Since 1989, OPPT has been routinely predicting the toxicity of every chemical submitted under Sec. 5 even when measured toxicity values were included in the PMN. We realized that this was the best way to improve the SAR analysis. Prior to 1989, predictions were not routinely done when measured toxicity values accompanied a PMN. Therefore, when the contractor found measured toxicity values but no predicted values during searches of the PMN ECOTOX DATABASE, a list of the PMNs with no or a partial set of predicted toxicity values was sent to Nabholz. Nabholz predicted the toxicity of these chemicals based solely on the chemical's physical/chemical properties and SAR methods used at the time of submission, and returned the predictions to the contractor for inclusion in the study.

During the actual calculation of validation ratios, two problems arose which needed resolution.

(1) When the predicted toxicity value was "greater than 100 mg/L", which indicated a low toxicity concern by OPPT, and the measured value was greater than 100 mg/L, then the "greater than" symbol was dropped and a ratio calculated using 100. This situation occurred with several chemical classes (Nabholz et al. 1993) which generally have low toxicity to fish and daphnids, have no QSAR, and whose SARs are characterized as generic evaluations or statements about their aquatic toxicity. For example, such classes include acid dyes with three or more sulfonic acid groups, amphoteric dyes, small molecular weight acids at pH 7.0 and which are not surfactants, polyanionic monomers, polyanionic polymers, nonionic polymers, and small molecular weight quaternary nitrogens at pH 7.0 and which are not surfactants. Likewise, when the predicted value was "greater than 100 mg/L" and the measured value was less than 100 mg/L, the "greater than" symbol was ignored and the ratio calculated.

(2) When the predicted value was "no effects at saturation" and a valid toxicity value was measured or when a toxicity value was predicted and the measured value was "no effects at saturation," no ratio was calculated because the numeric value that should be assigned to "no effects at saturation" was not known. This situation occurred most frequently with predictions of chronic toxicity. Many QSARs for chronic toxicity proceed from predictable ChVs to "no effects at saturation" as K_{ow} increases. Therefore, concentrations of a chemical which cause chronic effects and concentrations which are not toxic at saturation for the exposure period of the test may be very close together, e.g. 10.0 µg/L (ppb) and 5.0 µg/L.

Sample Size

Test data from 462 chemicals were included in the validation analysis. There were seven sources of information: (1) TSCA Sec. 5 PMNs, (2) TSCA Sec. 5 Low Volume Exemptions (LVE) which are new chemicals whose production volume will be less than 1000 kg/year, (3) TSCA Sec. 5 Test Market Exemptions (TME) which are new chemicals which will be test marketed before commercial production, (4) TSCA Sec. 8(e) submissions which are chemicals which may present a substantial risk of injury to the environment, (5) TSCA Sec. 8(e) submissions which are toxicity data for the Agency's information only, i.e. For Your Information (FYI), (6) TSCA Sec. 5 Pre-Notice Communications (PC) which are information packages submitted prior to submission of a PMN, and (7) toxicity information from

the Organisation for Economic Co-operation and Development (OECD) high production volume (HPV) Screening Information Data Set (SIDS) program. The distribution of chemicals by source and year is:

1	PMN (FY 1981);
1	PMN (FY 1982);
6	PMN (FY 1938);
4	PMN (FY 1984);
8	PMN (FY 1985);
7	PMN (FY 1986);
45	PMN (FY 1987);
88	PMN (FY 1988);
1	LVE (FY 1988);
51	PMN (FY 1989);
3	LVE (FY 1989);
3	TME (FY 1989);
50	PMN (FY 1990);
2	LVE (FY 1990);
1	TME (FY 1990);
84	PMN (FY 1991);
3	LVE (FY 1991);
38	PMN (FY 1992);
14	Sec. 8(e);
2	Sec. 8(e) FYI;
1	Sec. 5 PC;
1	SIDS; and
48	Toxicity data for analogous chemicals or positive control chemicals submitted with PMNs.

The distribution of chemicals by chemical class is listed below. Descriptions of these chemical classes have been discussed by Nabholz et al. (1993).

70	Neutral organic/solvent-like chemicals;
124	Organic monomers with excess toxicity;
8	Acids;
19	Polyanionic monomers;
75	Dyes:
20	Cationic dyes,
50	Acid (anionic) dyes,
3	Neutral (nonionic) dyes, and
2	Amphoteric dyes;
77	Surfactants:
9	Cationic,
42	Anionic,
25	Nonionic, and
1	Amphoteric;

79 Polymers:

35 Cationic,
37 Anionic,
4 Nonionic, and
3 Amphoteric; and

10 Metals.

Results

Distribution of Validation Ratios

Ratios for 920 effective concentrations (EC) were calculated and their distribution by type of effective concentration was:

414 Fish 96-h LC_{50};
307 Daphnid 48-h LC_{50};
145 Green algal 96-h EC_{50};
8 Fish ChV;
14 Daphnid ChV; and
32 Algal ChV.

The frequency distribution of ratios was plotted on an arithmetic scale (**Figure II.1**). As expected, this histogram showed that the distribution was strongly skewed to the larger ratios; therefore, the ratios were plotted on a logarithmic scale (**Figure II.2**). The skewed distribution was expected because all ratios of less than 1.0 would be summarized in the ratio class of 0.0 to 1.0. This transformation approached a normal distribution. The ratio class with the highest frequency was 0.95 to 0.05 or 1.0. Eighty-five per cent (782/920) of the ratios were >0.1 and <10.0. Only 9 per cent (83/920) of the ratios were <0.1 and only 6 per cent (55/920) were >10.0.

Mean Validation Ratios

The geometric mean ratio was calculated for each EC in the OPPT standard toxicity profile:

0.64 Fish 96-h LC_{50};
0.79 Daphnid 48-h LC_{50};
0.81 Green algal 96-h EC_{50};
0.24 Fish ChV;
0.39 Daphnid ChV; and
1.07 Algal ChV.

The most accurately predicted effect was the algal ChV and the most inaccurately predicted effect was fish ChV. The fish ChV was also the endpoint with the smallest sample size, having only eight ratios. The grand mean based on all 920 ratios was 0.72. On the average and with one exception, toxicity was over-predicted, although only four times.

The mean ratios for each toxicity endpoint for 60 chemical classes are listed in **Table II.1**. As was expected, ratios were much more variable with a range from 0.01 to 410.0.

Discussion

The results of this validation study were both surprising and expected. We were surprised because the results came out better than anticipated. We expected the results to support our SAR methods, because for years we knew we were doing well predicting toxicity. We frequently received positive feedback from the chemical industry about how well we had predicted the toxicity of their chemical(s), but this feedback was only on a case-by-case or chemical-by-chemical basis. It was easy to remember the accurate predictions, but much harder to figure out why some chemicals were significantly under- or over-predicted.

The great majority of ratios were within ten times of perfection, i.e. >0.1 to <10.0, and the grand mean was 0.72. The grand mean was close to 1.0 and it was slightly less than 1.0 which, as a regulatory program, is where it should be if it cannot be 1.0. The mean ratios for the toxicity endpoints indicated that we predicted the environmental base set of toxicity values, i.e. the fish acute value, the daphnid acute value, and the algal toxicity value, with more accuracy than the chronic values for fish and daphnids. Given hindsight, this is exactly the result we should have anticipated because this analysis reveals that we had 33 times more information about acute toxicity to fish and daphnids than about chronic toxicity. OPPT has much more experience with acute toxicity than with chronic toxicity, and Clements et al. (1993b) shows that OPPT has many more acute toxicity QSARs for fish and daphnids than chronic toxicity QSARs.

Only 6 per cent of the chemicals were significantly under-predicted and only 9 per cent of chemicals were over-predicted. These percentages were smaller than expected. Some of the reasons for under-predicting toxicity include: excess toxicity, missing fragment constants used to predict the octanol/water partition coefficients (K_{ow}), lack of knowledge about the exact chemical structure, and no physical/chemical properties available to relate to toxicity. Some of the reasons for over-predicting include: no QSAR for a class of chemicals, comparing predicted "greater than" toxicity values with actual measured values, comparing fish 96 h LC_{50} values with 72 h LC_{50} values, comparing daphnid 48 h LC_{50} values with 24 h LC_{50} values, comparing green algal 96 h EC_{50} values with 72 h EC_{50} values, predicting toxicity for chemicals with molecular weights greater than 600 or with large cross-sectional diameters, and predicting toxicity for neutral organic chemicals which are solids at room temperature.

Figure II.1 Histogram of validation ratios on an arithmetic scale

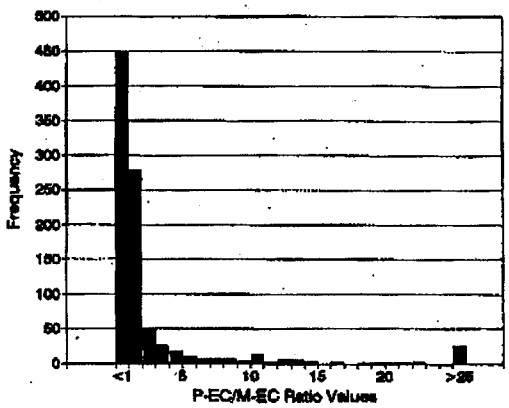

Figure II.2 Histogram of validation ratios on a logarithmic scale

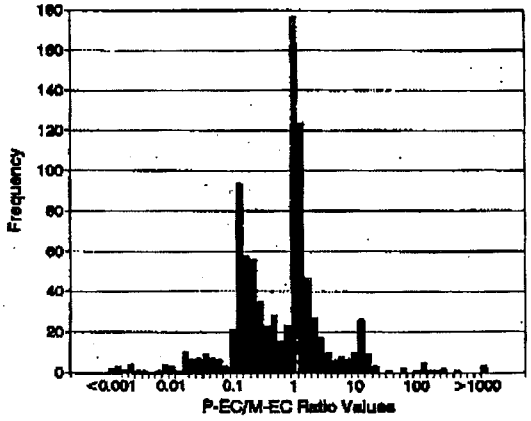

Table II.1 Validation ratios by chemical class. Ratios are geometric means. Dashes indicate that no ratio was available. For classes which are subdivided, e.g. acid dyes, the class ratio is reported along with ratios for each subclass. S# indicates the number of sulfonic acids in the dye.

Classes	P-EC/M-EC Ratios					
	Fish 96h LC_{50}	Daphnid 48h LC_{50}	Green Algae 96h EC_{50}	Fish ChV	Daphnid ChV	Green Algae ChV
Acid Chlorides	0.7	1.0	-	-	-	-
Acids	0.6	0.9	2.0	-	-	-
Acrylamides	0.2	0.2	-	-	-	-
Acrylates	0.7	0.3	2.9	-	-	10.0
Aldehydes	-	1.0	-	-	-	-
Alkyloxysilanes	3.1	0.1	0.6	-	-	-
Allyl Ethers	1.4	-	-	-	-	-
Allyl Ketones	2.4	-	-	-	-	-
Aliphatic Amines	0.9	0.5	2.7	-	-	0.3
Anilines	1.2	0.6	-	-	-	-
Benzotriazoles	2.0	-	1.4	-	-	-
Benzotriazoles	0.6	0.3	2.3	-	-	-
Carbamates	2.0	1.0	0.2	-	-	1.1
Chlorosilanes	0.2	0.1	-	-	-	-
Diazos	1.0	-	-	-	-	-
Dinitrobenzenes	0.1	0.2	20.0	-	-	15.0
Dithiocarbamates	1.0	1.0	-	-	-	-
Diepoxides	0.1	-	-	-	-	-
Dyes						
Acid Dyes	0.3	0.3	0.4	-	0.1	0.01
S1, S2	0.4	0.3	0.4	-	0.1	-
≥ S3	0.2	0.3	0.3	-	-	0.01
Cationic Dyes	0.9	0.8	3.0	2.3	-	-
Amphoteric Dyes	0.8	0.1	-	-	-	-
Neutral Dyes	1.0	1.0	-	-	-	-
Esters	1.0	1.0	1.6	-	-	-
Halo Allyls	3.2	111.0	-	-	-	-
Halo Amides	1.1	0.5	-	-	0.4	-
Hydrazines	-	0.1	-	-	-	-
Isocyanates	410.0	0.01	0.01	-	-	-
Melamines	0.4	0.8	1.0	1.0	1.0	1.0

Table II.1, continued

Classes	P-EC/M-EC Ratios					
	Fish 96h LC_{50}	Daphnid 48h LC_{50}	Green Algae 96h EC_{50}	Fish ChV	Daphnid ChV	Green Algae ChV
Metals						
Iron	0.1	0.8	-	-	-	-
Silver	-	2.0	-	-	-	-
Tin	1.3	0.7	-	-	-	-
Titanium	0.1	-	-	-	-	-
Vanadium	0.9	-	-	-	-	-
Zinc	0.2	1.5	5.4	-	-	7.7
Zirconium	0.1	-	-	-	-	-
Methacrylates	0.7	1.4	-	-	-	-
Monomers						
Polyamphoteric	23.0	1.2	0.3	-	-	-
Polyanionic	0.2	0.3	0.7	0.3	0.4	11.0
Neutral Organics	1.2	1.1	1.2	0.2	0.3	3.1
Peroxy Acids	80.0	14.0	-	-	-	-
Phenols	1.6	1.1	2.1	0.1	0.4	0.4
Phosphates	7.1	19.0	0.4	-	-	-
Phosphites	1.0	-	-	-	-	-
Polymers						
Polyamphoteric	0.2	0.1	0.03	-	-	0.4
Polyanionic	0.3	0.4	0.2	0.3	1.6	0.1
Polycationic	0.2	2.5	0.5	-	-	3.3
C backbone	0.2	1.0	0.4	-	-	3.3
Si backbone	0.2	24.0	19.0	-	-	-
Polynonionic	0.7	1.0	-	-	-	-
Salicylates	0.2	2.9	2.5	-	-	-
Sulfonamides	13.0	4.1	13.0	-	-	-
Sulfonates	-	0.3	0.05	-	-	-
Surfactants						
Amphoteric	58.0	5.4	1.4	-	-	-
Anionic	0.6	1.1	0.9	-	-	3.3
Cationic	0.9	5.3	10.0	-	-	-
Nonionic	0.9	1.0	1.1	2.5	0.2	0.6
Thiols	-	1.0	-	-	-	-
Triazines	27.0	14.0	2.2	-	-	-

Excess Toxicity

Chemicals with excess toxicity (Auer et al. 1990; Lipnick 1991; Nabholz et al. 1993) have probably been the largest cause of under-estimating toxicity since 1979. Chemicals are assumed to have only baseline toxicity (or narcosis) until measured toxicity data show significant excess toxicity, i.e. a measured toxicity value at least ten times more toxic than predicted by the SARs for neutral organic chemicals (Auer et al. 1990, Nabholz et al. 1993). If a chemical has a chemical structure which suggests a high probability of excess toxicity, e.g. a chemical which could belong to a class of chemicals used as pesticides (Lipnick 1991), OPPT may divide the toxicity prediction by an excess toxicity factor, say 10, but more likely a "less than" symbol is used with the toxicity prediction and a larger assessment factor (Nabholz 1991; Nabholz et al. 1993; Zeeman et al. this volume) is used during risk assessment. Once measured toxicity data are obtained for a chemical suspected of having excess toxicity, the excess toxicity is quantified and, if large enough, a new SAR is developed for that class of chemical.

Some chemicals are never suspected of having excess toxicity, yet when measured toxicity data are obtained, are shown to have excess toxicity. For example, schiff bases were considered no more toxic than aliphatic amines; however, when the first fish acute toxicity study was obtained for a schiff base, it had a significant amount of excess toxicity. Therefore, OPPT developed a SAR for schiff bases and classified them as a subclass of aliphatic amines.

Some reactive chemicals were assumed to be of little concern because they were predicted to be transformed quickly to less toxic chemical(s). For example, aromatic diazoniums were assumed to react so quickly to form a phenol that aquatic organisms were not expected to be exposed to the diazonium, only the resulting phenol. However, when OPPT received its first toxicity data for diazoniums through TSCA Sec. 8(e), diazoniums were shown to be significantly more toxic than the resulting phenol.

Missing Fragment Constants

OPPT uses calculated K_{ow} and molecular weight (MW) in most of its QSARs. The computer program, CLOGP Ver. 3.3, is used to calculate K_{ow}. The computer program, CLOGP, calculates the K_{ow} of organic chemicals using chemical structure and fragment constants for each portion of the chemical structure (Hansch and Leo 1979). When a missing fragment does not permit CLOGP to complete the calculation, OPPT staff must estimate a value for the missing fragment or find some test data for an analog. If the K_{ow} is inaccurate for a chemical, then the resulting toxicity prediction will also be inaccurate.

Exact Chemical Structure

Lack of sufficient knowledge about the chemical structure, especially for reaction products and dyes, can lead to significant under-estimations of toxicity. A toxicity prediction is only as good as the knowledge of a chemical's structure. If you change the structure, you must repredict toxicity. Many reaction products and some dyes are only known by their representative structure. For example, the range of fish acute (96 h LC_{50}) toxicity values for 27 cationic dyes with delocalized cationic charge varies 1,200 times. Many of these dyes are described by their representative structure only, tested products may contain several chemical

structures and numerous impurities, and there is no physical/chemical property identified which is strongly correlated to toxicity. OPPT uses the nearest analog SAR method to predict toxicity for these types of chemicals.

Chemical Reactivity

OPPT must attempt to estimate chemical reactivity from structure alone. OPPT has neither super computers that can be easily accessed to estimate exotic molecular descriptors of chemical reactivity nor widely-accepted laboratory tests that can be used to measure reactivity. OPPT has to use simple physical/chemical properties, such as K_{ow}, MW, number of reactive groups per molecule, e.g. epoxides, charge density of cationic polymer, number of aliphatic carbons in the hydrophobic component of a surfactant, or number of ethoxy groups in a nonionic surfactant, in its SARs. For example, if monoepoxides and diepoxides are used in the same SAR without accounting for the extra reactivity due to the second epoxide, predictability using K_{ow} and MW alone is poor. However, if an SAR is developed using only monoepoxides and another SAR is developed using diepoxides, excellent predictability can be achieved using only K_{ow} and MW. This approach requires that OPPT have more SARs for a chemical class and will make OPPT more dependent on measured toxicity information, but, as this validation study has shown, excellent predictability can be obtained using simple methods.

Classes Lacking a QSAR

Some chemical classes lack a QSAR (Auer et al. 1990; Nabholz et al. 1993) and the nearest analog method may also be a poor method for predicting toxicity. Some of the chemical classes falling into this category are polyanionic monomers, polyanionic polymers, acid dyes, and metal-complexes with organic acids and/or aliphatic amines. Polyanionic monomers and polyanionic polymers are toxic only to green algae because of over chelation of nutrient elements; acid dyes with more than three sulfonic acids per molecule are toxic only to algae through shading of visible light; acid dyes with only one or two sulfonic acids may be toxic to fish and daphnids as well as shading algae from growing; and metal-organic acid/chemical complexes may be toxic to one or all groups of aquatic organisms depending on the stability of the complex and/or the metal's bioavailability. Toxicity is difficult to predict for all of these groups because OPPT does not have a physical/chemical property that can be easily derived from the chemical's structure and used to predict toxicity. For example, polyanionic monomers chelate nutrient elements, such as calcium, magnesium, and iron, to such an extent that green algae are inhibited from growing in the algal bottle assay. The chelation ability of polyanionic monomers depends on the number of acids per molecule, the type of acid (e.g. carboxylic or phosphoric), the arrangement of the acids on the molecule, the presence of nitrogen in the molecule, and the type of salt. It is very difficult to accurately predict the algal 96 h EC_{50} value just by comparing structures. Therefore, when a close match between a chemical with known toxicity and a new chemical cannot be made, OPPT generally assumes that the new chemical will be just as toxic as the average polyanionic monomer which is about 3.0 mg/L as an algal 96 h EC_{50}. If the new chemical is tested and shows no inhibition of algal growth at 1000.0 mg/L, then the toxicity has been over-predicted by over 300 times. However, the database has been increased with one more analogous chemical for future use.

Molecular Weights Greater Than 600

OPPT predicts the toxicity of neutral organic chemicals with molecular weights up to 1000 daltons even though it has been reported that uptake decreases exponentially with MWs >600 (Zitko 1981). SAR analysis has shown that toxicity decreases as MW increases at a given K_{ow} value (Clements 1988, 1993b) and OPPT has only a few test data in its PMN ECOTOX DATABASE which show significant decreases in toxicity when MWs are greater than 600. Toxicity data for organic chemical monomers with MWs >600 and <1000 are rare. Predicting the effect of MW on toxicity when most of the chemicals in a SAR have MW <300 is difficult. OPPT knows that toxicity will decrease but the extent of the decrease is not known.

Cross-Sectional Diameter

The effect on toxicity of increasing the minimum cross-sectional diameter of a neutral organic chemical above the cross-sectional diameter of hexabromobenzene is analogous to the discussion on increasing MW. Minimum cross-sectional diameter is difficult to predict without the use of a mainframe computer. While it is known that increasing cross-sectional diameter will decrease toxicity, this property is rarely taken into account when doing predictions because the relationship between the cross-sectional diameter and toxicity cannot yet be determined with adequate accuracy and, if errors are made, they have to be on the side of safety.

Solids

Most of the organic chemicals used in OPPT SARs are liquids at room temperature. As the melting point (MP) of a chemical increases, the water solubility (WS) is known to decrease (Banerjee et al. 1980). When OPPT predicts the toxicity of solids, it also predicts the WS if no measured WS is available and if the MP is known. The WS limit is compared to the predicted toxicity value. If the WS limit is higher than the EC, then the prediction is considered accurate. However, if the WS limit is lower than the EC, then the certainty about the ability of the organic chemical to cause a toxic effect within the exposure period associated with the EC decreases. As a general rule, if the EC is ten times higher the WS limit for a chemical, then OPPT predicts "no effects at saturation". However, if the EC is within ten times of the WS of the chemical, then a toxicity prediction is made because of the uncertainty associated with predicting and measuring the WS limit of organic chemicals. For example, OPPT recently assessed a series of dinitrobenzenes for acute and chronic toxicity. All were solids at room temperature and only some MPs were known. Predictions of toxicity and WSs suggested that most of the dinitrobenzenes would be toxic in acute toxicity tests. Therefore, acute toxicity testing was recommended. When the test results were reviewed, it was apparent that the WS estimates were higher than measured values and most of the acute toxicity testing resulted in no effects at saturation. These new data permitted the reformulation of the SARs for dinitrobenzenes with respect to the environmental base set of tests.

Conclusions

In spite of the uncertainties inherent in predicting and measuring the toxicity of industrial chemicals, this study reveals that OPPT does an acceptable job in developing and using SAR Analysis for most classes of chemicals. OPPT will continue to validate its environmental SARs and continue to strive toward perfection in its SAR toxicity predictions. Although OPPT appears to be doing an adequate job in predicting environmental toxicity, measured toxicity data for chemicals are always preferred to predicted toxicity data in assessments as long as the measured data are valid and appropriate for the assessment.

Accurate SAR methods are dependent on accurate toxicity testing. OPPT's major source of toxicity data is the chemical industry and commercial testing laboratories and these data are used to develop new SARs and validate existing SARs. It is important that the test data submitted accurately reflect the toxicity of a chemical under a defined set of testing conditions. OPPT's entire SAR program is dependent on accurate and valid environmental test data. This study indicates that in order to improve and extend SAR analysis to all chemical classes, there is a need for standard testing methods and greater knowledge about the physical/chemical properties of chemicals prior to testing. Chemical structure needs to be clearly defined. The nature of the chemical substance to be tested has also to be known with as much certainty as possible, especially with respect to reaction products that have no definite chemical structure. The WS limit of chemicals with low WS should be measured and the dispersibility limit of surfactants should also be determined. The highest treatment concentration of toxicity tests should not exceed these WS/dispersibility limits. The amount of background dissolved organic carbon (DOC) in dilution water has to be measured when testing cationic chemicals. The reactivity of reactive organic chemicals should be determined with greater accuracy before toxicity testing. The hardness of dilution water should always be known and controlled because the average hardness of surface waters in the United States is about 120.0 mg/L as $CaCO_3$ and this fact should be kept in mind when testing chemicals for use/release in the U.S. For this reason, OPPT recommends that toxicity tests with fish and daphnids be done at a hardness of less than 180.0 mg/L. The pH should be known and controlled depending on the purpose of the toxicity testing. For example, if an acid is being tested for normal industrial/commercial use and release to the environment through the sewer, then it should be tested near neutral pH. If, however, the toxicity testing is to determine the environmental concern from an accidental spill, then the acid should be tested without pH adjustment.

Recommendations

Our advice to testing laboratories: Know what you are testing! Know why the sponsor is testing the chemical, e.g. normal use or the impact of a transportation spill. Recommend testing which corresponds to the physical/chemical properties of the chemical and control those factors, e.g. DOC when testing cationic chemicals, which you know will have the greatest influence on the toxicity of the chemical. Do not test an industrial chemical above its WS limit unless there is a specific purpose for the testing.

Our advice to the chemical industry: Tell your testing laboratory about your chemical! The more they know about your chemical, the more they can help you to test it properly. Testing laboratories have a lot of experience in testing many different types of chemicals. Remember that not much experience is gained by a testing laboratory by blindly testing unknown materials.

Disclaimer

This document has been reviewed by OPPT, US EPA and approved for presentation. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Annex III

Classification of Chemicals

Structural Requirements for Narcosis, Less Inert Compounds, Reactive or Specific Toxicity (from Verhaar and Hermens, 1991; see also Verhaar et al., 1992)

Class I chemicals (narcosis baseline toxicity)

We will consider for inclusion in our overview of requirements for narcosis toxicity only those organic compounds that consist of carbon, hydrogen, nitrogen, oxygen and/or halogens (excluding iodine). In our opinion, so little is known about compounds containing other elements, other than that they show tremendous variation in toxic action, that it is well-nigh impossible to construct definite rules for determining whether such compounds will act by narcosis or not, other than a complete enumeration of all known compounds known to act by narcosis.

Furthermore, we will define as narcosis only the type I, or non-polar narcosis. Please note that, according to Veith and Broderius (1990), type I and type II narcosis are generally indistinguishable for compounds with a Log K_{ow} higher than 2.7. This notion can be used to calculate narcosis type toxicity of those compounds that are known to exhibit type II narcosis (see for instance the overview of compounds known to be more toxic than baseline toxicity, in this report), and that have a Log K_{ow} that is higher than 2.7. Note that it is always possible to calculate the baseline toxicity for a compound; for compounds that do not act by non-polar narcosis this enables one to predict the range in which the effect concentration will be found, using the approach mentioned under "Aim of this study".

As type I narcosis chemicals can be classified all those compounds that:

A
- have a Log K_{ow} that lies between 0 and 6. Although there are compounds with Log K_{ow} values lower than 0, that act by narcosis, it is considered unlikely that these compounds would exhibit acute toxic action towards biota in aqueous environments because of the unrealistically high concentrations that will be required for this to happen; compounds that have a Log K_{ow} higher than 6 and that fulfil the requirements for narcosis, do not normally exhibit this. This is thought to reflect the notion that these structures are generally too bulky to be taken up through membranes, and/or that the time needed for these compounds to reach a steady state is too long to reach the internal concentrations that correspond with the aqueous concentrations considered within the time span that defines acute toxicity. An example of this is tetradecanol, which is considered essentially nontoxic. This rule does not mean that compounds with Log K_{ow} values that lie outside this range are to be considered nontoxic, but only that we do not recommend modelling their toxicity using narcosis-type QSAR equations.

– AND

have a molecular mass (MW) of not more than 600 Daltons. Generally speaking, compounds having a MW of over 1000 Daltons are too bulky to be taken up across membranes. Because of the fact that a high proportion of all chemicals with an MW higher than 600 Daltons are reactive chemicals or compounds acting by a specific mechanism, we define a limit of applicability of narcosis-type QSAR equations at MW = 600 Daltons.

– AND

have a (dimensionless) Henry's law constant of not more than 10^{-2}. Compounds having a Henry's law constant of more than 10^{-2} will not be considered aquatic hazards, because of their high rate of evaporation from water. This is not to say that compounds with a Henry's law constant will not act as narcotic chemicals, but that they normally will not exhibit this effect in aquatic environments, due to their very high evaporation rate.

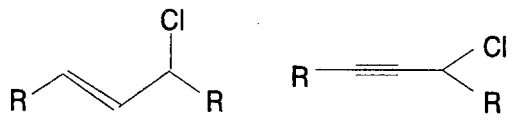
B
- do NOT contain I.[1] Organic compounds containing covalently bound I are in general potent alkylating agents
- do NOT contain ionic groups

AND

C
- contain only C and H

OR
- If they contain only C, H and halogen:

D
- are acyclic compounds NOT containing halogen at ß-positions from unsaturations (e.g. allylic/propargylic halogens)

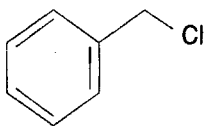


[1] Organic compounds containing covalently bound F are to be considered equivalent with the H-analogues; **but please note that F compounds are non-metabolisable if F substitutes for metabolically important H atoms**. This can give rise to chronic specific toxicity. Compounds containing Cl or Br atoms should not be activating these halogens or be activated by them. Activated Cl or Br can be found in e.g. allylic/propargylic halogenides, activating Cl or Br can be found in e.g. trichloroethanol or pentachlorophenol.

OR

E

- are monocyclic compounds substituted with halogens
- are monocyclic compounds that are unsubstituted or substituted with acyclic structures containing only C and H, or complying with rule D. Note that compounds containing benzylic halogens do NOT comply with rule D, and thus cannot be considered narcotic chemicals



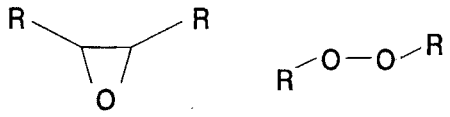
- are polycyclic compounds that are unsubstituted or substituted with acyclic structures containing only C and H, or complying with rule D. Note that compounds containing benzylic halogens do NOT comply with rule C, and thus cannot be considered narcotic chemicals. Note also that many of these polycyclic compounds, besides working as narcotics in acute toxicity experiments, have chronic toxicities based on specific modes of action.

OR

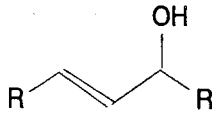
- If they contain C, H and O

F

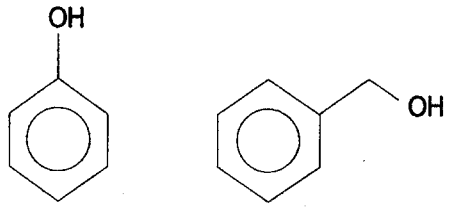
- are linear ethers or monocyclic non-ethers, but NOT epoxides or peroxides



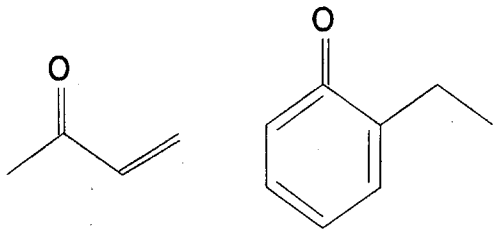
- are aliphatic alcohols, but NOT allylic/propargylic alcohols



- are alcohols with aromatic moieties, but NOT phenols or benzylic alcohols



- are ketones, but NOT α,ß- unsaturated ketones (e.g. 1-butenone or acetophenone)

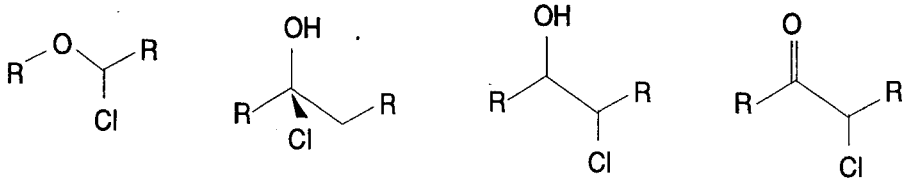


OR • If they contain C, H and N

G • are aliphatic secondary or tertiary amines

OR • If they contain C, H, O and halogen

H • are halogenated type F compounds, but NOT α or ß halogen-substituted compounds



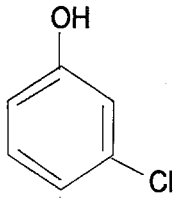
N.B.: It may be possible that some compounds, which are known to be more toxic than baseline toxicity, do classify as class 1 compounds.[2] If this be the case, DO NOT treat these compounds as baseline toxicants. As an aside, it is always advisable to check whether a specific compound is listed under specifically acting compounds, even if it classifies as class 1.

We would appreciate it if you would notify us of any such compounds that you encounter. This may be used to update the decision rules for classifying compounds in future versions of this document.

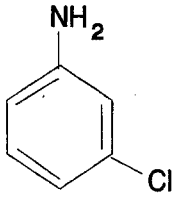
[2] An example of this would be lindane, or γ-hexachlorocyclohexane, which is much more toxic than the other hexachlorocyclohexanes.

Class II chemicals – Less inert (Verhaar and Hermens, 1991)

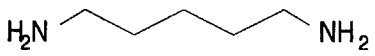
- non- or weakly acidic phenols; NOT phenols with two or more nitro substituents or four or more halogen or halogenlike (e.g. cyanide) substituents, e.g.



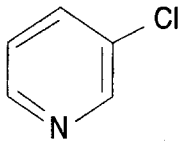
- aromatic amines and anilines; NOT anilines with two or more nitro substituents or four or more halogenlike (e.g. cyanide) substituents, e.g.



- aliphatic primary amines, e.g.



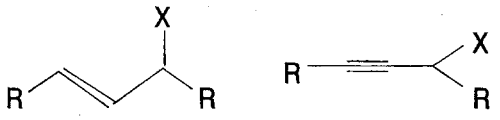
- weakly basic pyridines, e.g.



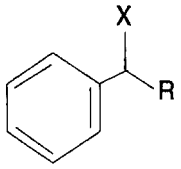
Class III chemicals – Unspecific reactivity (Verhaar and Hermens, 1991)

N.B.: The remaining groups are (small) structures that are sufficiently stable (under certain conditions) that they can stabilise an isolated negative charge, such as for instance halogen (chlorine, bromine, iodine), cyanide or, under acidic or basic conditions, the hydroxyl group.

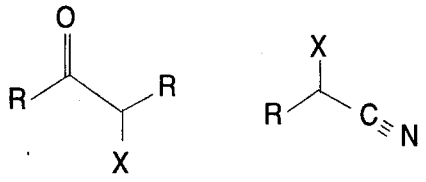
- allylic/propargylic activation. Compounds with a (good) leaving group at a ß-position of a carbon-carbon double or triple bond



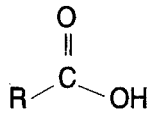
- benzylic activation. Compounds with a (good) leaving group at a ß-position of an aromatic bond



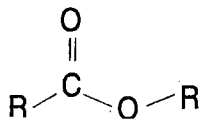
- general π-electron system activation. Compounds with a (good) leaving group at an α- position of a double or triple bond fragment, like a carbonyl or nitrile function



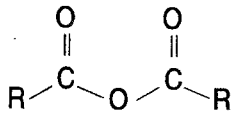
- carbonylic acides



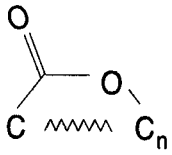
- carbonylic acid esters



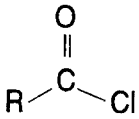
- acid anhydrides



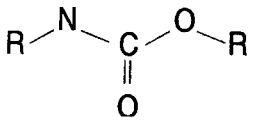
- lactones



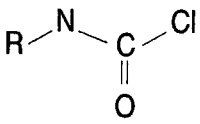
- acid halides



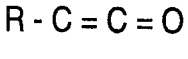
- carbamates



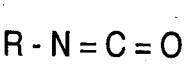
- carbamoylhalides



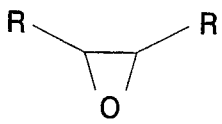
- ketenes

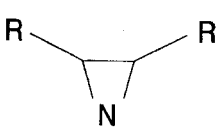


- isocyanates

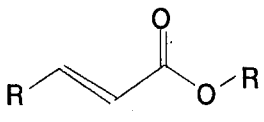
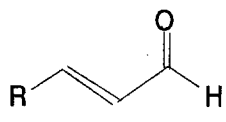
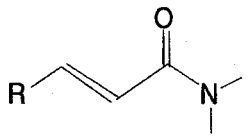
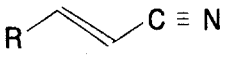
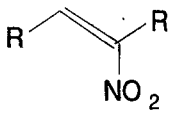
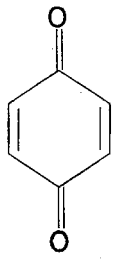


- three-membered heterocyclic rings. Compounds containing an epoxide or azaridine function

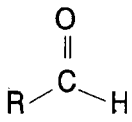




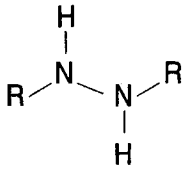
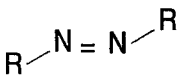
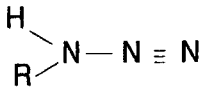
- activated carbon-carbon double/triple bond. Compounds containing a polarisable substituent (carbonyl, nitrile, amide, nitro, etc.) at an α-position of a double or triple bond. This enables a Michael type addition of nucleophiles across the double/triple bond, e.g.

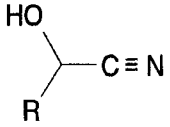
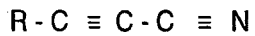
- aldehydes



- hydrazines and other compounds with a single, double or triple nitrogen-nitrogen linkage

- activated nitriles, like α-hydroxynitriles (cyanohydrins) or allylic/propargylic nitriles

Class IV chemicals – Acting by a specific mechanism (Verhaar and Hermens, 1991)

- alkyl/aryl-dodecadienoates
- aromatic sulphonates/sulphonate esters
- atropin and analogues (tropates)
- (aziridine)phosphide oxydes
- (aziridine)phosphide sulphides
- barbiturates
- (benzene/toluene)sulphonamides
- benzimidazoles
- benzoylphenylureas (insects only)
- biogenic lactones (avermectins)
- bipyridilium derivatives (diquat, paraquat)
- camphenes
- carbamates
- cyanates
- DDT and analogues (DDD, DDE, DDMU, Dicofol)
- (di)phenylacetic acid derivatives
- (dialkyl)formamidines
- (dioxo)pyrazolidines
- "drins"
- ether derivatives of hydroxyacetic acid
- griseofulvin
- hydantoins
- coumarins
- inorganic propionates
- isobornanes
- isocyanates
- isothiocyanates
- kepone & mire
- Lilly 18946
- lindane
- methylenedioxobenzenes
- nicotin analogues (nicotin, anabasin)
- norbornanes/norbornenes
- organometallics (organotin compounds; organocopper compounds; organomercury compounds; organolead compounds, etc.)
- organophosphate esters
- organophosphorothionate esters
- pentachlorophenol
- (pheno)thiazines
- phosphate esters
- phosphoric triamides
- phosphorocyanidates
- phosphorofluoridates
- phosphorotrithioites
- piperazines
- pyrethroids/chrysanthemates
- SKF-525A
- strychnine
- sulphinimides
- thiocyanates
- triazatriphosphorirnes(apholate)
- triazines (atrazine, diuron, bentazon)
- triorganophosphine oxydes
- (1,2)-dithiolanes
- 2-phenyl-3-pyrazolones (aminopyrine)
- (2-thione) thiadiazines

Annex IV

QSARS For Class I Chemicals

Survey of QSAR equations for chemicals that act by narcosis [data are expressed in mol/l[a] (OECD, 1992a)]. Relationships were recalculated with new K_{ow} values.

Species	BE	Endpoint	Log K_{ow} range	a	b	n	r^2	s.e.
Bacteria								
Clostridium botulinum	NOEC	24h pop. growth	0.77 - 6.11	-0.82	-0.29	14	0.94	0.46
Bacillus subtilis	EC_{50}	30m spore germ.	-0.77 - 4.57	-0.64	-1.03	14	0.92	0.33
Pseudomonas putida	NOEC	6h pop. growth	-0.25 - 2.72	-0.64	-1.60	5	0.98	0.14
Microcystis aeruginosa	NOEC	192h cell mult.	-0.25 - 2.72	-0.62	-2.33	4	0.84	0.51
Photobacterium phosphoreum	NOEC	15m luminescence	-1.31 - 4.14	-0.68	-1.52	20	0.90	0.60
Photobacterium phosphoreum	EC50	15m luminescence	-0.77 - 4.66	-1.01	-0.73	25	0.94	0.39
Protozoans								
Tetrahymena pyriformis	EC_{50}	48h proliferation	-0.77 - 5.58	-0.80	-0.80	26	0.93	0.40
Fungi								
Saccharomyces cerevisiae	NOEC	24h glucose util.	-0.77 - 1.56	-0.78	-0.35	5	0.90	0.29
Algae								
Skeletonema costatum	EC_{50}	96h pop. growth	1.48 - 4.60	-0.72	-0.94	9	0.72	0.45
Scenedesmus subspicatus	EC_{50}	48h cell multipl.	0.76 - 3.53	-0.86	-0.93	8	0.83	0.44
Selenastrum capricornutum	EC_{50}	72/96h pop. growth	2.19 - 4.05	-1.00	-1.23	10	0.93	0.17
Coelenterates								
Hydra oligactis	NOLC	48h survival	-0.25 - 2.72	-0.86	-1.35	5	0.92	0.45
Molluscs								
Lymnea stagnalis	NOLC	48h survival	-0.25 - 2.72	-0.86	-1.38	5	0.96	0.30
Arthropods								
Nitocra spinipes	LC_{50}	96h survival	-0.77 - 5.13	-0.78	-1.14	16	0.95	0.39
Daphnia magna	NOEC	18-21d reprod.	-0.24 - 5.18	-1.04	-1.70	17	0.98	0.25
Daphnia magna	NOEC	18-21d growth	-0.24 - 5.18	-1.07	-1.75	10	0.97	0.40
Daphnia magna	EC_{50}	48h survival	-1.36 - 5.18	-0.95	-1.19	17	0.99	0.21
Aedes aegypti	LC_{50}	48h&4h survival	-1.36 - 2.72	-1.09	-0.36	14	0.96	0.27
Aedes aegypti	NOLC	48h&4h survival	-0.25 - 2.72	-0.69	-1.42	5	0.91	0.37
Culex pipiens	NOLC	48h survival	-0.25 - 2.72	-0.86	-1.28	5	0.95	0.33
Fish								
Alburnus alburnus	LC_{50}	96h survival	-1.77 - 4.57	-0.75	-1.12	14	0.95	0.36
Brachydanio rerio	NOEC	28d larval growth	-2.90 - 5.18	-1.06	-1.42	6	0.97	0.17
Pimephales promelas	LC_{50}	96h survival	-1.24 - 5.13	-0.85	-1.41	68	0.94	0.34
Pimephales promelas	NOEC	28d larval growth	-0.46 - 4.07	-1.04	-1.96	7	0.96	0.30
P. promelas/B. rerio	NOEC	28-32d growth	0.46 - 5.24	-0.87	-2.35	27	0.90	0.35
Poecilia reticulata	LC_{50}	7&14d survival	-1.36 - 5.18	-0.87	-1.19	50	0.96	0.31
Amphibia								
Ambystoma mexicanum	NOLC	48h survival	-0.25 - 2.72	-0.88	-1.19	5	0.94	0.36
Rana catesbiana	LC_{50}	96h survival	-0.68 - 4.14	-0.86	-1.31	5	0.96	0.40
Rana temporaria	NOLC	30m survival	-0.77 - 2.97	-1.09	-0.77	11	0.98	0.23
Xenopus laevis	NOLC	48h survival	-0.25 - 2.72	-0.90	-1.09	5	0.94	0.38
Xenopus laevis	LC_{50}	48h survival	-1.36 - 2.83	-0.85	-1.84	12	0.52	1.16

[a] Equations are expressed as Log BE = a log K_{ow} + b, where BE stands for the LC_{50}, EC_{50}, NOLC or NOEC for a certain endpoint of toxicity, a is the regression coefficient, b is the y-intercept, n is the number of data, r^2 is the coefficient of determination, s.e. is the residual standard error.

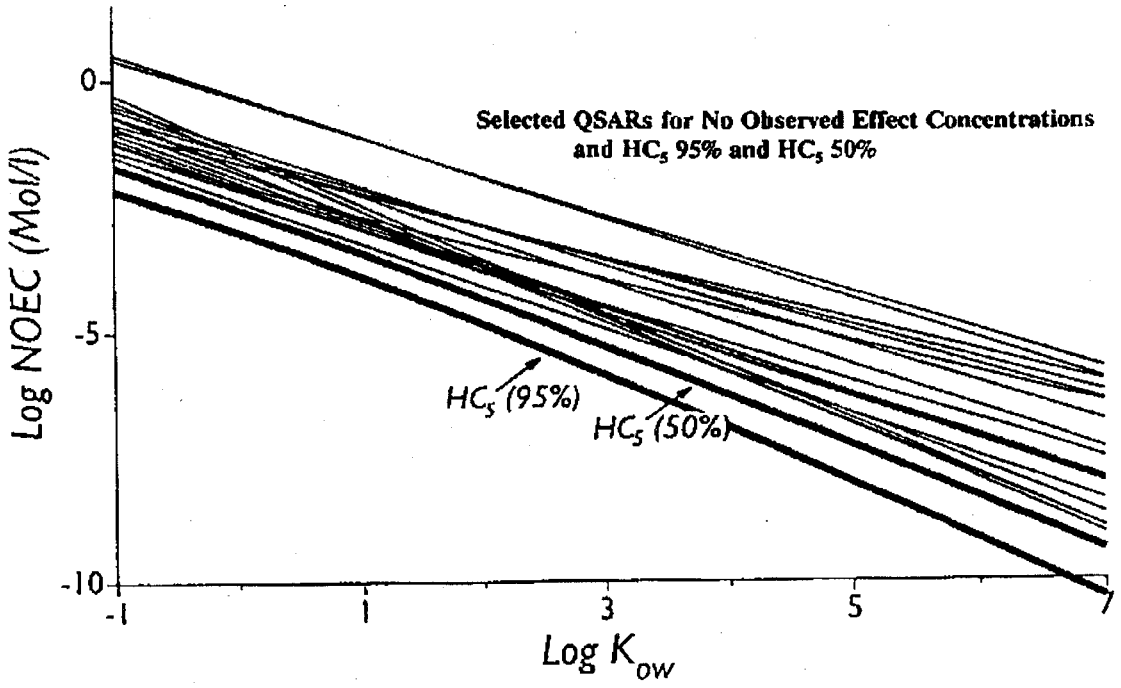
Annex V

QSAR Calculated MTC Values

For Class I chemicals, QSARs are available for a large number of species (see Annex IV). Estimated chronic toxicity data can be used as input in extrapolation models (see Section 5.1). This procedure was carried out for a selection of 19 species and more than 100 Class I chemicals. For each substance a hazardous concentration (HC_5, i.e. 95 per cent protection level) can be derived, and in this way a connection was made (via the QSAR toxicity estimate) between log K_{ow} and the hazardous concentration for Class I chemicals (Van Leeuwen et al., 1992). HC_5 is calculated under the assumption of a log-logistic distribution according to Aldenberg and Slob (1993) for narcotic chemicals as a function of K_{ow}. As explained in Section 5 of this report, the extrapolation methods may be applied with either 95 per cent or 50 per cent confidence for the hazardous concentrations. HC_5 values with 95 per cent and 50 per cent left confidence limits are presented in **Tables V.2 and V.3** for log K_{ow} values ranging from -1 to 7 at intervals of 0.1.

The HC_5 values are given for water (dissolved), for a "standard" sediment with an organic carbon content of 5 per cent (see Section 9) and for surface water, including suspended matter at a "standard" concentration of 30 mg/l (total). The relationship is shown in **Figure V.1**. The HC_5 (K_{ow}) may be used as an MTC for priority setting.

Figure V.1 Relationship between log K_{ow} and the Maximum Tolerable Concentration derived from the Hazardous Concentration HC_5 for 95 and 50 per cent confidence levels (Van Leeuwen et al., 1992)



In addition to the extrapolation method of Aldenberg and Slob (A&S), other methods as presented in Section 5 may also be used. In **Table V.1**, the QSAR estimates for the 19 species were used as input for the methods of Wagner and Løkke (1991; W&L) and Stephan et al. (1985). When the results of the three methods are compared (see **Figure V.2**), it may be concluded that the differences are rather small when the underlying assumptions are comparable (e.g. 50 per cent confidence level for A&S, W&L and the Stephan method).

Table V.1 Maximum Tolerable Concentrations calculated from log K_{ow} for the various extrapolation methods described in Section 5.

A&S: Aldenberg and Slob, 1991; W&L: Wagner and Løkke, 1991; Stephan et al., 1985

log K_{ow}	log HC_5				
	95% conf. level		50% conf. level		FAV
	A&S	W&L	A&S	W&L	Stephan
-1	-2.15	-2.09	-1.69	-1.71	-1.50
0	-2.97	-2.91	-2.52	-2.54	-2.32
1	-3.88	-3.82	-3.40	-3.43	-3.18
2	-4.85	-4.79	-4.34	-4.37	-4.05
3	-5.89	-5.82	-5.31	-5.35	-4.95
4	-6.97	-6.89	-6.32	-6.35	-5.89
5	-8.08	-7.98	-7.34	-7.38	-6.96
6	-9.20	-9.10	-8.37	-8.42	-8.05
7	-10.34	-10.23	-9.41	-9.47	-9.15
k-value	2.528	2.423	1.684	1.736	–

Figure V.2 Maximum Tolerable Concentration as a function of log K_{ow}: comparison of various methods

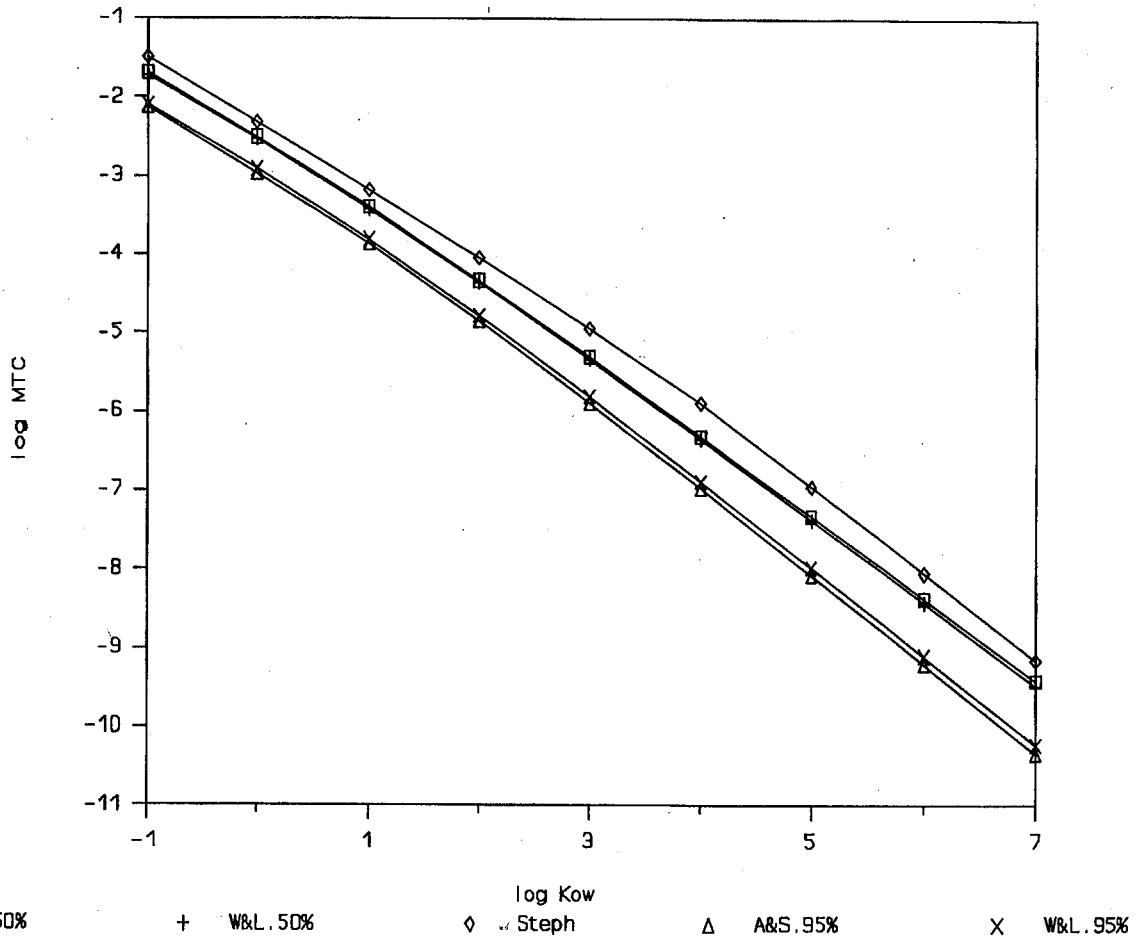


Table V.2 MTC values derived from HC_5 values (95 per cent confidence) for chemicals with baseline toxicity (Van Leeuwen et al., 1992)

log K_{ow}	log HC_5			log K_{ow}	log HC_5		
	dissolved (mol/l)	sediment (mol/kg)	total (mol/l)		dissolved (mol/l)	sediment (mol/kg)	total (mol/l)
-1.00*	-2.15	-4.68	-2.15	3.00	-5.89	-4.41	-5.89
-0.90*	-2.23	-4.65	-2.23	3.10	-6.00	-4.42	-6.00
-0.80*	-2.31	-4.63	-2.31	3.20	-6.10	-4.43	-6.10
-0.70*	-2.39	-4.61	-2.39	3.30	-6.21	-4.43	-6.21
-0.60*	-2.47	-4.59	-2.47	3.40	-6.32	-4.44	-6.31
-0.50*	-2.55	-4.57	-2.55	3.50	-6.42	-4.45	-6.42
-0.40*	-2.63	-4.56	-2.63	3.60	-6.53	-4.46	-6.53
-0.30*	-2.72	-4.54	-2.72	3.70	-6.64	-4.46	-6.64
-0.20*	-2.80	-4.52	-2.80	3.80	-6.75	-4.47	-6.74
-0.10*	-2.89	-4.51	-2.89	3.90	-6.86	-4.48	-6.85
0.00	-2.97	-4.50	-2.97	4.00	-6.97	-4.49	-6.96
0.10	-3.06	-4.48	-3.06	4.10	-7.08	-4.50	-7.07
0.20	-3.15	-4.47	-3.15	4.20	-7.19	-4.51	-7.17
0.30	-3.23	-4.46	-3.23	4.30	-7.30	-4.52	-7.28
0.40	-3.32	-4.45	-3.32	4.40	-7.41	-4.53	-7.39
0.50	-3.41	-4.44	-3.41	4.50	-7.52	-4.54	-7.49
0.60	-3.50	-4.43	-3.50	4.60	-7.63	-4.55	-7.60
0.70	-3.60	-4.42	-3.60	4.70	-7.74	-4.56	-7.70
0.80	-3.69	-4.41	-3.69	4.80	-7.85	-4.57	-7.80
0.90	-3.78	-4.40	-3.78	4.90	-7.96	-4.59	-7.90
1.00	-3.88	-4.40	-3.88	5.00	-8.08	-4.60	-8.00
1.10	-3.97	-4.39	-3.97	5.10	-8.19	-4.61	-8.10
1.20	-4.07	-4.39	-4.07	5.20	-8.30	-4.62	-8.19
1.30	-4.16	-4.38	-4.16	5.30	-8.41	-4.63	-8.28
1.40	-4.26	-4.38	-4.26	5.40	-8.52	-4.65	-8.36
1.50	-4.36	-4.38	-4.36	5.50	-8.64	-4.66	-8.44
1.60	-4.45	-4.38	-4.45	5.60	-8.75	-4.67	-8.51
1.70	-4.55	-4.38	-4.55	5.70	-8.86	-4.69	-8.58
1.80	-4.65	-4.38	-4.65	5.80	-8.98	-4.70	-8.64
1.90	-4.75	-4.38	-4.75	5.90	-9.09	-4.71	-8.70
2.00	-4.85	-4.38	-4.85	6.00	-9.20	-4.73	-8.75
2.10	-4.95	-4.38	-4.95	6.10*	-9.32	-4.74	-8.79
2.20	-5.06	-4.38	-5.06	6.20*	-9.43	-4.75	-8.84
2.30	-5.16	-4.38	-5.16	6.30*	-9.54	-4.77	-8.87
2.40	-5.26	-4.39	-5.26	6.40*	-9.66	-4.78	-8.91
2.50	-5.37	-4.39	-5.37	6.50*	-9.77	-4.79	-8.94
2.60	-5.47	-4.39	-5.47	6.60*	-9.89	-4.81	-8.96
2.70	-5.57	-4.40	-5.57	6.70*	-10.00	-4.82	-8.99
2.80	-5.68	-4.40	-5.68	6.80*	-10.11	-4.84	-9.01
2.90	-5.78	-4.41	-5.78	6.90*	-10.23	-4.85	-9.03

* Values for HC_5 are given over the interval of Log K_{ow} = -1 to 7; note, however, that due to a number of complicating factors the simple uptake and equilibrium partitioning models that were used for determining these values do not necessarily hold valid below Log K_{ow} = O and above Log K_{ow} = 5-6.

Table V.3 MTC values derived from HC_5 values (50 per cent confidence) for chemicals with baseline toxicity (Van Leeuwen et al., 1992)

log K_{ow}	log HC_5			log K_{ow}	log HC_5		
	dissolved (mol/l)	sediment (mol/kg)	total (mol/l)		dissolved (mol/l)	sediment (mol/kg)	total (mol/l)
-1.00*	-1.69	-4.21	-1.69	3.00	-5.31	-3.84	-5.31
-0.90*	-1.77	-4.19	-1.77	3.10	-5.41	-3.84	-5.41
-0.80*	-1.85	-4.17	-1.85	3.20	-5.51	-3.84	-5.51
-0.70*	-1.93	-4.15	-1.93	3.30	-5.61	-3.83	-5.61
-0.60*	-2.01	-4.14	-2.01	3.40	-5.71	-3.83	-5.71
-0.50*	-2.09	-4.12	-2.09	3.50	-5.81	-3.84	-5.81
-0.40*	-2.18	-4.10	-2.18	3.60	-5.91	-3.84	-5.91
-0.30*	-2.26	-4.09	-2.26	3.70	-6.01	-3.84	-6.01
-0.20*	-2.35	-4.07	-2.35	3.80	-6.11	-3.84	-6.11
-0.10*	-2.43	-4.05	-2.43	3.90	-6.22	-3.84	-6.21
0.00	-2.52	-4.04	-2.52	4.00	-6.32	-3.84	-6.31
0.10	-2.60	-4.03	-2.60	4.10	-6.42	-3.84	-6.41
0.20	-2.69	-4.01	-2.69	4.20	-6.52	-3.84	-6.51
0.30	-2.78	-4.00	-2.78	4.30	-6.62	-3.84	-6.61
0.40	-2.86	-3.99	-2.86	4.40	-6.72	-3.85	-6.70
0.50	-2.95	-3.98	-2.95	4.50	-6.83	-3.85	-6.80
0.60	-3.04	-3.97	-3.04	4.60	-6.93	-3.85	-6.90
0.70	-3.13	-3.95	-3.13	4.70	-7.03	-3.85	-6.99
0.80	-3.22	-3.94	-3.22	4.80	-7.13	-3.86	-7.08
0.90	-3.31	-3.94	-3.31	4.90	-7.24	-3.86	-7.18
1.00	-3.40	-3.93	-3.40	5.00	-7.34	-3.86	-7.26
1.10	-3.49	-3.92	-3.49	5.10	-7.44	-3.86	-7.35
1.20	-3.59	-3.91	-3.59	5.20	-7.54	-3.87	-7.43
1.30	-3.68	-3.90	-3.68	5.30	-7.65	-3.87	-7.51
1.40	-3.77	-3.89	-3.77	5.40	-7.75	-3.87	-7.59
1.50	-3.87	-3.89	-3.87	5.50	-7.85	-3.88	-7.65
1.60	-3.96	-3.88	-3.96	5.60	-7.96	-3.88	-7.72
1.70	-4.05	-3.88	-4.05	5.70	-8.06	-3.89	-7.78
1.80	-4.15	-3.87	-4.15	5.80	-8.17	-3.89	-7.83
1.90	-4.24	-3.87	-4.24	5.90	-8.27	-3.89	-7.88
2.00	-4.34	-3.86	-4.34	6.00	-8.37	-3.90	-7.92
2.10	-4.43	-3.86	-4.43	6.10*	-8.48	-3.90	-7.96
2.20	-4.53	-3.85	-4.53	6.20*	-8.58	-3.91	-7.99
2.30	-4.63	-3.85	-4.63	6.30*	-8.69	-3.91	-8.02
2.40	-4.72	-3.85	-4.72	6.40*	-8.79	-3.91	-8.04
2.50	-4.82	-3.85	-4.82	6.50*	-8.90	-3.92	-8.06
2.60	-4.92	-3.84	-4.92	6.60*	-9.00	-3.92	-8.08
2.70	-5.02	-3.84	-5.02	6.70*	-9.10	-3.93	-8.09
2.80	-5.12	-3.84	-5.12	6.80*	-9.21	-3.93	-8.11
2.90	-5.21	-3.84	-5.21	6.90*	-9.31	-3.94	-8.12

* Values for HC_5 are given over the interval of Log K_{ow} = -1 to 7; note, however, that due to a number of complicating factors the simple uptake and equilibrium partitioning models that were used for determining these values do not necessarily hold valid below Log K_{ow} = O and above Log K_{ow} = 5-6.