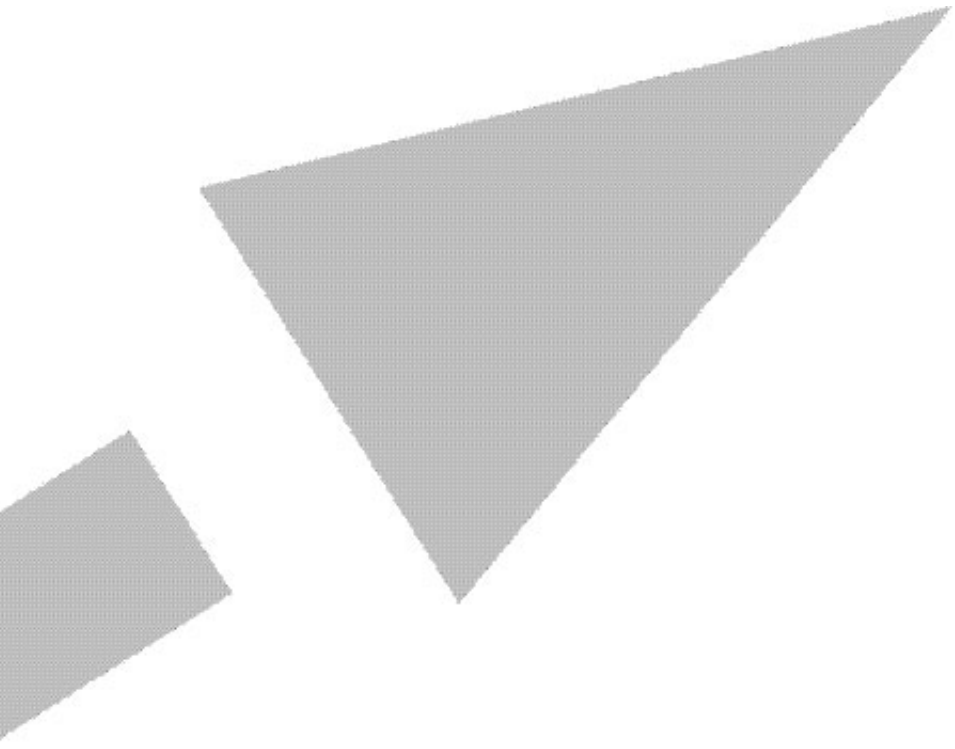

Environmental Risk Assessment of Difficult Substances

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Environmental Risk Assessment of Difficult Substances

CONTENTS

SUMMARY

Experience gained from the EU environmental risk assessments has shown that certain substances have properties that complicate the assessment of exposure and effects. Such substances, often referred to as 'difficult substances', are the subject of this report. Also included are naturally occurring essential and multi-ionic elements, as these represent chemicals for which further scientific debate between the scientific community and the Regulatory Authorities is required, in order that realistic risk assessments can be performed.

The properties or attributes of the so-called 'difficult substances' have been analysed in relation to the problems arising from the assessment of the effects and/or exposure. Pragmatic guidance is presented to address these issues, so that more realistic assessments can be conducted. This report was produced with the intention that it should be a contribution to the revision of the EC Technical Guidance Document.

Properties and/or attributes that were specifically considered for this report are:

- Unstable and highly reactive;
- poorly water soluble;
- sorptive;
- surface active;
- volatile;
- naturally occurring, essential and multi-ionic elements.

It is clear that some substances may display more than one of these characteristics and thus, no single property should be considered in isolation. Therefore, a holistic approach should be applied when conducting a risk assessment for a 'difficult substance', taking into account the influence of all properties or attributes that can complicate the exercise.

1. INTRODUCTION

Environmental risk assessment in the European Union (EU) is required for both 'new' and 'existing' substances according to EC Directive 93/67/EC (EC, 1993) and Commission Regulation (EC) No. 1488/94 respectively (EC, 1994). In addition under a proposal in the EC White Paper entitled 'Strategy for a Future Chemicals Policy' (EC, 2001), a stringent time line is proposed for risk assessment of all chemicals marketed in quantities greater than one tonne per annum.

A satisfactory outcome of all such risk assessments will require that the ratio of the Predicted Environmental Concentration (PEC) and the Predicted No Effect Concentration (PNEC), that is referred to as the Risk Characterisation Ratio (RCR), be less than 1.0. Details on how to derive PECs and PNECs are given in the EC Technical Guidance Document (TGD) (EC, 1996) and by van Leeuwen and Hermens (1996). In view of the risk management implications of a RCR greater than 1.0, it is important that the environmental risk assessment process should be appropriate for all types of substances. It was first recognised in 1993 (Whitehouse and Mallett, 1993), that some substances, which were termed 'difficult substances', presented problems for aquatic toxicity testing, and thus for their classification and labelling. Substances falling in this category included those which are:

- Sparingly water soluble;
- volatile;
- adsorptive;
- unstable;
- complex mixtures.

More detailed guidance on the subject was published by ECETOC (1996) and this later formed the basis of an official OECD guidance document (OECD, 2000a).

The term 'difficult substance' has been applied to a substance that is difficult to test using standard internationally recognised protocols. For the purposes of this report, it also refers to substances for which, because of their properties, the conventional TGD approach does not generate environmentally realistic PEC values. This may therefore have a significant impact on the RCR. The Task Force has also extended the term 'difficult substance' to include naturally occurring, essential and multi-ionic state elements, where it is recognised that the current TGD is not appropriate. Given the objective of the Task Force to provide timely guidance relevant to the TGD revision process, the scope of this report was confined to addressing key difficulties that have arisen in applying past TGD guidance to substances that have undergone risk assessment in the EU. As a result, only a cursory discussion is provided on potential challenges arising in risk assessment of complex mixtures (c.f section 3.4). Moreover, complications that occur for substances that give rise to persistent metabolites were also excluded from the scope of the present report. However, recent perspectives on this latter topic are provided by ECETOC (2003) and Fenner *et al* (2002).

The EU risk assessment model (EUSES, 1997) relies heavily on partitioning coefficients between the various environmental compartments, for example:

- Air - water;
- air - aerosol;
- water - suspended matter;
- water - sewage sludge;
- water - sediment;
- water - soil.

Together with measured or, more frequently, predicted data on degradation processes such as:

- Photodegradation in the atmosphere (OH radicals or ozone) and in water;
- biodegradation in surface water, sediment and soil;
- chemical transformation/reaction.

This information is used as inputs in multimedia box models (Mackay, 1991) to derive the PEC. For most substances, measurements on partition coefficients are not available and estimation methods based on fragment methods are often used (Lyman *et al*, 1990; Hansch and Leo, 1995; Boethling and Mackay, 2000). Similarly, data are often lacking on degradation rate constants, and estimations using models have to be made of the distribution of the substance in the environment, from a few substance-specific data including, for example:

- Molecular weight (mol wt);
- water solubility;
- vapour pressure;
- octanol - water partition coefficient.

If a substance has properties that do not allow the reliable estimation of partition coefficients or environmental degradation rate constants, the models may fail to predict realistic environmental concentrations.

Failure to take account of these unique properties may similarly influence the PNEC, since aspects such as test design and interpretation, or bioavailability, are not taken into consideration.

An indication of the need for a revised approach to risk assessment of substances with difficult properties is illustrated by a review of the 4th EU priority list of chemicals for risk assessment, which indicates that at least half of the substances assessed to date have one or more of these properties (see Appendix I).

With these considerations in mind, the objectives of this report are to:

- Clarify how the properties of a difficult substance impact environmental risk assessment;
- describe how these properties influence PEC, PNEC or both;
- illustrate the problem with the help of case studies;
- make reference to published literature on existing methodology;
- where necessary, make proposals for improved methodology;
- provide decision making schemes and guidance.

2. UNSTABLE SUBSTANCES

In conducting an environmental risk assessment of an unstable substance, difficulties arise because intermediates and degradation products that are formed require additional specific consideration in addition to the parent substance. The aquatic effects testing that forms the basis for risk assessment relies on experiments designed primarily for stable chemicals and does not necessarily include consideration of intermediates or degradation products. A consequence of the possible accumulation of intermediates in the test systems is that the results obtained may be difficult to interpret, particularly in terms of quantitative risk assessment.

This section deals with the testing and risk assessment of substances that are unstable in experimental test systems and in the environment, with particular emphasis on those substances that degrade rapidly in the aquatic compartment via abiotic or biotic mechanisms (e.g. hydrolysis, oxidation, photolysis, biodegradation).

2.1 Property description

2.1.1 Hydrolysis

Hydrolysis is one of the most common degradation pathways in the environment. Hydrolysis refers to a reaction of a chemical with water, with a net exchange of the group X with OH at the reaction centre:

$$RX + H_2O \longrightarrow ROH + HX$$

There are many functional groups that undergo significant hydrolysis in aquatic test media; typical examples are carboxylic acid esters, amides, carbamates, phosphoric acid esters, halogenated alkanes and epoxides.

Hydrolysis is commonly catalysed by hydrogen ions or hydroxide ions. Therefore, the rate of hydrolysis depends directly on pH. With the introduction of the hydroxyl group, additional polar products are formed, which are more water soluble and less lipophilic than the parent compound.

Hydrolysis kinetic rates are usually determined experimentally according to OECD guideline No. 111 (OECD, 1981a). This test guideline is applicable predominantly for soluble substances. Quantitative structure activity relationship (QSAR) models for hydrolysis kinetic rate are described in the literature for some specific chemical classes.

2.1.2 Photodegradation

Photodegradation refers to the reaction of a chemical after the absorption of (sun) light leads to an electronically excited state with increased reactivity and subsequent transformation. Two different types of photodegradation can occur:

Direct photodegradation

Direct photodegradation involves the transformation of a chemical resulting from the direct absorption from solar photons; the parent chemical degrades either directly or via short-lived reactive intermediates (radicals) which result in stable degradation products.

Indirect photodegradation

Indirect photodegradation refers to the transformation of a chemical due to the transfer of excess energy from another photosensitive molecule. This involves electronically excited triplet states and reactions with transient oxidants such as hydroxyl radicals, singlet oxygen, and peroxy radicals resulting from the absorption of solar photons by chromophoric dissolved organic matter and nitrate ions.

Since the rates of all photochemical reactions are proportional to light intensity, the rate of photodegradation will be influenced by factors such as time of the day or year, climatic conditions and the weather. In the aquatic environment, an important fraction of sunlight is absorbed by dissolved and particulate organic matter, influencing photodegradation rates in relation to the depth in water.

Kinetic photodegradation rates in aqueous media may be determined according to a proposed new OECD draft guideline (OECD, 2000b; ECETOC, 1984). Currently, there are no valid QSAR methods for either direct or indirect photodegradation in the aquatic medium.

2.1.3 Biodegradation

Microbial degradation can present serious challenges in aquatic toxicity testing due to the reduction in aqueous exposure concentrations. Often this problem can be overcome by static renewal or flow-through test designs. However, in the case of poorly water-soluble substances where test substances may be at or near the aqueous solubility limit, the rate at which test substance is biodegraded may exceed the rate at which the test substance can practically be re-supplied to the aqueous test media. As a consequence, aqueous concentrations can decline significantly during the test, thereby confounding interpretation. This is a problem particularly in chronic tests where test organisms are fed, as the addition of food provides an excellent carbon source for promoting the proliferation of microbes in the test system.

2.1.4 Other causes of instability

There may be other causes of instability in the aquatic environment for specific types of chemicals, e.g. oxidation/reduction, polymerisation, reaction of oxidisers with other materials, in addition to degradation by the test organisms.

Oxidation in water has not been described in the literature as extensively as hydrolysis. Functional groups susceptible to oxidation include phosphines, alkylated phenols, aromatic diols, metals in low oxidation states and aldehydes. Testing the rate of oxidation follows the same principles as those for hydrolysis, but with the use of constantly aerated solutions.

Polymerisation may be a relevant process only for a limited number of substances (e.g. alkoxysilanes and isocyanates), and is therefore not covered in this report.

It is thought that the general principles and proposals presented are also applicable for chemicals susceptible to other potential modes of instability. However, specific problems associated with such groups of chemicals may need consideration on a case-by-case basis.

2.1.5 Current testing and risk assessment procedure of unstable substances

2.1.5.1 OECD / EC Testing Guidelines / OECD Guidance Document on Testing

Current OECD / EC ecotoxicity testing guidelines do not provide specific guidance on the testing of unstable substances. In toxicity tests, maintenance of 80% of initial test concentrations is required whenever possible. The problems associated with maintaining test concentrations have been recognised by different authors and numerous strategies have been proposed, (ECETOC, 1996; UK DOE, 1996). These include semi-static or flow-through testing, minimising media preparation time, or in the case of photo-labile substances by testing, where feasible, with red light or in the dark.

Aquatic toxicity testing of unstable substances is specifically covered in a OECD Guidance Document (OECD, 2000a). A preliminary stability study in water is usually recommended under simulated test conditions, but in the absence of the test organisms. If losses are due to the inherent instability of the test article, the protocol of the final study should be modified in order to maintain exposure to the parent compound, or, if the degradation is too rapid, decision parameters are proposed as to whether to test the parent substance and/or its degradation products.

For substances that photolyse, working in a darkened environment or using red light is recommended in short-term fish and Daphnia studies. For algae studies, three strategies are proposed: selective removal of wavelengths to reduce photolysis but still enabling photosynthesis of algae, testing by using dark/light cycles, or testing of the product after pre-illumination. Despite the inherent problems of testing unstable substances in algae studies, no clear decision criteria and guidance on how to use the results for hazard/risk assessment are given.

For substances that hydrolyse, exposure concentration of the parent substance should be maximised by keeping duration of media preparation to a minimum and by adjusting pH and temperature within the range permitted in the test systems.

2.1.5.2 Risk assessment according to the Technical Guidance Document (TGD)

Risk assessment according to the TGD (EC, 1996) focuses primarily on the parent compound. Nevertheless, it is stated that "if stable degradation products are formed, these should be assessed as well". For chemicals that hydrolyse or photodegrade with a half-life <12 hours, potential environmental effects are normally attributed to degradation products rather than to the parent substances. Substances with a parent half-life <12 hours are expected to have aqueous exposure concentrations reduced by more than an order of magnitude during a standard 48-h acute aquatic toxicity test. Consequently, for such short-lived substances, assessment of parent toxicity using standard toxicity test guidelines is not appropriate.

The current TGD stipulates that both parent compound and its degradation products should be studied. The difficulties that this can present are discussed in section 2.1.5.

In the generic scenario for PEC_{local} calculation, instability due to hydrolysis or photolysis is currently not considered. Only if there are specific environmental monitoring data available, can this be incorporated into the risk assessment. On a regional and continental scale, abiotic and biotic half-lives of chemicals are converted to pseudo first-order rate constants, using the following equation:

$$k_x = \frac{ln2}{DT_{50}}$$

This can then be used as valuable input into multimedia environmental fate models.

Hydrolysis

For risk assessment purposes, a pH of 7 and a temperature of 285°K, which conform to the standard environment conditions, are normally used for generic calculations. Where the use of an alternative pH would affect the environmental distribution and toxicity significantly, this should be included in the risk assessment.

Photolysis

Due to large seasonal variations in light flux, only average values for photochemical reactions are used in the generic calculations. Methods to calculate these are described by Zepp and Cline (1977), but are not included in the EC modelling approach. Indirect photochemical reactions are included in the overall degradation rate in aquatic media, only if there is clear evidence that this is a significant pathway in comparison to other processes. For facilitating the complex calculation of phototransformation processes in natural waters, computer programmes have been developed and may be applied in addition (e.g. Frank and Klöppfer, 1989).

2.1.6 Case studies

2.1.6.1 Existing chemicals: risk assessment of dimethyl sulphate

- Dimethyl sulphate is an example of an Existing Chemical for which a risk assessment has been performed by a Competent Authority under the Existing Chemicals Work Programme and the framework set out in Commission Regulation (EC) 1488/94 (TNO, 1999);
- dimethyl sulphate hydrolyses rapidly in water leading to methanol/sulphuric acid under neutral or acidic conditions and to methanol/monomethyl sulphate under basic conditions with a DT_{50} in water of <1 day. Ecotoxicity data were predominantly generated on the parent compound, which was supposed to display the principal aquatic hazard;
- the PNEC for dimethyl sulphate (14 μg/l) in the aquatic compartment was based on the lowest value derived from acute toxicity tests. Due to the rapid degradation of the parent compound also occurring during ecotoxicity testing, nominal concentrations only were used in effects assessment, and no account was taken of the decrease of parent and accumulating degradation products in the test systems;
- the hydrolysis products were not considered to be of particular concern for the environment due to the known low environmental exposure and supposed toxicity. Nevertheless, a full base set of data is lacking for these entities. The PNEC evaluation for the hydrolysis products, based on the only available study, resulted in a figure of >10 mg/l for monomethyl sulphate and 630 μg/l for sodium sulphate;
- due to control measures in force during manufacturing/application of dimethyl sulphate, releases of the parent substance were considered insignificant, and thus, PECs were calculated only for the two major hydrolysis products, i.e. monomethyl sulphate and sodium sulphate. A risk characterisation was therefore conducted for these substances only;
- the risk assessment of dimethyl sulphate gives an example of a substance that rapidly degrades in the environment and also during testing. In the ecotoxicity studies conducted, this fact has not been adequately considered. However, due to its short half-life, and the control measures applied during its use, there are no significant releases of the parent compound to the environment. Significant exposure is only to be expected to the degradation products. As the main focus has been on testing of the parent substance, only few ecotoxicological data on the degradation products were available for risk assessment purposes.

2.1.6.2 Existing chemicals: hydrogen peroxide (based on: Cefic Peroxygene Sector Group, 1997).

- The industrial use for hydrogen peroxide is mainly in the production of chemicals, for bleaching of cellulose pulp and textiles and for other purposes such as wastewater treatment. Hydrogen peroxide also occurs naturally as a consequence of physiological and photochemical processes. In water and soil, it may also be formed by oxidation of iron and copper ions. Hydrogen peroxide is also naturally produced in water and soil, the amount depending on light intensity, the presence and concentration of catalysts, and dissolved oxygen. Natural hydrogen peroxide concentrations in sea and fresh water range from 0.3-30 μg/l. Decomposition in water and soil takes from minutes to several hours, depending on the concentration of microorganisms and oxygen in the water and the mineral content. Under experimental test conditions with clean water and culture media, hydrogen peroxide

is relatively stable, but in the presence of organic matter it degrades rapidly. Furthermore, it is biologically degradable. In the water of the river Saone, the half-life was determined to be 2-20 days depending on the initial concentrations. In eutrophic lakes, the half-life is even shorter. Thus, under typical environmental conditions, a half-life of 1-3 days may be regarded as a representative annual average. Under test conditions, concentrations can be maintained satisfactorily, although depending on the type of test, achieving 80% of the nominal concentration is not always possible, for example in algal studies, where the high intensity light catalyses the degradation.

- Hydrogen peroxide is a strong oxidant and, because of its high reactivity, only causes local toxicity to organisms. Most aerobic species have defence mechanisms allowing them to inactivate hydrogen peroxide and other reactive oxygen species. In addition, under environmental conditions, many natural substances such as metals and dissolved organic carbon can minimise the oxidising potential of hydrogen peroxide. Acute (L/EC_{50}) values for fish, Daphnia and algae are 30-42 mg/l, 7.7-15 mg/l and 1.6-4.3 mg/l respectively. In addition, no effects have been observed on zebra mussels with concentrations up to 2 mg/l after 56 days exposure.
- Thus, despite the instability of hydrogen peroxide, test concentrations can be maintained sufficiently to permit testing, and the risk assessment to be conducted using the ecotoxicity studies available on the designated three trophic levels and applying the current guidance procedures. As the degradation products are of no concern, no specific consideration is deemed necessary.

2.1.6.3 Existing chemicals: Fluorescent whitening agents (FWAs); photoisomerisation / photodegradation (based on Kramer 1996; Kramer *et al* 1996; Stoll, 1997; Poiger, 1994)

- FWAs are widely used in detergents, paper and textiles and consequently are distributed ubiquitously in the environment. The most important worldwide FWAs are the stilbenic-type, which are produced only as the fluorescent trans-isomer. Based on the standard OECD test methods (e.g. 301 series), FWAs are not regarded as "readily biodegradable". When the FWAs are exposed to sunlight, the first step in the degradation process is photoisomerisation. Experimental studies demonstrate that on exposure to sunlight, FWAs dissolved in water are converted within minutes from the trans-isomer into the non-fluorescent cis-isomer, significant concentrations of which may already be present in the influent of a wastewater treatment plant (WWTP). As the physico-chemical properties of FWAs vary depending on their isomeric form, this will influence their behaviour in WWTPs. During wastewater treatment, the concentration of the cis-isomer increases due to further photoisomerisation of the trans-isomer combined with and adsorbed onto the sludge. The different adsorption properties of the cis- and the trans-isomer favour a higher relative concentration of the cis-isomer compared to the trans-isomer during the later stages of the treatment. Activated sludge is stabilised and may be incinerated or used as fertiliser. Incineration of FWAs yields CO_2, H_2O, SO_2 and some NO_x. The fate of FWAs in soil is presently being monitored under a contract with EAWAG Dübendorf (Switzerland); final results will be available 2003.
- In a second step, FWAs undergo photodegradation. This has been shown to be significant in the photic zones of lakes and rivers. The kinetic data of the photodegradation step are well known, and enable a prediction to be made of photolysis under various light conditions. Depending on the chemical type, the resulting metabolites may be biodegradable. Thus some FWAs show photodegradation followed by biodegradation of the metabolites, thereby achieving > 70% (DOC) within 28 days.

- The photodegradation of FWAs also affects the determination of the PNEC of the parent compound. For studies where luminescence is required, a significant proportion of the FWAs will be transformed due to photoisomerisation and photodegradation. As some of the subsequent degradation products are biodegradable, this will have a marked influence on the test results.
- Unfortunately, clear guidelines for a classification of the combination of abiotic / biotic degradability are still lacking. These findings contribute to the scientific basis that will enable an amendment or a precise definition of this classification. Such an approach supports the assessment that FWAs which show abiotic/biotic degradation of >70% (DOC) within 28 days, are ready biodegradable.
- FWAs represent compounds that undergo rapid photoisomerisation under light conditions. It is therefore only possible to test the parent substance in those ecotoxicological studies not requiring illumination. In the algae study, only photoisomers can be tested. For a long-term risk assessment, additional considerations on the degradation products might be envisaged. However, as degradation products are biodegradable, they might be of no specific concern.

2.1.6.4 New chemicals: photodegradable substance

The data were generated for a 'full notification' of α-hydroxyketone photoinitiator of proprietary chemical structure, used for UV catalysis of polymer coatings,

Stability in Water

Biodegradation:	not readily biodegradable (<10%, 28 days)
Hydrolysis in algae medium:	stable (half-life > 1 year at different pH)

Ecotoxicity

Fish, LC_{50}, 96 hours:	>100 mg/l under light/dark cycle
Daphnia, EC_{50}, 48 hours:	>100 mg/l light/dark cycle
Algae EbC_{50}, 72 hours:	9 mg/l nominal, degraded completely

Risk Assessment

- For notification purposes the ecotoxicity studies were performed under standard light conditions. The substance proved essentially non-toxic in short-term daphnia and fish studies. The PNEC was derived based on the results of the most sensitive species, i.e. algae, with an EC_{50} of 9 mg/l based on measured concentrations at time zero. Complete photolytic degradation was observed during the course of the test. Since the test substance is not readily biodegradable in the Modified Sturm test, the default TGD model assumes an insignificant degradation rate for the local risk assessment. As a result, the full risk assessment indicates concern on the local scale.
- Subsequently, stability studies were performed both in sunlight and under standard algal study conditions. After illumination, two major stable degradation products were identified in algal medium. Based on their chemical structure, they are predicted to be at least inherently biodegradable. Two short-term toxicity algal studies were also performed with and without a pre-illumination step prior to the standard test. The results of these studies were:

Stability in water

Photodegradation half-life:	3-10 min	pre-illumination of test medium
	7 hours	standard algal study light conditions (300-800 nm, 400-765 W/m^2, 7'700 Lux)

Ecotoxicity

- Algae EbC_{50} 72 hours: 35 mg/l after 24-h pre-illumination in medium;
- full recovery of algae after 120 hours illumination;
- it is apparent that the parent compound is very unstable in the presence of sunlight. Therefore, its direct effect could be investigated only in fish and daphnia and not in algae. No ecotoxicological hazard potential of the parent compound itself was identified towards fish, daphnia and bacteria. Due to its inherent instability in the presence of light, it is proposed that the transient photodegradation intermediates are responsible for the algaestatic effect as a consequence of their inherent reactivity. Any toxic effects on algae are transient in nature and decline if pre-illumination occurs. Therefore, and due to the highly transient nature, these intermediates are not considered to present a long-term risk to the aquatic environment.

2.2 Environmental risk assessment

2.2.1 Environmental effects assessment

There are two main problems associated with the testing and derivation of PNECs for unstable chemicals:

Firstly, unstable chemicals may degrade during testing. Maintenance of the concentration as required in the guidelines can be almost impossible if degradation is very rapid or where semi-static or flow-through testing is not possible (e.g. in an algae study). A strong reduction of parent substance concentration during testing may lead to increased uncertainty or an error in a quantitative risk assessment, since the (no)-effect concentrations cannot be directly related to the concentration of test substance.

Secondly, degradation of a substance during testing may lead to the formation of stable degradation products, whose toxicity may differ from those of the parent substance, and which may have varying residence times in the environment. As a consequence, a main challenge with regard to risk assessment of unstable chemicals is to relate toxicity quantitatively to the different entities generated in the course of degradation, i.e. parent substance versus stable degradation product(s) if formed. Additionally, degradation of the parent substance could impose on the test organisms secondary effects that need specific consideration, e.g. effects arising from salt formation or changes in the test medium such as oxygen depletion and pH fluctuation.

2.2.2 Environmental exposure assessment

The most critical factor in the derivation of local, regional and continental PECs for a chemical is the identification and quantitative assessment of the different chemical entities generated during the potential degradation process, particularly as these are often more water soluble than the parent material. Since the toxicity and the half-life of the parent, intermediate and degradation products may vary significantly, careful consideration must be given to their respective relevance for the environment.

Instability is a factor considered for the $PEC_{regional}$ estimation, where multimedia models are applied. However, it is not considered in the calculation of PEC_{local} for the aquatic compartment, due to the short distance between the point of effluent discharge and the exposure location. Even in the sewage treatment plant (STP) model, no mode of degradation other than biodegradation is considered. Although the default residence time in STPs is 6 hours, in reality it can be up to 32 hours in industrial plants. At the local scale, this may result in an unrealistic focus on parent compound toxicity, by disregarding potential degradation products, especially where measured environmental concentration data are lacking.

2.3 Recommendations

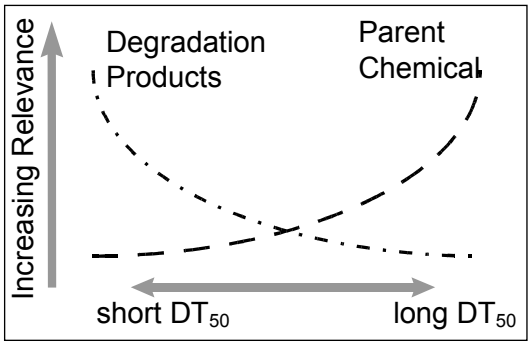
2.3.1 Recommendations for improving effects assessment

Based on known information on a substance, particularly its physico-chemical properties and the behaviour of structurally related compounds, a stability test may be necessary prior to eco-toxicity testing. Before initiating full ecotoxicity tests for any substance that is predicted or known to degrade during testing, the determination of degradation rate under the respective test conditions, or according to specific guidelines, with appropriate analytical support, is recommended in accordance with the OECD guidance document on testing of difficult substances (OECD, 2000a). Maintenance of test concentrations is crucial for the assessment of continuous exposure, which could occur locally at point emission sources.

For very rapidly degrading substances, compliance with current guidelines may not always be possible, e.g. in the algae study, as semi-static or flow-through testing is not feasible, and results generated are considered of limited value with regard to risk assessment. The following example may serve as an illustration: a substance degrading with a half-life of 6 hours tested in an algal study will be at one thousandth of the nominal test concentration at the end of the study but there may be a concomitant increase in degradation products. Data generated under conditions of rapid degradation may be appropriate for hazard assessment but of little value for risk assessment, since the results can be related neither to a chemical entity, nor to a specific test substance concentration. Some of these problems arising specifically in the algae study, could in principle be circumvented by using semistatic technique proposed by Radetski (1995), by using repeated spiking, or by testing higher plant species (US-EPA, 1996). The interpretation of test results for risk assessment purposes generated under these conditions might be difficult. Since such test systems have not yet been fully validated, they cannot be generally recommended.

In the case of certain substances (e.g. readily biodegradable substances), unstable degradation products are not expected to occur. However, in other cases stable degradation products may result, and warrant independent assessment. A well-known example is nonylphenol ethoxylates. As outlined in Figure 1, it may be postulated that the overall relevance of a chemical depends strongly on its residence time in the environment, being directly influenced both by abiotic and biotic degradation rates. For highly unstable substances, parent environmental toxicity could lead to local adverse effects only, due to the rapid dissipation of parent chemical and concomitant generation of degradation products. For a risk assessment of such a highly unstable chemical, excluding accidents and incident situations, main emphasis could therefore be put on degradation products. For chemicals reacting spontaneously with water (e.g. certain acid chlorides or isocyanates) testing of the parent chemical may not be possible except as its decomposition products. On the other hand inherent stability leads, at least on a local scale, to the emphasis being mainly on parent chemical. Between these two extreme situations, the relevance would shift from parent chemical to stable degradation products or vice versa.

Figure 1: Illustration of relationship between parent chemical degradation products and DT_{50}



Based on these considerations, common sense should be used to choose a pragmatic testing and risk assessment approach for those specific cases where the degradation half-life of a chemical is very short and compliance with testing guidelines is not possible. A specific framework for testing and risk assessment is recommended and outlined in the scheme below.

Substances degrading during testing

If a substance is highly unstable, and maintenance of test concentrations by secondary measures, as recommended in the OECD guidance document (OECD, 2000a), is not possible within appropriate limits, the degradation half-life in the test system may serve as criteria to inform decisions for further testing and subsequent risk assessment.

For such substances, testing may be achieved by degradation of the test substance over a certain period of time (e.g. six times its instability half-life), and exposing the test organisms to its breakdown products. Subsequently, a targeted risk assessment is performed with the main emphasis on the degradation products.

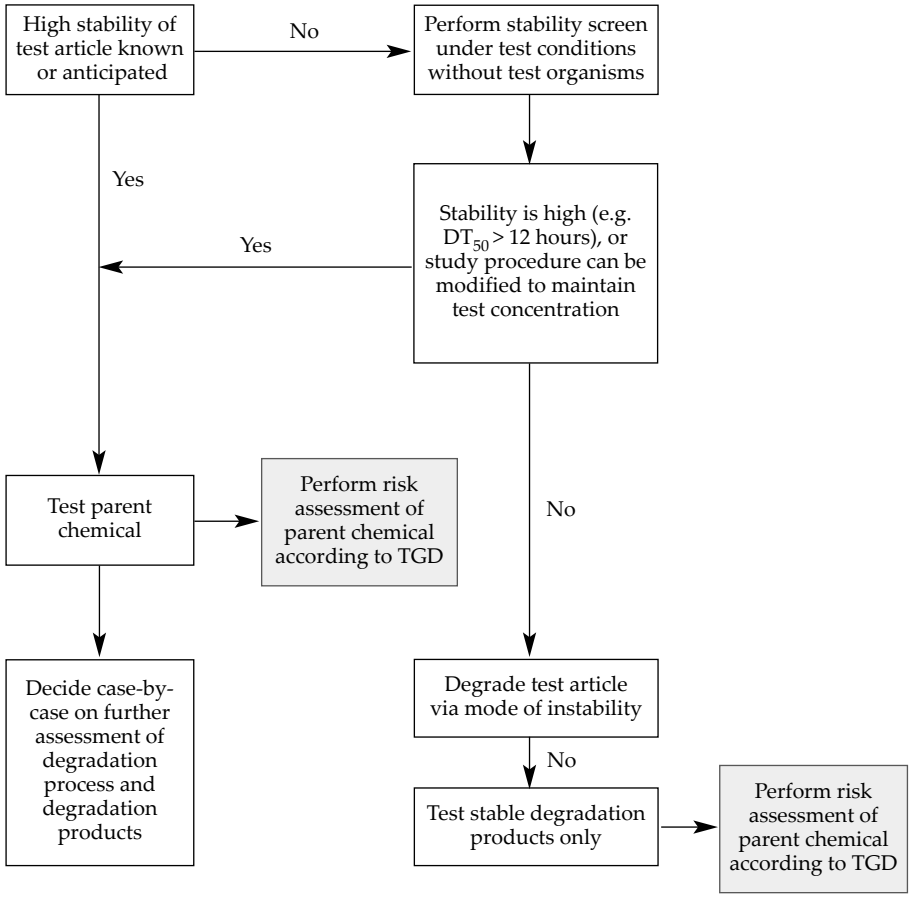
A distinct threshold might be defined as decision criteria for priority setting of testing and risk assessment. A DT_{50} of approximately 12 hours is advocated, reflecting a point where approximately 80% of the nominal concentration can be maintained in a flow-through test, and >1% of nominal concentration in a static short-term ecotoxicity test. A DT_{50} of 12 hours is also supported in the current TGD, where it is stated that for substances with a DT_{50} <12 hours, environmental effects are likely to be attributed to degradation products, rather than to the parent substance itself.

Substances stable during testing

For substances with reasonable stability in the test systems (i.e. DT_{50} >12 hours) or where study procedure can be modified in order to maintain the test concentration satisfactorily, it is reasonable to assume that the results from ecotoxicity testing are directly applicable in the risk assessment process related to continuous exposure.

For substances with reasonable stability in the test systems, but decomposing at an intermediate rate, it should be decided on a case-by-case basis whether potential degradation products are to be included in a full risk assessment, and whether its mode of instability, the kinetics, and the ecotoxicity hazard of resulting degradation products require a further specific evaluation on a regional and continental scale. Such decisions may be based on a tiered approach; considering the tonnage of the substance, the potential that degradation products may exert significant toxicity (known from specific testing or expected from QSAR) or other relevant properties of specific concern (stability, bioaccumulation potential). The principles described in the TGD are considered appropriate for this process. The recommendations are summarised in Figure 2.

Figure 2: Proposed scheme for ecotoxicity testing and risk assessment of unstable substances



2.3.2 Recommendations for improving exposure assessment

With respect to the PEC, it is recommended that for the PEC_{local}, instability should be included in the modelling process for those highly unstable substances (e.g. DT_{50}< 4 hours) where the reduction of the concentration in the aquatic compartment at a local scale is considered to be significant.

Given large uncertainties in applying default TGD procedures and EUSES model algorithms to unstable substances, field measurements may serve as an alternative for exposure assessment. General guidance on the design and conduct of field monitoring studies has been provided by ECETOC (1999).

3. POORLY WATER SOLUBLE SUBSTANCES

3.1 Property description

Poorly water-soluble substances (PWSSs) have been defined as substances with a limit of water solubility below 100 mg/l (OECD, 2000a). This definition reflects the upper concentration used in standard tests to assess ready biodegradability, as well as the upper limit of aquatic toxicity used for environmental classification of substances in the European Union (EC, 1988). However from a risk assessment perspective, practical difficulties are encountered typically for substances with water solubility below 1 mg/l, which is consistent with the definition used in the TGD (EC, 1996). Nevertheless, many existing and new substances fall within this narrower definition. Examples of commercially important PWSS include chlorinated paraffins, brominated flame retardants, hydrophobic dyes, phthalate plasticisers, methylsiloxanes, triaryl phosphates, nonionic detergents, fatty amines and alcohols, liquid crystals, as well as many hydrocarbon solvents.

PWSS can be divided into two general classes:

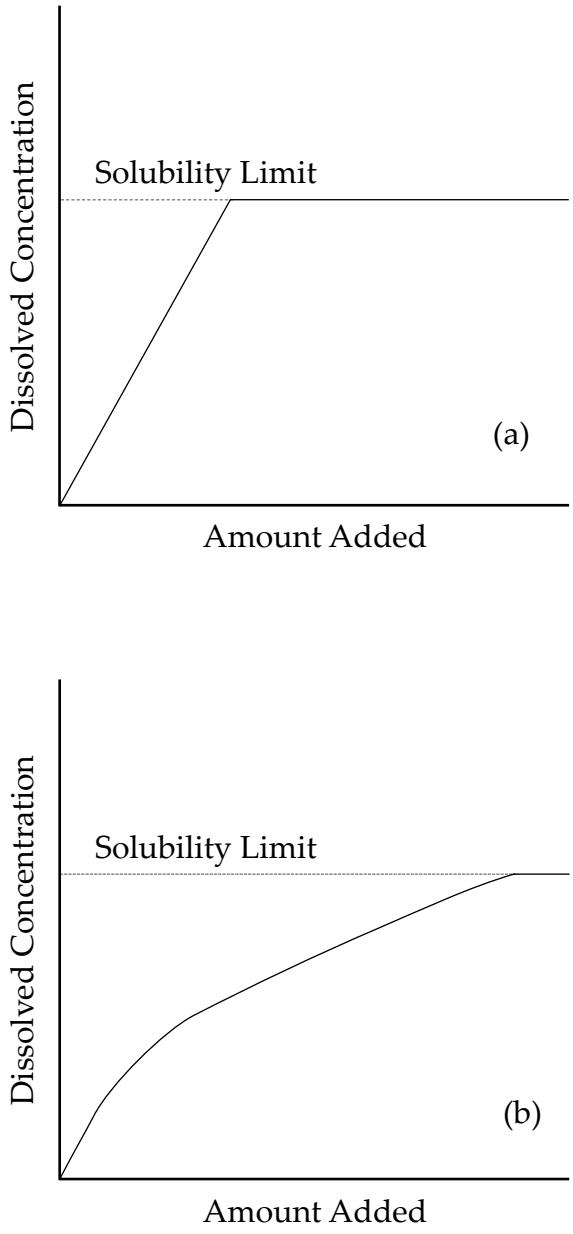
- Single component or simple multi-component mixtures;
- complex multi-component mixtures.

For the purpose of this report, simple multi-component mixtures refer to substances that are comprised of components that are structurally-related, and do not show a wide range of physico-chemical properties (e.g. water solubility). For example, a commercial product (e.g. di-isononyl phthalate) comprised of various isomers having a similar alkyl chain length but differing primarily in branching pattern, can be considered to be a simple PWSS, despite the structural diversity of the isomeric constituents. In contrast, complex multi-component mixtures refer to substances with components that exhibit large differences in physico-chemical properties. Many petroleum substances (e.g. gasoline) fall into this second category. Substances included in this class would be considered PWS if the water solubility of at least one component was below 1 mg/l.

The significance of dividing PWSSs into these two groups can be understood better by considering the different behaviour when added to water (see Figure 3). As the amount of a pure (or simple multi-component) substance is added to water, the aqueous concentration increases linearly until the solubility limit is attained. Above this point, further addition of test substance results in a two-phase system, and no further increase in the truly dissolved concentration is observed. Now consider the same situation with a complex multi-component PWSS. Initially, as test substance is added in amounts below the solubility limit of the least soluble component, the aqueous concentration increases proportionally, in a manner similar to the pure substance. However, as the solubility limit of the least soluble component is reached, only the more soluble components continue to dissolve, and a two-phase system forms. Further addition of the test substance results in an aqueous concentration that is a non-linear function of the amount added. Moreover, the relative composition of the aqueous phase no longer remains constant as a function of the amount added.

At low loading (mass of substances per volume water) the relative importance of the less water soluble components dominate. In contrast, at high loading the more water soluble components become increasingly important. This fundamental difference in the aqueous partitioning behaviour of these two classes of PWSS has important implications for risk assessment. As a result the limitations of current technical guidance for performing risk assessments and suggested future methodology improvements are discussed separately for each PWSS class.

Figure 3: Behaviour of a simple (a) and a complex multi-component (b) poorly water soluble substance when added to water



3.2 Environmental risk assessment - nonionic organic substances

The current EC risk assessment is based on a risk quotient approach in which the PNEC, derived from ecotoxicity tests, is compared to the PEC, that is derived from joint consideration of exposure modelling calculations and actual field measurements (EC, 1996). The limitations of applying this approach in both the effect and exposure assessment of simple PWSSs are described. Practical recommendations for improving current approaches/methodologies are also discussed.

3.2.1 Limitations in effects assessment

The difficulty in applying the current guidance for conducting effect assessments for simple PWSSs involves both practical testing issues, as well as concerns regarding test interpretation that may lead to the potential for false positive and false negative conclusions.

3.2.1.1 Experimental design of aquatic toxicity tests

From a methodology perspective, the key criteria for performing aquatic toxicity tests with a PWSS are:

- Expose test organisms to aqueous concentrations as close as possible to the water solubility limit that reflects only truly dissolved test substance;
- analytically confirm the low exposure concentrations tested;
- maintain aqueous concentrations reasonably constant over the exposure period.

The first challenge requires a reliable estimate of the water solubility to be available. Cousins and Mackay (2000) have outlined an approach for assessing the reliability of experimental water solubility, and other physico-chemical measurements, for a given substance using quantitative structure property relationships (QSPRs). If experimental data are not available, QSPRs can be used to estimate water solubility based on theory (Karickhoff *et al*, 1991) or semi-empirical correlations (Meylan and Howard, 1995). A limited comparison of model predictions, with reliably-determined experimental measurements, is provided in Table 2 for selected simple PWSS. This preliminary analysis suggests that model predictions are approximately equal to, or greater than, measured values. Thus, QSPR models may provide a logical first step in assessing available data, or deciding if an experimental measurement of water solubility is possible, given the limits for analytical detection of the substance.

For solids, a generator column is usually the preferred approach for measuring aqueous solubility (Billington *et al*, 1988). For liquids, a slow-stir technique is preferred (Ellington, 1999; Varaprath *et al*, 1996). Further experience in applying this method with biodegradable test substances indicates that the aqueous test system should be poisoned to prevent the water solubility from being underestimated as a result of biotic loss processes during the equilibration period (Letinski *et al*, 1999). If it is determined that the aqueous solubility of the PWSS cannot be quantified due to a lack of analytical sensitivity, it is obvious that it will not be possible analytically to confirm aqueous concentrations in an aquatic toxicity test.

However, even if it is possible to confirm analytically aqueous concentrations at the solubility limit of the PWSS, it may not be possible to maintain low aqueous concentrations sufficiently constant during a toxicity test, due to abiotic and biotic loss processes. This is especially true in the conduct of chronic toxicity studies with readily biodegradable PWSSs since the addition of food often promotes bacterial growth and can result in significant loss of test substance even under flow-through conditions, or in algal tests that are conducted under static conditions. Recognising such concerns, OECD (2000a) recommended that a preliminary assessment of the stability of the test substance be performed. This test should be performed in the test medium and at the test temperature used in the aquatic toxicity test. For nonpolar organic chemicals, lower temperature and higher ionic strength of dilution water is expected to lower, by a factor of two to four, the solubility that can be achieved relative to that obtained in distilled water at 25º C.

Several methods have been used for introducing PWSSs into aqueous media for aquatic toxicity testing which include:

- Direct addition (no stirring);
- mechanical mixing (slow-stirring; vigorous-stirring);
- heating;
- sonication;
- water miscible co-solvents (e.g. acetone);
- dispersants (e.g. Tween 80);
- generator column systems.

The thermodynamic basis of various methods used to facilitate dissolution of PWSSs into aqueous test media, has been reviewed by Sijm (1996). Practical guidance in applying the various methods are also provided by Bowmer and Hooftman (1995), ECETOC (1996) and OECD (2000a). One important generalisation from these reviews is that presence of undissolved test material should be avoided. For this reason, the use of dispersants and vigorous mechanical mixing that promote emulsion formation is not recommended. Co-solvents, if used, must be mixed homogeneously with water to prevent local supersaturation. Furthermore, due to potential interactions with the test substance, and influence on accumulation by test organisms, use of co-solvents is generally recommended only for hydrolytically unstable and highly viscous PWSSs (OECD, 2000a). Recent research on passive generator systems (partition driven administration systems) seems to offer the most technically promising and cost effective strategy for maintaining constant concentrations of truly dissolved PWSSs in aquatic tests (Urrestarazu Ramos *et al*, 1997; Mayer *et al*, 1999; Mayer, 2000).

3.2.1.2 Interpretation of aquatic toxicity tests

Historically the interpretation of aquatic toxicity test results with PWSSs has been difficult for two principle reasons. First, early toxicity tests, that were often conducted with emulsions of PWSSs at concentrations that were orders of magnitude higher than the true aqueous solubility, were sometimes found to harm test organisms. Several possible explanations to account for such findings have been discussed by ECETOC (1996).

The most common explanation is direct physical impairment of test organisms, caused when substances are tested well in excess of their true aqueous solubility. This problem seems to occur most frequently for hydrophobic liquids in tests with *Daphnia*, unfortunately, one of the most commonly tested aquatic species. For example, irreproducible effects on *Daphnia* have been reported for di-2-ethylhexyl phthalate and polydimethylsiloxane, when tested at concentrations several orders of magnitude above their respective solubility limits. These unexplained effects do not exhibit a consistent concentration-response relationship and have been attributed to physical coating and subsequent entrapment (Rhodes *et al*, 1995; Sousa *et al*, 1995). Physical impairment limits feeding ability, and/or may lead to suffocation if the test organism gets trapped in the surface film, as has been observed in tests with these substances. As a result, the unexplained effects are an artifact, not related to the concentration of the test substance, but rather to the physical form of the emulsion formed under unrealistic laboratory test conditions. Such problems can be identified readily by a consideration of QSARs (Parkerton and Konkel, 2000). The inherent toxicity hazard posed by PWSSs may also be understood better by consideration of internal tissue concentration-effect relationships (van Egmond *et al*, 1999).

In addition to physical effects, there may be other explanations to account for effects that occur above the water solubility limit including the presence of a water-soluble impurity or degradation product. For these reasons, aquatic toxicity data which show effects at concentrations above the water solubility of a test substance, should be regarded as not interpretable and rejected for use in risk assessment (Robertson, 1995). Fortunately, current test guidelines for PWSSs clearly stipulate that water solubility should not be exceeded (OECD, 2000a). In the future, therefore, such improved approaches should prevent the occurrence of observed effects that are simply a laboratory artifact associated with excess undissolved test substance.

3.2.1.3 Adequacy of aquatic toxicity tests

While effects reported above the water solubility limit may be regarded as false positives, thereby confusing proper test interpretation as discussed above; of equal concern is that absence of effects at the solubility limit, may represent a false negative (de Bruijn and Herremans, 1995). False conclusions regarding the lack of aquatic toxicity for PWSSs may arise because:

- The duration of the toxicity test is not sufficient to allow the test organism to approach steady-state with the aqueous exposure concentration;
- sorption to test vessels may reduce actual exposure below the solubility;
- dietary exposure via bioaccumulation of the substance in the food chain is not adequately considered.

Both of the above considerations could lead to higher internal concentrations in field organisms than may be realised in laboratory toxicity tests. Therefore, careful consideration is needed to ensure appropriate data are available to conclude that a PWSS does not pose a chronic toxicity hazard.

A key parameter that can be used to assess if the above concerns may confound aquatic toxicity test results is the half-life of the PWSS in fish. The time in days required to achieve 95% of steady-state is given by:

$$T_{95} = 4.3 * T_{50}$$

Where T_{50} is the first-order half-life in days, calculated from the elimination rate obtained from a fish bioaccumulation test. The T_{95} provides an estimate of the time required for test organisms to approach equilibrium. This concept is currently applied in the OECD bioaccumulation test guideline No. 305 (OECD, 1996) for selecting time intervals for fish sample collection during uptake and depuration phases of the bioconcentration test. This same approach can be used to design a study of chronic fish toxicity of sufficient duration to ensure lack of observed effects is not due to a non-steady state condition.

For a fish early life stage test with trout (>60 days post-hatch exposure; OECD, 1992a), substances with $T_{50} < 15$ days would achieve 95% of steady-state. Similarly for a 28-day juvenile growth test (draft OECD guideline), substances with a $T_{50} < 7$ days would approach steady-state. Appendix II provides additional guidance on how the half-life of a PWSS in fish can be determined and applied in a risk assessment context. Based on the information presented, it is concluded that this parameter serves as a key decision trigger for judging the adequacy of aquatic effects data in assessing chronic hazard, and the potential for secondary poisoning.

3.2.2 Proposal for improving effects assessment

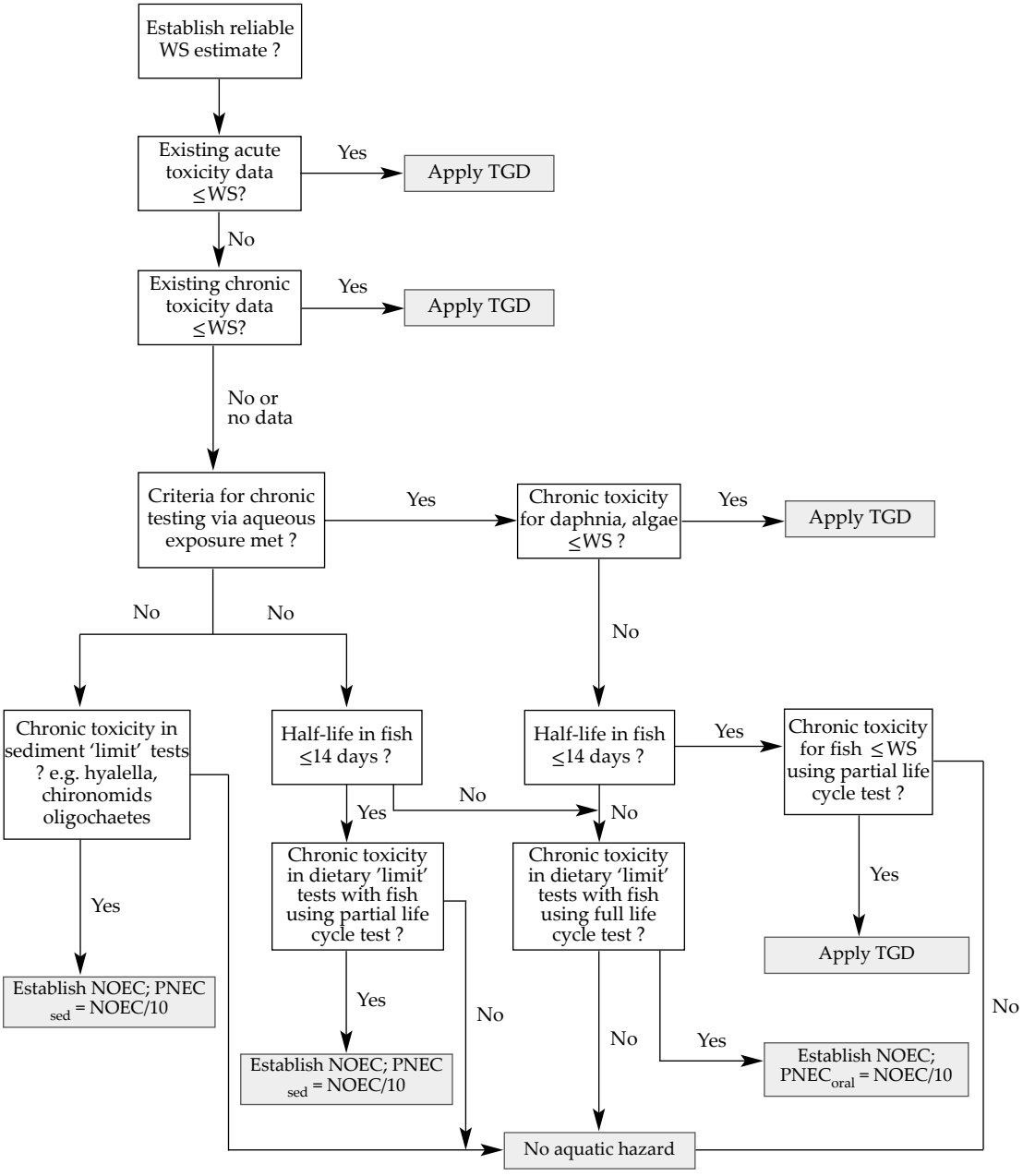
A proposed scheme illustrating important concepts that can be used to improve the current approach for effect assessment of PWSSs in the aquatic environment is provided in Figure 4.

The scheme begins by determining the water solubility of the substance. As previously discussed QSPR models can be used to provide an initial estimate to determine if experimental measurements should be attempted. Once a reliable water solubility estimate (or upper bound) is identified, available acute and chronic toxicity data should be evaluated relative to this benchmark. If valid aquatic toxicity data exist below the water solubility limit, then standard TGD procedures can be followed for PNEC derivation. However, if no data are available, or if available data indicate no effects or are equivocal, the log K_{ow} of the substance, as estimated from an automated fragment contribution method (Meylan and Howard, 1995), is calculated.

Based on past experience, substances with estimated log $K_{ow} > 9$ are expected to be too insoluble to enable aquatic testing to be feasible in practice. Furthermore, measured bioconcentration factors have been shown to be below 100 for substances with log $K_{ow} > 9$, indicating that such substances are unlikely to pose a bioaccumulation concern as a likely consequence of bioavailability constraints (Meylan *et al*, 1998). For such substances, testing resources are most effectively directed to an assessment of chronic toxicity on sediment-dwelling organisms. Currently, chronic sediment toxicity test protocols are available for three benthic species representing different important taxonomic groups and feeding behaviours (Hill *et al*, 1994):

- *Hyalella azteca* - amphipod; epibenthic feeder;
- Chironomus sp. - insect larvae; suspension feeder;
- Tubifex or Lumbriculus - oligochaete, deposit feeder.

Figure 4: Proposed scheme for risk assessment of poorly water soluble substances



Such testing can proceed in a tiered fashion first by performing limit tests at an upper-bound sediment concentration to determine if any effects are observed. Options for selecting an upper-bound sediment concentration in limit tests will be discussed later. If no effects are evident a qualitative conclusion is reached indicating that the PWSS poses no chronic hazard. If effects are observed, a definitive study is undertaken to establish the NOEC for $PNEC_{sediment}$ derivation.

The critical decision point in the above scheme is whether chronic testing via aqueous exposure is experimentally feasible. This determination can be made based on the results of inherent substance properties (e.g. log K_{ow} > 9, water solubility below analytical detection) or a preliminary assessment of substance stability in the aqueous test systems. If it is deemed appropriate to test the PWSS in an aqueous-based test system, chronic 'limit' tests for daphnids and algae are performed at the water solubility of the test substance in the aquatic test media used. If chronic effects are observed, definitive chronic studies are performed to establish NOEC for $PNEC_{aquatic}$ derivation in accordance with current TGD procedures. However, if no effects are observed, further limit testing with fish is performed to determine if the PWSS poses a chronic toxicity hazard.

The appropriate experimental design for a fish chronic toxicity test using either an aqueous or dietary exposure is dictated by the half-life of the PWSS in fish. For example, if the half-life in trout is less than 15 days, a conventional partial life cycle test with this species is adequate for assessing chronicity. Since the observed half-life is expected to be proportional to the lipid content of the fish tested, it is suggested that the experimental half-life be adjusted to a standard lipid content (e.g. 5%) before comparison to the proposed cut-off value. If effects are observed in a limit test, the existing TGD guidelines can be applied; this would require performing a follow-up study with multiple concentrations to establish a definitive NOEC for $PNEC_{aquatic}$ derivation. However, if no effects occur in the limit test, it is concluded that the substance does not pose an aquatic toxicity hazard.

If the half-life in the fish is greater than 15 days, an extended chronic life cycle test is warranted. Guidance on the duration of this test can be determined based on steady-state considerations using the fish half-life. Due to the practical difficulties and costs associated with the conduct of long-term tests with PWSSs, a dietary toxicity test should be considered as a possible alternative to conventional aqueous exposure, even if it is possible to perform fish toxicity tests using this route. Generally, the diet is expected to provide the dominant source of exposure of poorly metabolisable hydrophobic substances to fish (Thomann, 1989). If this generalisation applies to the substance under investigation, then use of a dietary route of administration is clearly justified in long term toxicity testing. Ideally a tiered testing scheme could be used in which an initial 'limit' test with a single upper-bound dietary concentration would be performed first to determine if adverse effects occurred relative to a control treatment. If no effects were observed in this test, it would be concluded that there was no aquatic hazard. In contrast, if adverse effects were realised, a definitive long-term dietary study with multiple concentrations would be required to establish a definitive NOEC for $PNEC_{oral}$ derivation. Due to time and cost considerations, long-term fish toxicity tests may be best focused on species such as zebrafish or medaka (Braunbeck *et al*, 1990; Patyna *et al*, 1999; Yokota *et al*, 2001).

The proposed scheme illustrates a decision framework for PWSS effect assessment that includes two central concepts:

- Application of a logical and pragmatic tiered-testing strategy;
- use of a qualitative assessment in cases where evidence shows no hazard.

A tiered testing strategy is used to identify the most appropriate toxicity tests based on key properties of the PWSS. This tiered approach minimises the use of vertebrate testing and introduces the concept of 'limit' toxicity tests as a cost-effective approach for deciding if further definitive testing is necessary. Limit tests involve comparing effects observed at a single upper-bound exposure concentration, to the control treatment, using analysis of variance methods. Therefore, results from a limit test provide a 'Yes' or 'No' answer with regard to hazard potential. A key consideration in a limit test is the selection of the PWSS concentration to be investigated. Since results will determine the need for further testing, limit tests should be based on an experimental design that minimises the potential for false negatives. This can be accomplished by testing a reasonable worst case exposure concentration and increasing replication to improve the detection of statistically significant differences relative to the control group.

As previously described,the limit concentration in aquatic toxicity tests is the water solubility limit (i.e. in terms of dissolved concentration) of the substance in the aqueous test medium. A limit concentration in sediment tests may be selected as follows. If an aquatic toxicity QSAR is available for the sediment-dwelling test species/endpoint exposed to substances acting by a nonpolar narcotic mechanism, the aqueous concentration corresponding to baseline toxicity is calculated. If the baseline toxicity estimate is below the water solubility of the PWSS, this estimate is used in the Equilibrium Partitioning (EqP) model to determine a corresponding sediment concentration. This concentration is expected to elicit toxicity for substances with the least toxic mode of action. If the toxicity estimate exceeds the water solubility, the water solubility is applied instead in the EqP model to provide a 'limit' concentration in sediment toxicity tests:

If $EC_{50} < WS$ then:

$$C_{sed,limit} = EC_{50} * K_{oc} * F_{oc}$$

If $EC_{50} > WS$ then:

$$C_{sed,limit} = WS * K_{oc} * F_{oc}$$

Currently baseline toxicity QSARs are not well characterised for 10 or 28-day sediment toxicity tests with freshwater organisms. However, future research may enable this approach to be applied.

Another possible option for defining a sediment limit concentration is by considering measured and predicted sediment exposure concentrations. For example, a limit concentration could be determined as:

- Maximum predicted local $PEC_{sediment}$ based on TGD default calculations;
- 90^{th} percentile of observed concentrations based on field monitoring data.

Limit concentrations for dietary toxicity tests with fish could also be selected based on a consideration of observed or predicted exposure concentrations in prey organisms, or mode of action based on internal effect concentrations (cf. Appendix II).

As in aqueous tests, once the limit concentration is selected it is necessary to confirm that exposure concentrations (either in sediment or diet) remain stable. For biodegradable substances, oxygen depletion may occur in sediment tests at elevated test concentrations causing potential indirect effects confounding proper test interpretation. In feeding studies, palatability may be a confounding issue due to the elevated concentrations of substances spiked. Therefore, such additional considerations should be taken into account in the experimental design of a limit lest.

A second important aspect of the proposed decision framework is the possibility that derivation of a numerical PNEC may not be necessary. In the current TGD, PNEC derivation relies on NOECs that represent the lowest concentrations above which adverse effects are expected. However, for PWSSs, NOECs may simply reflect the highest test concentration (e.g. water solubility, limit concentration in a sediment or dietary test) investigated. Consequently, definitive PNECs for PWSSs cannot be derived credibly from such data. Rather, if chronic effects are not observed in limit tests, it is concluded that the PWSS does not pose a hazard, and a qualitative assessment is used as the technical basis supporting the risk assessment conclusion (i.e. the substance poses a negligible risk). This avoids the difficulty of incorrectly applying the current paradigm to PWSSs for which a reliable PNEC cannot be established due to lack of effects in laboratory toxicity tests.

3.2.3 Limitations in exposure assessment

Difficulty in exposure assessment for PWSSs arises from:

- Inappropriate extrapolation of laboratory tests to define compartment half-lives;
- conservatism in model algorithms used to estimate indirect exposure (e.g. bioaccumulation in the aquatic and terrestrial food chain);
- limitations of conventional field measurements (based on total concentrations) to quantify the bioavailability of PWSSs.

3.2.3.1 Extrapolation of environmental fate test results

The fate of a substance in the aquatic environment is characterised by results obtained in standardised aqueous-based test systems. For example, ultimate biodegradation potential is determined by various OECD 301 protocols (OECD, 1992b). These tests require test substance concentrations in the range of 2 to 100 mg/l, which are above the solubility limit for PWSSs. Thus, both the rate and extent of observed biodegradation for PWSSs is confounded by bioavailability limitations and dissolution kinetics (Aichinger *et al*, 1992). Recent data reported for the linear alkane, n-octadecane, provide a good example (Battersby, 2000). Application of the OECD 301 F test (manometric respirometry) with direct addition, resulted in a mean degradation of 18% after 28 days. In contrast, octadecane was extensively biodegraded, if dosed as an emulsion in tetrapropylbenzene sulfonate, or on a filter support yielding 88% and 87% respectively after 28 days. While such approaches clearly enhance the bioavailability of PWSSs in standard biodegradation tests and indicate the likely conservatism imposed by solubility limitations, it is uncertain if results using such dosing methods can be appropriately extrapolated to characterise biodegradation behaviour at environmentally realistic concentrations (Hales *et al*, 1997).

In the aquatic environment, PWSSs will exist principally in forms that are complexed to dissolved and particulate organic carbon. Such partitioning mechanisms may reduce bioavailability to microbes (thereby potentially increasing substance persistence) and aquatic organisms (thus mitigating ecotoxicity and indirect exposure via bioaccumulation in the food chain). Further reductions in bioavailability of particle-bound forms may occur as a result of hysteresis or aging-effects (Steinberg *et al*, 1987; Kan *et al*, 1994a,b; Alexander, 1995; Loehr and Webster, 1996; Kesley and Alexander, 1997). These considerations introduce additional uncertainty in the extrapolation of laboratory soil and sediment biodegradation and bioaccumulation tests to the field. While the examples cited above focus on biotic fate processes, such factors apply equally to abiotic transformations.

According to the TGD, substances are classified with respect to biodegradability based on the results of standardised tests and specific pass criteria (e.g. >60% biodegradation after 28 days). A further distinction is made based on the rate at which biodegradation is achieved (i.e. the so-called "10-day window criterion"). Given the biodegradation classification of the substance, default half-lives for WWTP and surface water are assigned as input for regional exposure modelling. As explained above, since the results of standardised biodegradation tests are misleading for PWSSs due to mass transfer limitations, this approach may seriously affect the estimation of the biodegradation half-life at environmentally relevant levels. This potential source of error is further compounded in the derivation of soil (and sediment) half-lives, by assuming that only the dissolved form of the substance in these compartments, is available to microbes. The dissolved fraction is determined by application of the EqP model. Since the estimated dissolved fraction for PWSSs is low, conservative default half-lives are obtained even for PWSSs that meet the stringent, ready biodegradation requirements. For example, despite the low water solubility of di-2-ethylhexyl phthalate (Table 1) this hydrophobic liquid has been shown to be readily biodegradable in standard biodegradation tests. Since the log K_{ow} for this substance is 7.5, application of the existingTGD default guidance results in an estimated half-life in soil of 30,000 days (= 82 years). However, half-lives in soil, based on the ultimate biodegradation endpoint in laboratory tests, are in the time scale of months (Staples *et al*, 1997). This example illustrates the potential over-conservatism in regional PECs that may occur when default TGD guidance is applied in specifying compartment half-lives for PWSSs.

3.2.3.2 Application of indirect exposure models

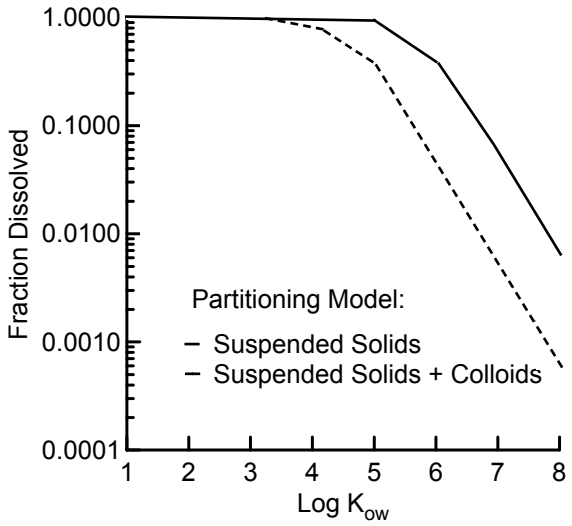
A second limitation in applying existing TGD procedures relates to indirect exposure assessment, especially for the local exposure scenario. Under the current guidance, conservative default calculations are used to calculate local exposure concentrations near production and processing facilities. Due to the conservative nature of the local exposure scenarios, (default emissions, limited dilution, no substance degradation) local PECs may often exceed the water solubility of the PWSS by orders of magnitude. (Note: this may also be true for air since predicted PEC_{local} may exceed the substance's solubility in air, i.e. vapour pressure). Conservatism in predicted biota concentrations may then be further exaggerated by multiplying default PECs that exceed solubility, by default bioaccumulation factors for plants, fish, meat and milk (estimated from model algorithms derived principally from poorly biotransformed substances).

3.2.3.3 Interpretation of field monitoring data / bioavailability

A third difficulty in performing exposure assessments for PWSSs is the proper interpretation of field monitoring data, since analytical measurements are traditionally based on total concentrations that ignore bioavailability considerations. In addition, while regional and local model calculations account for partitioning to suspended solids in the derivation of a surface water PEC, these models ignore partitioning to dissolved organic carbon. Previous work has shown the importance of DOC complexation in mitigating the aquatic toxicity and bioconcentration of PWSSs (McCarthy, 1983; McCarthy and Jimenez, 1985a,b; Landrum *et al*, 1987; Black and McCarthy, 1988; Arthur and Pawlisszyn, 1990; Day, 1991; Larson *et al*, 1992; Gobas and Zhang, 1994; Hermens *et al*, 1998). These studies support the generally accepted hypothesis that the freely dissolved fraction of a nonionic organic chemical represents the bioavailable fraction.

To illustrate the importance of DOC in exposure assessment, the predicted freely-dissolved fraction of a nonionic organic PWSS is compared using both two- and three-phase EqP models (Figure 5). The former model considers only partitioning to suspended solids while the latter model, as described by Eadie *et al* (1990) and Mitra and Dickhut (1998), includes sorption to both particulate and dissolved organic carbon phases. Results suggest that the bioavailability of PWSSs with log $K_{ow} > 5$ is overestimated by about one order of magnitude, using the two-phase EqP model that is applied in the current TGD.

Figure 5: Comparison of the freely dissolved fraction of a nonionic organic chemical with and without consideration of dissolved organic carbon complexation. (Model assumptions are as follows: $K_{poc} = K_{doc} = K_{oc}$; Suspended solids and dissolved organic carbon = 15 mg/l; Organic carbon fraction in suspended solids = 0.1)



3.2.4 Proposal for improving exposure assessment

A tiered approach for exposure assessment of PWSSs is proposed. If default PECs obtained from the EUSES model are below the compartment-specific PNEC (or 'limit' concentration at which no adverse effects are elicited), further refinement of the exposure assessment may not be warranted. However, if this is not the case, the large uncertainties that occur in applying default TGD procedures and EUSES model algorithms to PWSSs must be recognised, such that a refined exposure assessment is warranted. As a possible first tier, additional laboratory studies may be conducted to help improve calibration/interpretation of the default TGD algorithms / EUSES model predictions.

For example, to improve the fate assessment of PWSSs in STPs, laboratory simulation studies using radiolabelled substance can be conducted at environmentally realistic concentrations well below the solubility limit (Federle *et al*, 1997). Another option is to conduct mass balance studies on existing STPs, as recently reported for selected commercial surfactants (Matthijs *et al*, 1999). Results from these studies can be used to improve both the structure and calibration of STP models for PWSSs, as demonstrated by Cowan *et al* (1993).

To refine model parameters related to indirect exposure, laboratory bioaccumulation tests may be needed to overcome conservative default algorithms. As described in Appendix II, the results of dietary bioaccumulation tests with fish can be used to assess the role of dietary exposure and estimate the fish bioconcentration factor. Bioaccumulation, in lower trophic organisms if necessary, may best be determined via sediment or soil bioaccumulation tests (US-EPA, 1994; Belfroid *et al*, 1994).

To refine the $PEC_{aquatic}$, a three-phase partitioning model could be used to translate predicted total concentrations to the freely dissolved fraction. Initially, the partition coefficient to dissolved organic carbon could be calibrated based on the octanol-water partition coefficient and literature correlations (Burkhard, 2000). This review also provides empirical data that can be used to extrapolate partition coefficients from reference humic acid (often used in laboratory studies) to DOC in the field. Recent work relating humic acid bulk properties to bioavailability reduction of PWSSs may allow a better mechanistic basis for such extrapolations in the future (Kile *et al*, 1999; Haitzer *et al*, 1999). Analytical methods that differentiate freely dissolved from complexed forms could also be applied on a limited number of field samples to improve the quantification of the fraction of the total concentration that is bioavailable (Sproule *et al*, 1991; Resendes *et al*, 1992; Gustafson and Dickhut, 1997; Urrestarazu Ramos *et al*, 1998; Freidig *et al*, 1998; Oomen *et al*, 2000).

If the results of laboratory tests can be used to improve EUSES model calibration/interpretation such that the revised PEC is below the PNEC (or C_{limit}), it is concluded that the PWSS does not pose an environmental concern. However, if this conclusion is not reached, the exposure assessment proceeds to a subsequent tier that focuses further refinement on the collection of actual field measurements. Field measurements serve as the definitive basis for the exposure assessment. General guidance on the design and conduct of field monitoring studies has been provided by ECETOC (1999).

For PWSSs, it is usually logical to focus field monitoring efforts on sediment (and soil) compartments, because analytical concentrations are expected to be much higher than in surface water due to partitioning behaviour. As a result, analytical detection will be much more practical. Furthermore, the sediment (and soil) compartment is not subject to the large potential temporal variations sometimes characteristic of surface waters. Therefore, monitoring data for these compartments provide a more time-integrated exposure metric than afforded by surface water. Characterisation of sediment/soil concentrations may be the only viable option for risk assessment of certain PWSSs if aquatic-based toxicity tests are not possible due to solubility/stability limitations, but chronic effects in sediment tests allow a $PNEC_{sediment/soil}$ to be established (cf. Figure 4).

As a first step in addressing the bioavailability of nonionic neutral organic PWSSs in sediments (or soils), organic carbon normalisation can be used. Therefore, field monitoring programmes should include determination of organic carbon. However, when applying organic carbon normalisation, it should be noted that this approach is only appropriate for soils/sediments with organic carbon contents above ca. 0.2% organic carbon, since below this value, other mineral phases become important in controlling partitioning behaviour (Di Toro *et al*, 1991). Further refinement in exposure assessment of field soils and sediments may be possible through future application of new analytical tools that are based on biomimetic extraction principles (Mayer *et al*, 2000).

Residues in field biota provide another means to quantify the bioavailability of a substance in the foodchain. If collected in conjunction with other monitoring data (e.g. sediment concentration measurements), such data can be used to calibrate bioaccumulation models that are specific for a given substance class (Thomann and Komlos, 1999). If residues are also measured independently in laboratory toxicity tests so that residue-effect relationships can be determined, bioaccumulation models can be used to provide the quantitative link between environmental exposure and ecological risk (McCarthy and Mackay, 1993; ECETOC, 1995; Sijm and Hermens, 2000).

3.3 Environmental risk assessment - ionic organic substances

Risk assessment of PWSSs that possess either anionic or cationic functional groups is more complicated than for nonionic organic PWSSs. This complexity is due to the pronounced influence of ionic interactions on the environmental fate and ecotoxicity of these substances. As a result, fate and ecotoxcity tests generated in a laboratory water matrix may be misleading if extrapolated to field conditions where radically different matrix interactions occur. Moreover, simple models, or analytical methods to estimate the bioavailable fraction of the total concentrations, are generally not available.

An example of a commercially important ionic PWSS is the cationic surfactant N,N-Dimethyl-N-octadecyl-1-octadecanaminium chloride (DHTDMAC). This substance has a molecular weight of 586 and an estimated log K_{ow} of 12.5 and water solubility of 4 pg/l based on the EPIWIN QSPR model (Meylan and Howard, 1995). Practical guidance on conducting environmental risk assessment for ionic PWSSs is discussed further in section 5.

3.4 Environmental risk assessment - complex organic substances

The principle means to distinguish complex from simple PWSSs is the non-linear nature of the observed aqueous concentration that occurs, as previously discussed, as a function of product loading (Figure 3). The difficulties highlighted with regard to conduct and interpretation of aquatic-based laboratory tests for simple PWSSs equally apply to complex PWSSs. However, since components exhibit different environmental behaviour, further complications arise in risk assessment.

For assessing the aquatic toxicity of a complex PWSS, the lethal loading approach is the recommended test procedure (Girling *et al*, 1992; OECD, 2000a). The principle advantage of this approach is that the composition of the aqueous phase varies as a function of the substance loading, depending upon the inherent partitioning behaviour of each component. While results from lethal loading tests can be used to characterise intrinsic hazard and are thus useful for environmental classification purposes, such data cannot be applied directly for risk assessment. This is due to the fact that distribution of components in an aquatic laboratory test is likely to be significantly altered as a result of environmental fate processes that act differently on individual components.

However, toxicity data generated using a lethal loading approach can be used to determine if an additive toxicity model can be applied for risk assessment of the complex PWSS. Given compositional data on a complex PWSS and equilibrium partitioning theory (Shiu *et al*, 1988), the concentration of individual components in the aqueous phase of an aquatic toxicity test can be calculated as a function of substance loading. If independent toxicity data are available for individual constituents for a given test species/endpoint so that an aquatic toxicity QSAR can be developed, an additive model can then be applied to predict the lethal loading in a laboratory test. If model predictions are confirmed by experimental results, this provides technical justification to apply an additive toxicity model in evaluating the risk posed by the environmental distribution of the complex PWSS. This approach is illustrated by Peterson (1994), who showed that application of equilibrium partitioning and additive toxicity models could be used to predict reliably algal toxicity of gasoline in lethal loading experiments. Recent examples illustrating the application of additive toxicity models in the risk assessment of complex mixtures include Schwarz *et al*, 1995; King *et al*, 1996; Brack *et al*, 1998; Fuchsman *et al*, 1998; van de Plassche *et al*, 1999; Di Toro and McGrath, 2000.

Table 1: Comparison of water solubility predictions with experimental data (µg/l) for selected substances

Compound	EPIWIN Model	SPARC Model	Experimental Measurement
1-Tridecanol	4533	791	393 ± 19
1-Pentadecanol	468	58	6.0 ± 2.0
Dodecane	110	4.4	3.5
Tridecane	27.5	3.6	0.33
Hexadecane	0.9	2.2	-
Chrysene	26.4	1.9	1.5 ± 0.4
Benzo(a)pyrene	10.4	1.9	1.8 ± 0.3
Benzo(ghi)perylene	2.8	0.7	0.14 ± 0.03
Coronene	0.28	2.3	0.1
Hexachlorobenzene	192	231	5
C10 alkylbenzene	18.6	57.7	~ 41
Di-n-octylphthalate	0.4	1.2	0.5± 0.1
Di-2-ethylhexylphthalate	1.1	5.3	1.9 ± 0.2
Di-2-ethylhexyladipate	0.5	13.1	3.2 ± 0.4
1,2,5,6,9,10-Hexachlorodecane	18.3	36.1	-
1,1,2,13,14-Pentachlorotetradecane	0.4	0.18	-
Pentabromodiphenylether	0.08	0.02	0.07 ± 0.05
PCB 52	86	17	170 ± 8
PCB 86	9.4	1.4	34 ± 5
PCB 153	1.3	0.4	-
PCB 163	3.8	0.3	5.3 ± 1.0
Hexamethyldisiloxane	2881	CNC	930
Decamethyltetrasiloxane	7.0	CNC	4.7
Dodecamethyltetrasiloxane	0.3	CNC	0.5
Octamethylcyclopentasiloxane	54.7	CNC	45
Decamethycyclopentasiloxane1	7.1	CNC	17

CNC = Could Not Calculate

4. SORPTIVE SUBSTANCES

4.1 Property description

Principles of sorption and desorption

Depending on their physico-chemical properties, organic and inorganic chemicals in the environmental compartments water, soil and sediment can sorb to particulate or dissolved matter that are:

- Neutral (e.g. neutral proteins, lignin and cellulose);
- negatively charged (e.g. humic acids, microorganisms, algae, clay and silica);
- positively charged (e.g. Si, Al or Fe oxides).

The mechanisms of sorption are quite different and not always simple (Westall, 1987), e.g.:

- Physical adsorption due to van der Waals forces;
- chemisorption due to a chemical bonding or surface coordination reaction;
- partitioning of an organic chemical into the carbon phase of particulates or DOC;
- ion exchange mechanism for cationic or anionic substances.

Various models have been established to explain the sorption and desorption of chemicals. These include straightforward ones, such as sorption/desorption isotherms according to Langmuir or Freundlich (Schwarzenbach, 1993) and the Ion Exchange Model (Schwarzenbach, 1993), or more complex ones, such as Diffuse Double Layer Model, Constant Capacitance Model or Triple Layer Model (Schnoor, 1996).

In many cases the straightforward equilibrium models work quite well, and the sorption constant K_p can be derived from property-property estimations via K_{oc} (e.g. K_{oc} from K_{ow}, (Karickhoff *et al*, 1979)) or by incremental procedures from the substance structure (Lyman *et al*, 1990; Syracuse, 1999).

However, in some cases, e.g. if sorption includes an ion exchange mechanism, desorption is very much slower than sorption, or is not fully reversible and leads to so-called bound residues (Schnoor, 1996; Schwarzenbach, 1993). In certain even more complicated cases, the desorption of a chemical depends on structure and/or the age of the particulate to which the substance is sorbed (Steinberg *et al*, 1987; Kan, 1994a,b).

4.2 Environmental risk assessment

The TGD (EC, 1996) describes the exposure and effects assessment for the environmental compartments. Exposure assessment leads to the different PEC, and effects assessment to the PNEC for the different compartments.

4.2.1 Environmental exposure assessment

4.2.1.1 Influence of sorption on fate and distribution - general considerations

Sorption and water solubility

Sorption is often negatively correlated to water solubility (Lyman *et al*, 1990; Boethling and Mackay, 2000). This means highly sorbing substances exhibit in most cases low water solubility. Difficulties in environmental assessment associated with extremely low water solubility are discussed and a detailed testing strategy is given in section 3.

Sorption and bioavailability

As bioavailability (Hamelink *et al*, 1994) describes the portion of a chemical, in the different environmental compartments, which is available for uptake by biota, it is evident that the sorption and desorption behaviour of a chemical can considerably influence its bioavailability in the environment. To determine the truly dissolved part of the substance requires advanced analytical methodology such as solid-phase microextraction (SPME) (Arthur and Pawlisszyn, 1990), which can be applied for nonionic non-polar substances (see section 3). No such analytical method is available however for other sorptive substances, such as long-chain alkyl quats (e.g. dihydrogen tallow dimethyl ammonium chloride, DHTDMAC).

As mentioned above, these effects are even stronger, especially for substances where sorption is fast and desorption slow and/or incomplete. Uptake of the bioavailable fraction of a substance can therefore lead to transformations, complete biodegradation, accumulation and/or toxic effects.

Sorption and transformation/ultimate biodegradation

Strongly sorbed substances, such as long-chain alkyl quats (e.g. DHTDMAC), which desorb very slowly, also biodegrade slowly, although the alkyl chain should be easily biodegradable in an analogous manner to fatty acids. Shorter chain alkyl quats, which do not sorb as strongly as DHTDMAC and which are more easily desorbed, are also more readily biotransformed / biodegraded (van Ginkel and Kolvenbach, 1991). From these findings it can be concluded that for this type of substance, desorption is a necessary step for biotransformation/biodegradation.

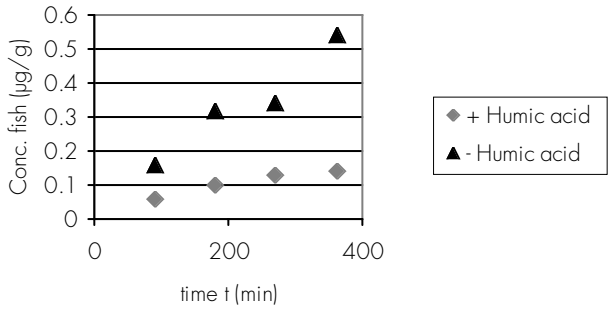
Sorption and bioconcentration/bioaccumulation

Sorption as an influencing factor on bioconcentration / bioaccumulation has been described briefly by ECETOC, (1995). Some more recent findings are described below.

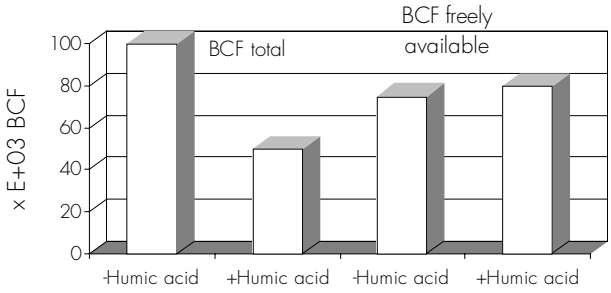
A lowered bioconcentration due to the presence of DOC has been reported for, among others, water flea (Day, 1991), bluegills (McCarthy and Jimenez, 1985b) and in pike (Larson *et al*, 1992). In a more recent study, Freidig *et al* (1998) demonstrated by kinetic solid phase extraction, that a humic acid concentration of 17.2 mg/l considerably decreased the uptake of hydrophobic hydrocarbons in guppies when compared to tests using DOC free water (Figure 6). A bioaccumulation study (Hermens *et al*, 1998) with *Daphnia magna*, with and without humic acid, demonstrated that the bioconcentration factor (BCF) based on total concentrations of the hydrophobic chemicals, is approximately halved when humic acid is added (see Figure 6). But it was also demonstrated with SPME (Arthur and Pawlisszyn, 1990), that the BCF based on freely available substance in the water, was more or less the same with and without humic acid. This is in agreement with the concept that only the dissolved fraction of a substance contributes to bioconcentration.

Figure 6: Influence of humic acid on bioconcentration potential

Conc. of Hexachlorobenzene in fish with and without humic acid (Freiding *et al*, 1998)



BCF of PCB No. 77 based on total and freely available substance with and without humic acid (Hermens *et al*, 1998)



It is important to note that bioconcentration/bioaccumulation (ECETOC, 1995) is also decreased by up to several orders of magnitude by other factors, such as ionic instead of neutral state (Saarikoski *et al*, 1986). Furthermore a molecule diameter of more than 10 Angström can influence bioconcentration/bioaccumulation, because transport across membranes can be hindered (Operhuizen *et al*, 1985).

4.2.1.2 Determination of sorption constants

Calculation of sorption constants

A wide range of QSPR are available for calculation of sorption constants (Boethling and Mackay, 2000; Lyman *et al*, 1990; Syracuse, 1999), but, as explained earlier, a check should be made to establish whether these methods can be applied to the substance under investigation.

Measurement of sorption constants

The bioavailable/dissolved fraction of a substance in water can be determined by sophisticated analytical methods e.g. SPME for non-polar non-ionic substances (Arthur and Pawlisszyn, 1990). When applied according to OECD Guideline 106 (OECD, 1981b) this technique can also be used to determine sorption constants for different matrices (soil, sewage sludge, suspended matter, sediment).

Special cases

For substances which have complex sorption characteristics (e.g. surface active substances which sorb at low concentrations via ion exchange, at medium concentrations via hemimicelles (Schwarzenbach, 1993; Chandar *et al*, 1983) but at CMC form micelles), the determination via sorption constants is not applicable. For strongly hydrophobic substances with low water solubility (see section 3) it can be very costly to carry out a sorption/desorption study using a classical approach, as radiolabelled substance is required for analytical purposes. In other cases it might not be possible to determine K_p because an adequate classical analytical method is lacking or is not sufficiently sensitive. For example, for the strongly sorbing quat (DHTDMAC), several K_p in the environment were measured (ECETOC, 1993) with a range from 3,833 - 85,000 l/kg. This variability of K_p cannot be explained purely by the variability of particulate matter in the environment. Recent measurements in Japan result in even higher K_p. This is a clear indication that the reported K_p values for DHTDMAC reflect the significantly improved quality of the analytical methodology over the years.

4.2.1.3 Aquatic exposure assessment

Sorption influencing the $PEC_{local,\ aquatic}$

The $PEC_{local,\ aquatic}$ is estimated from release, fate and distribution data and takes into account the sorption of a substance to particulate matter. $PEC_{local,\ aquatic}$ includes the dissolved background concentration $PEC_{regional}$, normally calculated with a multimedia fugacity model.

Therefore $PEC_{local,\ aquatic}$ is the total dissolved, hence the bioavailable, part of the substance in the aquatic environment. It is calculated from the total (bulk) concentration as follows (EC, 1996):

$$C_{local,\ aquatic\ diss} = C_{local,\ aquatic\ bulk} * 1 / (1 + K_p * c_{part.\ matter})$$

Table 2 shows the influence of K_p on the ratio of $C_{dissolved}/C_{total}$ assuming a particulate matter concentration of 15 mg/l for the EU region (EUSES, 1997).

Table 2: Influence of K_p on $C_{dissolved}$ / C_{bulk} for C_{susp} = 15 mg/l

K_p (l/kg)	$C_{dissolved}/C_{bulk}$
10^1	1
10^3	0.99
10^4	0.87
10^5	0.40
$5x10^5$	0.12
10^6	0.06
10^7	0.01

Table 2 also clearly shows that for K_p = 10 l/kg the substance is completely dissolved, whereas for $K_p = 10^7$ l/kg 99% is sorbed and only 1% is dissolved. This means that $PEC_{local, aquatic}$ is considerably decreased for substances exhibiting high sorptivity.

$$PEC_{local, aquatic} = C_{local, aquatic\ diss} + PEC_{regional}$$

$PEC_{local, aquatic\ bulk}$

If the sorption constant or the freely dissolved fraction of a strongly sorbing substance cannot be determined easily, $PEC_{local, aquatic\ bulk}$ together with the $PNEC_{aquatic\ bulk}$ is an alternative for the aquatic risk assessment. $PEC_{local, aquatic\ bulk}$ covers the total amount of the substance (bulk) in the aquatic compartment and if appropriate also the corresponding $PEC_{regional, aquatic\ bulk}$. To avoid double counting in mass load, the regional concentration should not to be added to the local concentration for diffuse emitted substances with wide dispersive use.

These local and regional $PEC_{aquatic, bulk}$ can be derived easily e.g. from EUSES (EUSES, 1997) or by direct measurement of the total substance.

$$PEC_{local, aquatic\ bulk} = C_{local, aquatic\ bulk} + PEC_{regional, aquatic\ bulk}$$

$PEC_{local, aquatic\ bulk}$ has the advantage that the value can be compared directly with monitoring data based on the total (bulk) substance in water.

Sorption influencing the bioconcentration factor

As previously indicated, the BCF depends on the reliable determination of the truly dissolved fraction of the substance (Hermens *et al*, 1998). If this cannot be determined reliably for a highly sorptive substance, an alternative is to determine the biomagnification factor (BMF) e.g. via fish feeding study (see section 3) or field BAFs.

4.2.1.4 Sediment exposure assessment

Sediment concentrations are calculated in most cases from the dissolved aquatic concentration, the partitioning coefficient suspended matters K_{susp} in the surface water, and for the regional assessment, an estimated dissipation time $DT_{50\ sediment}$ (EC, 1996). Only if it is possible to determine reliably the dissolved substance concentration, K_{susp} as well as $DT_{50\ sediment}$, can a realistic $PEC_{sediment}$ be calculated. If a reliable calculation is not possible, a direct measurement of the substance concentration in sediments could be carried out.

If possible, the calculated $PEC_{sediment}$ should be compared with reliably measured data. For example, for DHTDMAC, the calculated $PEC_{sediment}$ of the EU risk assessment is in reasonably good agreement with recent measurements of DHTDMAC in sediments of Swiss lakes (Alder *et al*, 2000).

4.2.1.5 Terrestrial exposure assessment

In most cases, soil concentrations are calculated and take into account such factors as wet and dry deposition from air, volatilisation to air, leaching to ground water and sewage sludge amendment to agricultural soil and grassland, as well as the dissipation by biotic degradation (EC, 1996). For strongly sorbing non-volatile substances, the most important transfer path to soil is the amendment of sewage sludge. The concentration of the substance in the sewage sludge is normally estimated from the calculated dissolved concentration of the substance in the waste water treatment system, using a sewage sludge sorption constant K_{sludge}, and for the regional assessment, an estimated dissipation time $DT_{50\ soil}$. Only if it is possible to determine reliably the dissolved substance concentration in the wastewater treatment system, K_{sludge} as well as $DT_{50\ soil}$, can a realistic $PEC_{terrestrial}$ be calculated. The measurement of the sludge concentration would be an alternative.

If possible, the calculated $PEC_{terrestrial}$ should be compared with reliably measured data e.g. soil or sludge concentration.

4.2.1.6 Secondary poisoning and indirect exposure

In the EU risk assessment, drinking water concentrations, BCF and BAF/BMF for different species, together with transfer factors, are used in the estimation of secondary poisoning in the bird/mammal food chain, and for indirect exposure assessment of humans via the environment (EC, 1996).

Drinking water from surface water and ground water

The drinking water concentration is derived from either the dissolved surface water concentration using a purification factor, or the dissolved porewater concentration of agricultural soil averaged over 365 days (EC, 1996). The highest value of these two is normally taken. For the calculation of the dissolved concentrations, the K_{susp} and the K_{soil} partitioning coefficients need to be calculated or measured reliably (see section 4.2.1.2).

Alternatively, the truly dissolved surface and soil porewater concentrations need to be determined. If this is not possible, a measurement of the total substance concentration after water purification (which typically reduces considerably the concentration of highly sorptive substances) should be carried out.

Bioconcentration factors (BCF) in surface water and soil

BCF in surface water and soil can be determined reliably only if the dissolved concentration in surface or soil porewater can be calculated using calculated or measured sorption constants K_{susp} or K_{soil}. It is also possible to measure directly the truly dissolved fraction with advanced analytical methods. If this is not possible or practical, bioaccumulation/biomagnification study/studies in water, soil and sediment are proposed (see section 4.2.2.2).

4.2.1.7 Measured concentrations in environmental compartments

Measured environmental concentrations of strongly sorbing substances have to be considered with great care. Often it is not clear what was determined (e.g. total or dissolved concentrations or something in between) as the extraction methods used might have desorbed the substance from particulate matter (i.e. in the environment, the truly dissolved concentration is lower). Studies that use advanced methods, e.g. SPME for non-polar non-ionic substances, (Arthur and Pawlisszyn, 1990), will produce data that are more reliable.

4.2.2 Environmental effects assessment

4.2.2.1 Influence of sorption on effects

Sorption and ecotoxicity

The bioavailable fraction of pyrethroids in surface water decreases with increasing dissolved organic carbon (DOC). Consequently, the toxicity to *Daphnia magna* relating to the total concentration of the pyrethroid is considerably reduced (Table 3 and Day, 1991).

Table 3: Decreasing acute toxicity of pyrethroids to Daphnia magna with increasing DOC (Day, 1991)

Pyrethroid	DOC in mg/l	LC50 (24h) in µg/l
Fenvalerate	3.3	1.1
	8.6	11
	15.5	19.5
Deltamethrine	2.2	0.6
	4.5	3.4
	10.1	4.7

Sorption and Standard Guideline Tests

The PNEC is derived from ecotoxicity data by applying appropriate application factors (EC, 1996). Normally these data for the aquatic compartment are determined according to standard test guidelines (e.g. OECD, ISO) in laboratory tests using tap water. These tests do not take into account environmental conditions, such as particulate matter or dissolved organic carbon (DOC), because sorption is normally taken into account in the exposure assessment. For some chemicals this can have serious consequences. For example, hydrophobic long-chain quaternary ammonium compounds and long-chain amines are extremely poorly soluble in water, and because they are positively charged, under environmental conditions, they will sorb strongly to test organisms such as algae that are negatively charged. This means that in a normal aquatic laboratory test with zero particulate matter and/or absence of DOC the test organism faces a stress by being coated with the test substance which, for example, may cause pH shifts or inhibit substance exchange with the environment and therefore might harm the organism, not only via the effect of the substance itself, but also by secondary effects.

4.2.2.2 Modified ecotoxicity test systems

Aquatic ecotoxicity test systems

To reduce the influence of secondary effects such as those mentioned in section 4.2.2.1, modifications of standard aquatic ecotoxicity tests can be used (Table 4).

Table 4: Modified aquatic ecotoxicity test systems

Aquatic Test System	**Reference**
Acute Fish Test Fish Acute Toxicity Mitigated by Humic Acid	US EPA OPPTS 850.1085
Acute Daphnia Tests Determination of the Inhibition of the Mobility of *Daphnia magna strauss* by polyelectrolytes in the presence of organic carbon in the form of humic acids - Acute Toxicity Test	AFNOR XP T90-380
OECD Guideline 202, Daphnia sp. Acute Immobilisation Test using natural water	OECD, 2002
Chronic Daphnia Test - Reproduction Test Modified OECD Guideline 211, *Daphnia magna* Reproduction Test using effluent from a wwtp simulation test	OECD Guideline 212 (1998b); Noack 2000c
Acute / Chronic Algal Test Modified OECD Guideline 201, Algal Growth Inhibition Test using two different river waters (river Böhme and river Elbe)	OECD, 1984; Noack, 2000a

When using river water or effluent from a wastewater treatment system, the possibility that biodegradation might occur during the ecotoxicity test has to be taken into account. To avoid biodegradation, the test water can be inactivated chemically (e.g. sodium azide) or by freezing and defrosting (Noack, 2000b). It is also necessary to check if the river water or effluent used meets the culturing conditions for the test organism (e.g. sufficient inorganic salts for algae). It is important that the media are characterised at least e.g. by DOC, DIC (Dissolved Inorganic Carbon) and pH (see also Table 6). For European rivers a suspended matter concentration of 15 mg/l is assumed for the regional and 25 mg/l for the continental setting (EUSES, 1997).

Results from such tests are shown in Tables 5 and 6. The adaptation of the ecotoxicity test to river water is relatively easy, whereas the use of effluent (e.g. from a WWTP simulation test as test medium) requires more effort.

Effluent has a particulate matter concentration (e.g. 30 mg/l) and a hydraulic retention time (e.g. 6 hours) (EUSES, 1997) which means that the substance will effectively be sorbed. An argument against the use of effluent diluted with river water, is that not all wastewater is discharged into a WWTP. But, as the EU Urban Waste Water Treatment Directive 91/271/EC (EC, 1991) requires all communities with more than 2000 inhabitants to have installed a biological STP between 1998 and 2005 at the latest, then this counter argument will soon no longer be valid.

Bioconcentration test systems

Test systems to determination bioconcentration (e.g. OECD, 1996) require the reliable measurement of the substance concentration in the water phase and the biota. If the truly dissolved fraction of the substance cannot be measured reliably the BCF could be overestimated (Hermens *et al*, 1998). Therefore, for highly sorbing substances, a dietary bioaccumulation/biomagnification study should be considered as a practical alternative (see section 3).

Bioaccumulation studies

For strongly sorbing substances it is more sensible to carry out a bioaccumulation (e.g. in sediment) or a biomagnification study (e.g. fish feeding study, see section 3). Unfortunately no official OECD test guidelines are yet available for such studies.

4.2.2.3 Aquatic effects assessment

Effects as function of suspended matter

Two case studies illustrate the influence of laboratory versus natural waters on the effects observed in aquatic ecotoxicity studies.

DODMAC/DHTDMAC

These are strongly sorbing quaternary ammonium salts which have been tested under various conditions (e.g. laboratory water, well water, effluent, river water) for different species. DODMAC/DHTMAC's truly dissolved concentration in water is not known but estimated < 1 μg/l (ECETOC, 1993). It should be noted that the concentrations tested and shown in Table 5 are above the truly dissolved concentration of DODMAC/DHTDMAC.

Algae and daphnia are the most sensitive species in ecotoxicity tests with DHTDMAC (see Table 5). For algae in particular, the difference in the results between laboratory water and effluent for the 96-h growth inhibition test is more than two orders of magnitude; for the 5-d test, the difference between laboratory and river water is less pronounced. For the Ceriodaphnia reproduction test a significant difference between river water and effluent was observed.

Testing DHTDMAC under environmentally realistic conditions has shown that bioavailability and hence ecotoxicity is lower than in tests using laboratory water (see Table 5).

Table 5: Ecotoxicity test results from a strongly sorbing quat DODMAC/DHTDMAC, CAS No. 107-64-2/61789-80-8 (ECETOC, 1993)

Species	Test method	Water quality	Suspended matter (mg/l)	NOEC C or EC_{50} (mg/l)	Comments / Impurities
Daphnia					
Daphnia magna	48-h acute	Lab	-	0.065-1.06	EC_{50}, more data avail. Relability unknown
		Well water	1	1.06	EC_{50}
		River water	9.2	2.1-3.6	EC_{50}
Daphnia	Reproduction	Lab	-	0.18	-
magna	21-d	River water	?	0.38	-
Ceriodaphnia	Reproduction	River water	?	0.2-0.78	EC_{50} adults
dubia	7-d	Effluent	?	4.53-10.7	Reproduction
Algae					
Selenastrum	Growth inhibit	Lab	-	0.006-0.12	Sonication/4% MHTTMAC
capricornutum	96-h	Effluent	-	10.7-20.3	-
	Growth inhibit	Lab	-	0.075-0.078	-
	5-d	River Water	68-139	0.062-0.25	-

C_{12-14} Alkyl dimethylamine

C_{12-14} Alkyl dimethylamine is a strongly sorbing tertiary amine used for the synthesis of many types of surface active materials such as amine oxides, betaines and quats. It is highly toxic to algae. An OECD 201 Algal Growth Inhibition Test was carried out using the usual OECD Test Medium and two different river waters. One water was collected from a highland river (Böhme, Harz) with municipal discharge only. The other was collected from the river Elbe, which is a river receiving industrial discharge. Table 6 shows parameters of the test media and the resulting EC_{50} (growth rate). The results show that the toxicity is reduced with increasing suspended matter concentration but for the Elbe, a much higher reduction in ecotoxicity should be expected due to the much higher content of suspended matter. The water of the river Elbe has a much higher hardness and conductivity compared to the water of the river Böhme. This could explain why the reduction in ecotoxicity is less pronounced when using Elbe water, although other factors could be responsible (e.g. unknown toxicants in the relatively more polluted river Elbe).

Unfortunately insufficient scientific knowledge is available and further research is needed.

Table 6: (Modified) OECD 201 algal growth inhibition test on C_{12-14} alkyl dimethyl amine, CAS No. 84649-84-3 using OECD Test Medium and two different river waters

Parameter	OECD Test Medium	River water Böhme	River water Elbe
Location	-	Dorfmark, Böhmegrund	Schnakenburg, Fähre
Sampling date	-	Sept. 10, 2000	Sept. 10, 2000
Weather on sampling day	-	cloudy	cloudy
Weather on day before Sampling	-	cloudy	cloudy
Colour of water	clear, colourless	yellowish	yellowish
pH value	8.2	7.4	8.2
Conductivity (µS/cm)	n.a.	475	1130
DOC (mg C/l)	n.a.	14	14
DIC (mg C/l)	n.a.	16	25
Ammonium N (mg N/l)	15	0.03	< 0.002
Nitrate N (mg N /l)	n.a.	1.3	2.5
Ortho-Phosphate P (mg P/l)	n.a.	0.05	0.1
Total Phosphate P (mg P/l)	n.a.	0.07	0.2
Total hardness (mg $CaCO_3$/l)	50 ($NaHCO_3$)	96	339
Suspended matter (mg/l)	0	1	36.5
EC_{r50} (µg/l)	14	56	92
Confidence Interval (95%)	n.a.	51-62	82-102
PNEC (ng/l) (application factor 1000)	14	56	92

n.a. = not available

$PNEC_{aquatic\ bulk}$

$PNEC_{aquatic\ bulk}$ is the $PNEC_{aquatic}$ which is derived from a modified aquatic ecotoxicity test (using for example humic acid, natural water or effluent) by applying the usual application (safety) factors (1000 to 10) depending on the available tests. $PNEC_{aquatic\ bulk}$ can then be used in the aquatic risk assessment for strongly sorbing substances when it is based on $PEC_{local,\ aquatic\ bulk}$.

$$PNEC_{aquatic\ bulk} = \frac{(LC_{50}/EC_{50}/NOEC)_{modified\ test}}{1000/100/10}$$

4.2.2.4 Sediment effects assessment

Strongly sorbing substances will sorb to suspended matter in surface waters and hence end up in the sediment of rivers, lakes and seas. The sediment tests described in section 3 are recommended, especially with endobenthic feeders which might face additional exposure via ingestion of the sediment. A bioaccumulation study resulting in a biota sediment accumulation factor $BAF_{sediment}$ should be carried out in conjunction with the ecotoxicity test.

Spiking an appropriate sediment is essential, which means that the sorption constant for this sediment needs to be determined and compared with measurements in the environment. Only if the sorption constant measured in the environment is similar to the sorption constant for the sediment used in laboratory testing will a meaningful result (i.e. NOEC) be obtained. For example, if the sorption constant in the environment is 10-fold of the measured value in the test, then this implies a 10-fold higher sediment porewater concentration in the laboratory than in the field. Since observed toxicity correlates to porewater concentrations (EC, 1996) the PNEC derived from this test should not be used directly for the calculation of the $PEC/PNEC_{sediment}$ as it would overestimate the true risk in the field. For the strongly sorbing quat DHTDMAC where sediment concentrations up to 80 mg/kg dry weight (dw) were found in lake sediments during the 1980s (Alder *et al*, 2000), the endobenthic feeding worm *Lumbriculus variegatus* was tested in spiked sediment resulting in a NOEC for 28-d reproduction of 5,000 mg/kg. The $BAF_{sediment}$ of 0.28 as well as the NOEC indicates that DHTDMAC is tightly bound to the sediment, and almost not bioavailable for uptake and effects (APAG, 1999).

4.2.2.5 Terrestrial effects assessment

Strongly sorbing substances will sorb to sewage sludge in biological wastewater treatment systems. If the sewage sludge is applied to soils, then terrestrial ecotoxicity testing with earthworm, nematodes or collembola as well as plants (according to OECD or US EPA Test Guidelines) are indicated. It should be mentioned that strongly sorbing substances often exhibit a hydrophobic action to soil when directly applied. This hydrophobic effect means that plant seedlings, for example, will not emerge due to insufficient water and/or air. In such cases it is advisable to use freeze dried sewage sludge, apply the substance onto the sludge and mix the sludge into the soil (APAG, 2000). As only

5000 kg/ha of a sewage sludge can be applied to soil according to EU legislation (EC, 1996), the concentration of sludge into soil to account for realistic conditions should not exceed 1.7 g sludge/kg dry weight soil (APAG, 2000). In addition, spiking of the substance to sewage sludge is also advisable because it ensures realistic sorption under test conditions, and which is similar to the environmental conditions (sludge amendment to soil). Otherwise, if there is a much lower sorption in the soil test than in the environment, a high soil concentration would result in an unrealistically high soil porewater concentration under test conditions. As a consequence the $PEC/PNEC_{soil}$ would overestimate the risk to the terrestrial compartment (see also section 4.2.2.4).

As mentioned in the sediment effects assessment, soil dwelling organisms may face additional exposure via ingestion of the soil (Belfroid *et al*, 1995). A bioaccumulation study resulting in a biota soil accumulation factor (BAF_{soil}) should be carried out in conjunction with the ecotoxicity test.

4.2.3 Environmental risk characterisation

4.2.3.1 Aquatic risk characterisation

According to the EU Technical Guidance Document (TGD), the risk for the aquatic environment is characterised by the quotient $PEC/PNEC_{aquatic}$, where $PEC_{aquatic}$ is the dissolved concentration in the aquatic compartment and the $PNEC_{aquatic}$ is derived from ecotoxicity tests normally carried out in test medium with no suspended matter.

PEC / PNEC $_{aquatic\ bulk}$ approach - an alternative for strongly sorbing substances

Problems may arise in the assessment of both the exposure and the effect of strongly sorbing substances (see sections 4.2.1 - 4.2.2). For the aquatic risk assessment of strongly sorbing substances, an approach based on $PNEC_{aquatic}$, bulk derived from a modified ecotoxicitiy test using humic acid, natural water or effluent and a $PEC_{local,\ aquatic\ bulk}$, which addresses the total substance concentration (dissolved and sorbed = bulk) can be a practical alternative. The risk quotient for the aquatic compartment is derived as usual:

Risk quotient aquatic compartment for the PEC/PNEC bulk approach

$$PEC_{local,\ aquatic\ bulk} / PNEC_{aquatic\ bulk}$$

When using this approach, it is not necessary to determine the truly dissolved fraction of a strongly sorbing substance in the exposure assessment and, in the effects assessment, it helps to avoid secondary effects in ecotoxicity test by e.g. coating the organisms. However, the limitations of this approach need to be considered carefully (see sections 4.2.2.3 and 4.3).

Comparison of $PEC_{local}/PNEC_{aquatic}$ and $PEC_{local,\ aquatic\ bulk}/PNEC_{aquatic,bulk}$

The following case study compares the EU TGD approach and the proposed bulk approach.

C_{12-14} alkyl dimethylamine

This is the starting material for, and also an impurity in (ca. 0.7% in average), detergent raw materials. Based on a consumption rate of 27,000 tonnes/annum amine for the EU, the release is 189 tonnes/annum as impurity in detergents. From this release, local and regional concentrations can be calculated and compared with different PNECs (see Table 7). For the regional situation, a connection degree to STPs of 70% (EC, 1996) and 90% (German situation) has been calculated (IC, 2000). The latter should soon be the situation across the EU because the Urban Waste Water Directive 91/271/EC requires that all communities with more than 2,000 inhabitants install biological wastewater treatment systems before 2005. The local concentration does not include the regional concentrations as this would mean double counting of the release for diffuse emitted substances.

Table 7: C_{12-14} alkyl dimethylamine PEC/PNEC calculations

	Local	**Regional (70% wwtp)**	**Regional (90% wwtp)**
$PEC_{dissolved}$ (ng/l) (OECD, 2000b)	3.8	50	17.2
PEC_{bulk} (ng/l) (OECD, 2000b)	4.2	55.6	19.1
$PNEC_{OECD}$ (ng/l)	14	14	14
$PNEC_{Böhme}$ (ng/l)	56	56	56
$PNEC_{Elbe}$ (ng/l)	92	92	92
$PEC_{dissolved}/PNEC_{OECD}$	0.3	3.6	1.2
$PEC_{bulk}/PNEC_{Böhme}$	0.08	0.99	0.3
$PEC_{bulk}/PNEC_{Elbe}$	0.05	0.6	0.2

The PEC/PNEC values in Table 7 based on the river water test result are a factor of 4 - 6 lower when compared to the PEC/PNEC based on the dissolved PEC and the PNEC derived from the OECD 201 Test using OECD Test medium (IC, 2000).

As mentioned earlier in this report, before sorption to DOC and suspended matter explain the reduction of the risk quotient, but other factors that are not yet fully understood may have a counter-effect.

4.2.3.2 Sediment risk characterisation

For the $PEC/PNEC_{sediment}$ the EU TGD approach should be used if $PEC_{sediment}$ can be calculated or measured. In addition, available biota sediment accumulation factor(s) $BAF_{sediment}$ should be used to check whether there may be a long-term risk via substance accumulation.

4.2.3.3 Terrestrial risk characterisation

For the $PEC/PNEC_{terrestrial}$ the EU TGD approach should be used if PEC_{soils} can be calculated or measured. In addition the available biota soil accumulation factor(s) BAF_{soil} should be used to check whether there may be a long-term risk via substance accumulation. These BAF_{soil} and measured transfer factors can also be used to evaluate the secondary poisoning (earthworm-eating birds) and indirect exposure (e.g. grass-eating cows, transfer to milk and meat (EC, 1996).

4.2.3.4 Secondary poisoning and indirect exposure risk characterisation

Bioconcentration factors (BCF) for water and soil (if the determination is possible) and bioaccumulation (BAF) or biomagnification factor (BMF) for the aquatic compartment (e.g. via fish feeding study), the sediment and terrestrial compartment, are indispensable parameters for evaluating the transfer of the substance to other organisms in order to check the risk via secondary poisoning and indirect exposure (EC, 1996).

4.3 Limitations of the PEC/PNEC Bulk Approach

Tests using natural waters are more environmentally relevant. However, the effects observed under these conditions are more like the results from mesocosm and field studies, and difficult to interpret compared with classical laboratory tests. It is difficult to judge the conditions for the environment as a whole and therefore, when these results need to be used for risk assessment purposes, all stakeholders must agree on the details of the approach before testing begins.

The composition of, for example, river waters varies with space and time. Factors such as rain events, runoff conditions, river type and seasonal variations determine such parameters as the content of DOC, suspended matter, water hardness, conductivity and pH. Currently, knowledge is insufficient to determine how these parameters and their variability influence the test result. Further research is needed into these aspects.

4.4 Recommendations

Sorption influences the bioavailability of a substance and hence the bioconcentration/bioaccumulation, transformation/ultimate biodegradation, ecotoxicity and concentrations in the different environmental compartments.

If sorption constants cannot be calculated or measured, then the reliable determination of the bioavailable fraction of a substance using advanced analytical techniques should be considered. The truly dissolved fraction of the substance is the key to reliable BCF, sorption constants K_p and concentrations in environmental compartments. Otherwise conflicting results may occur (see section 4.2.1.1).

If the determination of the truly dissolved substance is not possible, other approaches are proposed e.g. bioaccumulation/biomagnification instead of bioconcentration studies, or an approach based on bulk instead of dissolved concentrations for the aquatic compartment.

Despite the known limitations of the PEC/PNEC Bulk Approach for the risk assessment of the aquatic compartment (see section 4.3), it can be a valuable strategy for strongly sorbing and therefore difficult substances in order to avoid unrealistic results from classical laboratory ecotoxicity test systems. Unrealistic exposure assumptions are also avoided because measurement of the truly dissolved substance concentration is not possible.

Guidance on environmental risk assessment of strongly sorbing substances

Decision charts for risk assessment and characterisation of strongly sorbing substances for the aquatic, sediment and soil compartments as well as for secondary poisoning and indirect exposure are provided in Figures 7 - 9.

Figure 7 shows a decision chart on the environmental risk assessment of strongly sorbing substances for the aquatic compartment.

Process aquatic compartment

If the truly dissolved concentration of the substance in the aquatic environment can be determined reliably by calculation or measurement, then the risk assessment can be carried out according to the EU TGD (EC, 1996).

If the truly dissolved concentration of the substance in the aquatic environment cannot be determined reliably, a PEC/PNEC$_{aquatic\ bulk}$ approach is an alternative. The PEC$_{aquatic\ bulk}$ is either calculated or measured and the PNEC$_{aquatic\ bulk}$ is derived from modified effects tests (with humic acid, river water or effluent) by applying the safety factors as proposed in the EU TGD (EC, 1996). For the aquatic risk characterisation, PEC$_{aquatic\ bulk}$ is divided by PNEC$_{aquatic\ bulk}$ in a way analogous to the TGD process.

Figure 8 shows a decision chart as guidance for the environmental risk assessment of strongly sorbing substances for the sediment and terrestrial compartment.

In principle for the risk assessment of the sediment and the terrestrial compartment the EU TGD approach can be applied if at least sorption constants can be calculated, or measured or environmental concentrations in sediment, sludge or soil are available.

Process sediment compartment

If K_{susp} can be calculated from QSPR, and the dissolved concentration of the substance in surface water is available, the TGD approach is directly applicable. If the calculation cannot be done, check if a measurement e.g. according OECD 106 (OECD, 1981b) is possible. If yes, application of the TGD is the next step, if not, direct measurement of the bulk sediment concentration is needed, otherwise a sediment risk assessment is not possible. This would also be necessary if the truly dissolved concentration in surface water is not available.

Process terrestrial compartment

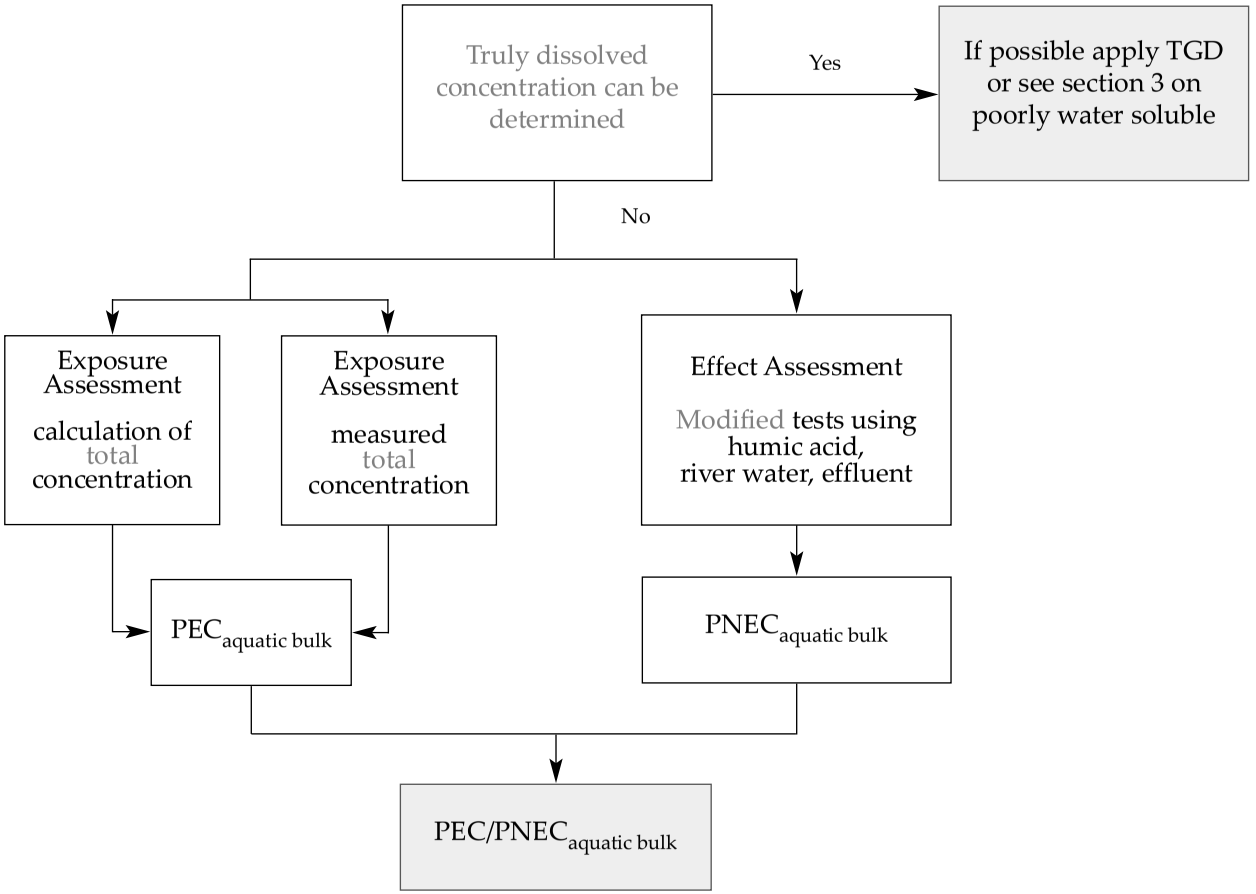
A risk assessment for the terrestrial compartment for strongly sorbing non-volatile substances is only necessary if the substance is expected to reach the terrestrial compartment via the application of sewage sludge.

If K_{sludge} can be calculated from QSPR and the dissolved concentration of the substance in the WWTP is available, the TGD approach is directly applicable. If the calculation cannot be done it has to be checked if a measurement e.g. according OECD 106 (OECD, 1981b) is possible. If yes, application of TGD is the next step, if not, direct measurement of the substance concentration in the sewage sludge or in soil is needed otherwise a sediment risk assessment is not possible. This would be also necessary if the truly dissolved concentration in the WWTP is not available.

Figure 9 shows a decision chart as guidance on the environmental risk assessment of strongly sorbing substances for secondary poisoning and indirect exposure.

If the truly dissolved concentration of the substance in surface or porewater is available, then a BCF in water (e.g. fish) or soil (e.g. earthworm) can be carried out, and the concentration in drinking water from surface and/or ground water determined according the EU TGD. If not, instead of BCF studies, bioaccumulation (BAF) or biomagnification (BMF) studies in the different compartment need to be carried out if necessary. BAF_{soil} for instance might only be indicated if the substance is expected to reach the terrestrial compartment via application of sewage sludge.

Figure 7: Decision chart for guidance on the environmental risk assessment of strongly sorbing substances for the aquatic compartment



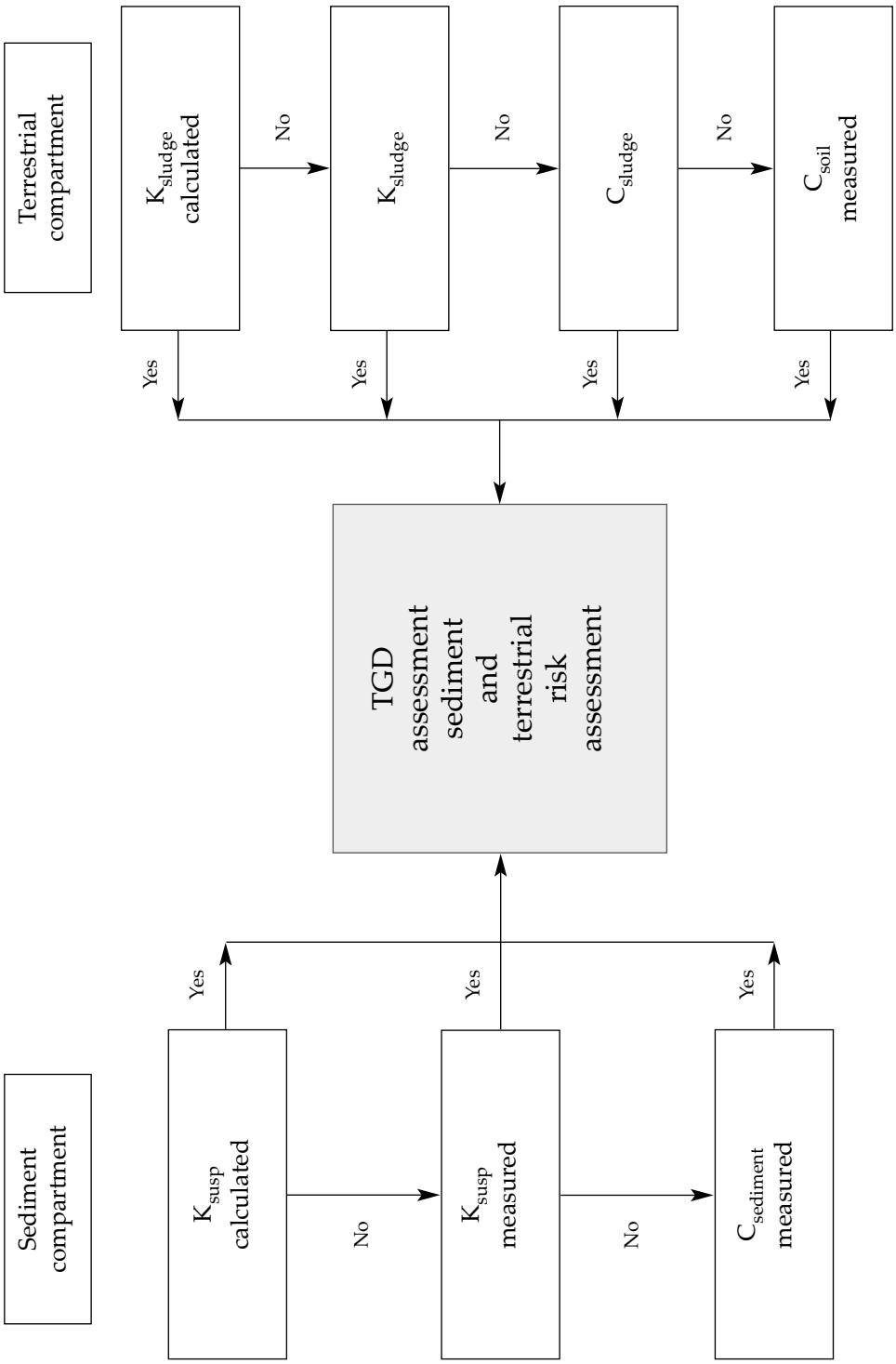


Figure 8: Decision chart for guidance on the environmental risk assessment of strongly sorbing substances for the sediment and terrestrial compartment

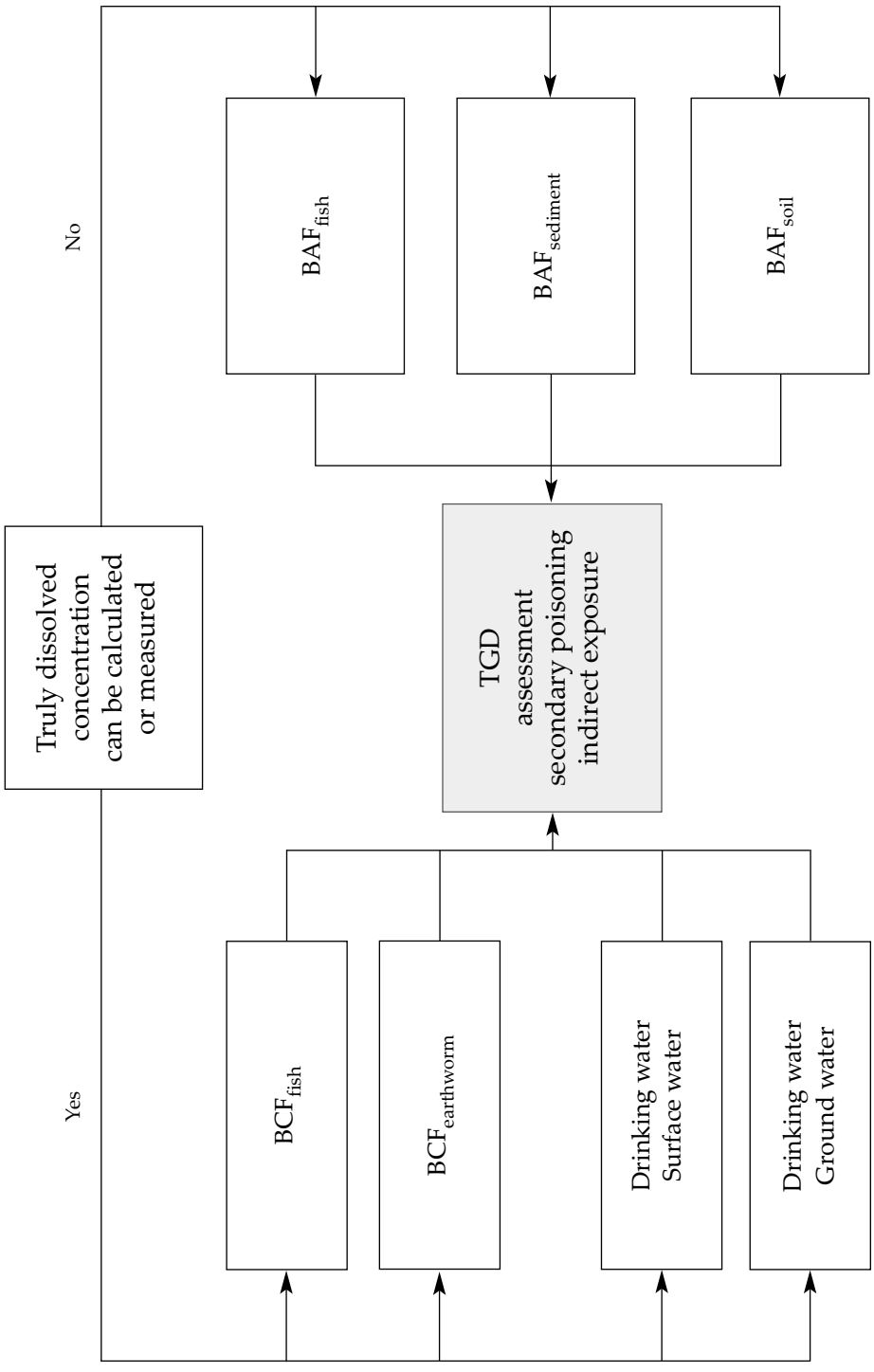


Figure 9: Decision chart for guidance on the environmental risk assessment of strongly sorbing substances concerning secondary poisoning and indirect exposure

5. SURFACE ACTIVE SUBSTANCES

5.1 Property description

Surfactants are chemicals that have surface active properties. Surface activity is a consequence of the unique structure of such chemicals, i.e. the co-existence of an apolar and a polar moiety within the same molecule, which commonly are referred to as the hydrophobic tail and the hydrophilic headgroup, respectively (Boethling and Mackay, 2000). Because of the bipolar structure, surfactants tend to accumulate at the interface of phases in order to shield the hydrophobic tail from the water molecules, whereas the hydrophilic headgroup has the strong tendency to be hydrated by water. Thus, water molecules are displaced by surfactant molecules at the water interface, with the result that the surface tension of the solution is reduced when compared to pure water. This effect can be measured as a decrease in the interfacial tension. Depending on the headgroup, surfactants (Römpp, 2000) can be classified as:

- Cationic;
- anionic;
- non-ionic;
- amphoteric.

Some types of surfactants, e.g. alkylamides, are difficult to classify precisely since in alkaline solution they act as nonionic surfactants and in acid solution they act as cationic surfactants. At higher concentrations, surfactants form spontaneously aggregates called micelles and lamellae, in order to minimise the energetically unfavourable interactions of the hydrophobic tail with the water molecules. The surface tension is lowered until the interface is completely filled with aggregates, and this concentration of the surfactant in water is called the critical micelle concentration (CMC). The CMC varies between 0.05 - 0.3 mMol/l for nonionics, between 1-10 mMol/l for anionics and around 20 mMol/l for cationics at 25°C (Rosen, 1989). The situation is further complicated, by the fact that the CMC is influenced by counterions and electrolyte composition, e.g. water hardness (Rosen, 1996).

The risk assessment of surfactants following the methodology laid down in the TGD (EC, 1996) may lead to erroneous conclusions because of inadequate consideration of three unique properties frequently associated with surfactant structures, i.e. high sorptivity (see section 4), poor water solubility (see section 3) and surface activity. The present section concentrates primarily on surface activity.

The bipolar structure of surfactants results in unique properties, namely:

- Strong tendency to accumulate at (compartment) interfaces;
- formation of heterologous phases (above the CMC);
- complete change of properties after primary degradation.

These properties have significant implications for the risk assessment of these substances. These implications are discussed in the following paragraphs, and recommendations are given for improvement of the methodology.

5.2 Environmental risk assessment

The TGD describes the exposure and effects assessment for the environmental compartments. Exposure assessment leads to the PEC, and effects assessment to the PNEC for the different compartments. Although the PEC/PNEC approach is generally accepted, recent experience with the risk assessment of existing high production volume (HPV) chemicals (e.g. DHTDMAC) has shown that the current methodology is not always suitable for the assessment of substances exhibiting surface activity.

5.2.1 General considerations

The environmental fate leads to a distribution of the substance between the different environmental compartments, i.e. air, sediment, soil and water. Therefore, the PEC has to be established for each (relevant) compartment. The standard distribution model for the EU risk assessment is based on certain physico-chemical properties of the substance as descriptors for the partition between the compartments, i.e. water solubility, volatility and octanol/water partition coefficient (K_{ow}). Although the model works quite well for substances with certain properties, it is generally not applicable for surface active substances. This is because the 'surface activity' adversely interferes with, or even precludes the determination of, the water solubility and in particular, the octanol/water partition coefficient. No vapour pressure or Henry's Law Constant data are available for ionic and nonionic surfactants. However, as surfactants (e.g. alcohol ethoxylates) have high boiling points and are relatively water soluble, negligible volatilisation can be expected (Boethling and Mackay, 2000) and the assessment of the air compartment is therefore not addressed in this section.

Influence of surface activity on water solubility

The phase diagram of surfactants consists of a triple point where the solid, the truly dissolved and the micelles coexist. The temperature of that triple point is called the Krafft temperature T_k and for most surfactants, T_k is below ambient temperature. This means that for these surfactants, the CMC can be regarded as their molecular solubility (Boethling and Mackay, 2000) and hence used in environmental modelling. However, for cationic surfactants such as the fabric softeners e.g. DHTDMAC (see section 4), T_k is above ambient temperature. The anionic surfactant LAS, which is a complex mixture of homologues, also has a T_K above ambient temperature. As most surfactants are mixtures, the solubilisation is even more complex as these mixtures deviate negatively from Raoult's Law, and this is triggered by headgroup effects (Scamehorn, 1986; Abe *et al*, 1992). In general, the least soluble component of the surfactant mixture will be solubilised by the more soluble ones. Exceptions to this rule are the cationic fabric softeners. In all these cases the CMC cannot be used as descriptor of the water solubility for environmental modelling.

Surface activity and octanol / water partition coefficient

A knowledge of the hydrophobicity of a substance is important for the prediction of its environmental fate. Highly hydrophobic substances are expected to sorb strongly onto soil and sediment, whereas the more hydrophilic substances are expected to partition in the water phase. Normally the octanol/water partition coefficient (K_{ow}) is used as a descriptor of the hydrophobicity of a chemical. The K_{ow} itself can either be derived from the molecular structure of the chemical by a QSPR (Lyman *et al*, 1990; Hansch and Leo, 1995; Boethling and Mackay, 2000) or by a direct experimental measurement (OECD, 1989, 1995). This concept works well for neutral organic compounds without surface activity (EC, 1996, 2.3.2, Data for exposure models). However, for surface active substances which accumulate at the octanol/water interface in order to shield the hydrophobic tail from the water molecules, leaving the hydrophilic headgroup still sticking into the water, no meaningful partition coefficient can be obtained. Even when measured, the results are highly dependent on the parameters chosen. Therefore fate models and property estimation methods relying on the K_{ow} as input parameter cannot be used for the assessment of surface active substances (Boethling and Mackay, 2000). This has been recognised in the TGD and, as a solution of the problem, it is stated that ''for surfactants, obtaining measured K_{oc} and BCF-values may be considered''.

Surface activity and partitioning between phases

Adsorption to solid surfaces is the main partitioning process that drives distribution in soil, surface waters, and sediment. The bipolar structure of surfactants result in their enrichment at interfaces. Therefore, surfactant sorption onto environmental solids is of major importance, especially if the ratio of water volume to water-solid interface is low (e.g. sludge from WWTP, soil). Measured sorption isotherms for all kind of surfactants are available (Boethling and Mackay, 2000). However, in most cases the sorption is non-linear (Brownawell, 1992), being stronger at lower substance concentration. Additionally, the sorption behaviour for a given surfactant can vary by up to three orders of magnitude depending on the sorbents (Ou *et al*, 1996). Only a few models exist for the prediction of sorption coefficients for surfactants (Kiewiet *et al*, 1996; Di Toro *et al*, 1991). Since log K_{ow} is not a suitable predictor for the hydrophobicity of surfactants, property estimation methods for sorption constants (e.g. $K_{oc\,soil}$) based on K_{ow} are not applicable to surfactants. This has been recognised in the TGD (2.3.2, Data for exposure models, 2.3.5 Partition coefficients) and, as a solution of the problem, it is stated ''for surfactants it may be considered to obtain measured K_{oc}- and BCF-values''.

Surface activity and transformation / ultimate biodegradation

In the EU, much progress has been made over the last decades to improve the biodegradability and hence the environmental impact of surfactants. Nowadays, all surfactants intended for down-the-drain use have to be > 90% primary biodegradable (Detergent Directive), and most surfactants already comply with the requirements of the forthcoming Revised Detergent Directive, i.e. they are readily biodegradable. For readily biodegradable substances, the TGD (EC, 1996) proposes default values for the half-life and the rate constant k in surface water of 15 days and 4.7×10^{-2} d^{-1} respectively.

Surfactants are frequently synthesised from natural precursors and readily breakdown via biotic degradation or hydrolysis due to predetermined breaking points (e.g. modern fabric softener quats). In most cases the true degradation rate in WWTP and environmental compartments is higher by far than the EU TGD default values (EC, 1996) (e.g. LAS half-life is 2 hours in rivers compared to TGD value of 15 days). Therefore, measured half-lives of the surfactant should be used to obtain a reliable assessment of the fate. This has been recognised in the TGD (section 2.3.6 Biotic and abiotic degradation rates), and it is stated that ''higher biodegradation rates may be justified if this can be confirmed by experimental data''.

Surface activity and bioaccumulation

Surfactants represent the largest group of surface active substances today, and most undergo > 90% primary degradation and metabolism in biota.

Property estimation methods are not applicable for the estimation of BCF of surfactants as they rely on K_{ow}. Additionally, such estimation methods do not take into account the possible transformation processes. Ignoring metabolism will lead to an overestimation of the bioconcentration (Tolls *et al*, 1994). From the available measured data for surfactants, the trend suggests that bioconcentration increases with decreasing CMC (Tolls and Sijm, 1995; Tolls *et al*, 1997).

Measurements using appropriate analytical techniques should be carried out when necessary.

5.2.2 Environmental exposure assessment

Aquatic, sediment and soil compartment

Local and regional PECs for surface water, sediment and soil are normally calculated with fate models using partitioning coefficients. For surfactants, these partitioning coefficients cannot be derived from K_{ow} by property estimation methods and therefore, measured partitioning coefficients need to be used. Whenever possible calculated PEC_{local} and $PEC_{regional}$ should be compared with measured data for the substance or for similar surfactants. Difficulties in the estimation of PECs associated with poor water solubility and/or high sorptivity are addressed in sections 4 and 5 respectively.

Measured concentrations in environmental compartments

Measured environmental concentrations of strongly sorbing substances have to be considered with great care. Often it is not clear what was determined (e.g. total or dissolved concentrations or something in between) because the extraction methods that were used may have desorbed the substance from particulate matter.

Secondary poisoning and indirect exposure

In the EU risk assessment, drinking water concentrations, BCF and BAF/BMF for different species, together with transfer factors, are used to estimate secondary poisoning in the bird/mammal food chain and for indirect exposure assessment of humans via the environment (EC, 1996).

Drinking water from surface water and ground water

For a realistic assessment, the water solubility and the sorption behaviour need to be known. As the water solubility of a surfactant is not always related to the CMC and the sorption behaviour often depends on the matrices, the use of appropriate measured data is advisable.

BCF in surface water and soil

The BCF cannot be estimated from K_{ow}, as explained earlier, so if necessary, it has to be measured. The measurement is complicated by the fact that a determination of the truly dissolved fraction in water is required (see also section 3). Additionally as surfactants are in most cases readily metabolised/biodegraded, only a fraction of the parent compound will be found in the tissue of the organism. To avoid difficulties in analyses, the use of radiolabelled substance is therefore required. It is essential however not to relate the BCF to the total radioactivity in the tissue otherwise it will not reflect the bioconcentration of the parent compound (Tolls *et al*, 1994; Tolls and Sijm, 1995; Tolls *et al*, 1997). With respect to bioaccumulation by soil or sediment organisms, measured data are preferred to account properly for metabolism.

Assessment of secondary poisoning and indirect exposure

To allow for a realistic assessment of secondary poisoning and indirect exposure, it is highly advisable to measure BCF, BAF and drinking water concentrations with state of the art methodology. If the surfactant is poorly water soluble a fish study using forced feeding should be carried out in order to determine the BMF instead of a BCF. For BCF, BMF and BAF it is essential that these factors are related only to the parent compound and not also to metabolites (see also sections 4 and 5).

5.2.3 Environmental effects assessment

General considerations

The PNEC is derived from ecotoxicity data by application of appropriate safety factors (EC, 1996). Normally the effects data are determined according to standard test guidelines (e.g. OECD, ISO etc) in laboratory tests. These tests are designed to mimic exclusively the compartment under consideration, excluding, as far as possible, interactions with other compartments. Therefore, in general these tests are considered to be appropriate for surface active substances. Exemptions are described below.

A $PNEC_{sediment}$ or a $PNEC_{soil}$ can only be estimated from the $PNEC_{aquatic}$ according the equilibrium partitioning method if measured sorption coefficients are available. Sorption coefficients calculated from K_{ow} are not reliable for surface active substances.

Advice for sound sediment or terrestrial effects testing of highly sorptive substances is given in section 4.

5.2.4 Environmental risk characterisation

The risk characterisation can be carried out as described in the TGD (EC, 1996) if PECs and PNECs are derived as explained in previous sections.

5.3 Recommendations

The current TGD methodology may not be adequate for surfactants since they exhibit unique properties, i.e. surface activity, high sorptivity and/or poor water solubility which might lead to erroneous results.

As the octanol/water partition coefficient K_{ow} for surfactants is dependent on the test conditions, it cannot be used for the estimation of sorption coefficients or bioaccumulation factors. The estimation of environmental concentration needs to be based on measured sorption coefficients, or preferably on measured environmental data.

If necessary BCF, BMF and BAF also need to be measured using state of the science analytical methods. As most surfactants are readily biodegradable, rapid metabolism may occur in tissues. As a consequence BCF, BMF and BAF have to be related to the parent compound only; if not, estimation of secondary poising and indirect exposure will be erroneous.

If the substance is highly sorptive, difficulties might occur in the determination of the $PNEC_{aquatic}$. Advice on how to overcome these problems is given in section 4.

Estimation of $PNEC_{sediment}$ and $PNEC_{soil}$ from aquatic effect data using the equilibrium partitioning method requires measured sorption coefficients.

If the surfactant is highly sorptive, the sediment and terrestrial effects testing requires special attention. Effort must be taken to ensure that the sorption under environmental conditions is similar to the test conditions in the laboratory, otherwise the risk characterisation will be wrong (see section 4.2.2.4).

It is essential to evaluate carefully the properties of the surfactant under consideration in order to ensure that the assessment is based on sound fate, distribution and effect data. Measured data (e.g. removal rates in STP, monitoring data in surface water, sediment and soil) of the substance or similar compounds are important when evaluating the reliability of the environmental risk assessment of surfactants.

6. VOLATILE SUBSTANCES

6.1 Property description

Volatilisation is defined as the process in which a chemical is transferred from a liquid or solid into the gas phase. The key parameters influencing this transfer are the vapour pressure and Henry's law constant. The vapour pressure *vp* [Pa] of a substance is the partial pressure above the pure solid or liquid phase at thermal equilibrium. Henry's law describes the partitioning of a chemical usually between air and water, and taking into account the fact that the solubility of the gaseous compound in the liquid is proportional to its partial pressure above the solution. The proportionality factor obtained for equilibrium conditions is represented by the Henry's Law Constant. It is expressed either as *H* (Pa.m^3/mol), i.e. the ratio of the partial pressure in the vapour phase, *Pv* (Pa), and the concentration in water, C_w (mol/m^3) in the equilibrium, or as the 'dimensionless' Henry's Constant or 'H'.

Henry's Constant can be measured experimentally but typically is estimated as the ratio of the solubilities in air and water (i.e. vapour pressure and water solubility). Quantitative structure property relationships are also available for calculating the Henry's Constant.

H = Pv /Cw
H' = Ca/Cw = (Pv /RT)/Cw

It should be recognised that measured values may differ significantly from estimates, and clearly the latter can not be applied to substances that are completely miscible with water. Similarly, for poorly water-soluble substances, there may be wide discrepancies; for example the measured value for octamethylcyclotetrasiloxane is between 3 and 17, while the calculated value is 487 (Mazzoni *et al*. 1997). For practical purposes any chemical with a Henry's Law Constant >1.0 will partition preferentially into the gas phase, for example oxygen has an 'H' value of ~ 3.

The other aspect of relevance for risk assessment is the sub-cooled liquid vapour pressure. This influences the potential for a substance to associate with aerosol particles. All parameters are temperature dependent. This is particularly relevant as, while most vapour pressure or H values are measured or estimated at 20 or 25°C, the TGD refers to 12°C as the standard temperature for exposure calculations.

6.2 Environmental risk assessment

The volatility of a compound may affect both the PNEC as well as the PEC calculation and, thus, should be considered during the process of risk assessment.

6.2.1 Environmental effects assessment

OECD and EU Test Guidelines require that test concentrations should be maintained at >80% of the nominal concentration throughout the duration of the test. In open test systems a Henry's Constant of 0.1 Pa m^3/mol will give rise to a loss of substance at rates that are important relative to the length of typical tests (Thomas, 1982; ECETOC, 1996). Thus, for volatile substances this means either testing with a flow through system or, in the case of very volatile materials, in a closed system.

In tests with *Daphnia* or fish, the use of a sealed system (vessels closed with parafilm, small headspace) is useful for the determination of the aquatic toxicity of volatile substances. However, there may also be methodological reasons why testing in a sealed system results in an inaccurate LC_{50} or NOEC. Algal tests for example are normally conducted in an open vessel in order to allow oxygen diffusion into the test medium. When testing octamethylcyclotetrasiloxane in an acute algal test, the test vessels were completely closed with no headspace. This resulted in lower than expected growth rates. The inclusion of additional reference controls allowed this to be taken into account when interpreting the results. Alternatively, lower initial biomass at the start of test and increased addition of bicarbonate in the algae medium have been suggested as necessary modifications for optimising algae growth rates in closed system tests (Nyholm *et al*, 2000). Another example also observed with testing octamethylcyclotetrasiloxane, where the use of a sealed system generated an atypically low NOEC, was sediment testing with *Chironomus* larvae. The absence of an air-water interface interfered with the organisms' normal behaviour (Kent *et al*, 1994). Higher than expected mortalities were observed in both the test and control organisms at the pupae stage. During this time, the organisms float to the water surface and obtain oxygen directly from the air. Therefore, careful consideration should be given to ensure that laboratory artifacts are not introduced as a result of sealed systems.

The risk assessment and PNEC calculation of volatile substances which are also poorly water soluble is discussed in section 3.

6.2.2 Environmental exposure assessment

Volatilisation is an important component of the $PEC_{regional}$ estimation, where multimedia fugacity models are applied (Mackay *et al*, 1992). However, in the calculation of PEC_{local} it is only considered at the level of the sewage treatment plant, but not for the aquatic (or sediment) compartment due to "the short distance between the point of effluent discharge and the exposure location" (EC, 1996). For highly volatile substances, (according to Thomas (1982) measured Henry's Law Constant > 1.0) this may overestimate the PEC_{local}. In the calculations described below, the potential impact that volatilisation losses contribute on the PEC_{local} has been assessed:

In accordance with the current TGD, the local predicted exposure concentration in the receiving water to which a substance is released is given by:

$$PEC_{local} = C_{total}\, F_d \qquad (1)$$

Where C_{total} is calculated from site-specific or default emissions and dilution data and F_d is the dissolved fraction of the substance that is calculated from the equilibrium partitioning model (see TGD section 3, eqn. 30). Thus, these calculations consider explicitly the effect of substance transfer from water to suspended solids in the local PEC derivation. However, PEC calculations ignore the potential transfer from water to air that similarly would reduce the local PEC in receiving water.

To address this current TGD limitation, equation (1) can be rewritten as:

$$PEC_{local} = C_{total} F_r \qquad (2)$$

where

$$F_r = F_d F_v \qquad (3)$$

and

F_r = fraction dissolved in water with volatilisation
F_d = fraction dissolved in water without volatilisation
F_v = fraction that is lost to air due to volatilisation

The dissolved fraction that remains in the receiving water if volatilisation occurs is given by Thomann and Mueller (1989):

$$F_v = \exp\left[-\frac{K_v F_d}{Z}\right]\frac{x}{U} \qquad (4)$$

where

K_v = volatilisation transfer coefficient (m/d)
Z = receiving water depth (m)
x = length of mixing zone (m)
U = receiving water velocity (m/d)

The volatilisation transfer coefficient can be estimated from the two-film resistance model:

$$\frac{1}{K_V} = \frac{1}{K_L} + \frac{1}{K_G H} \qquad (5)$$

where:

K_L = liquid film mass transfer coefficient (m/d)
K_G = gas film mass transfer coefficient (m/d)
H = dimensionless Henry's Constant

Equations to estimate the liquid and gas film mass transfer coefficients are proved by Thomann and Mueller (1989):

$$K_L = 0.013 \left(\frac{32}{M}\right)^{0.25} \left(\frac{U}{Z}\right)^{0.5} \quad (6)$$

$$K_G = 168 \left(\frac{18}{M}\right)^{0.25} U_W \quad (7)$$

where

M = molecular weight of substance (g/mol)
U_W = wind speed (m/s)

Thus, the quantitative importance of volatilisation in reducing local PECs depends upon both the properties of the substance (K_v, H, M) and environment (U, U_{wind}, X, Z).

Based on a simple flow balance the dilution factor of an effluent discharge is:

$$\text{Dilution Factor} = \frac{Q_{wwtp}}{Q_{wwtp} + Q_{river}} \quad (8)$$

Based on the default TGD assumptions of a dilution factor of 10 and an STP flow of 2000 m^3/day, this implies a Q_{river} of 18,000 m^3/day.

The TGD default river flow can be compared to typical values given by de Greef and de Nijs (1990) for different types of receiving waters:

Table 8: Summary of receiving water characteristics

Surface Water Type	Depth (m)	Width (m)	Velocity (m/sec)	Flow (m^3/day)
Tributary	0.75	3	0.05	9,720
River	2	15	0.1	259,200
Large River	4	100	0.3	10,368,000
TGD Default	1	3.6	0.058	18,000

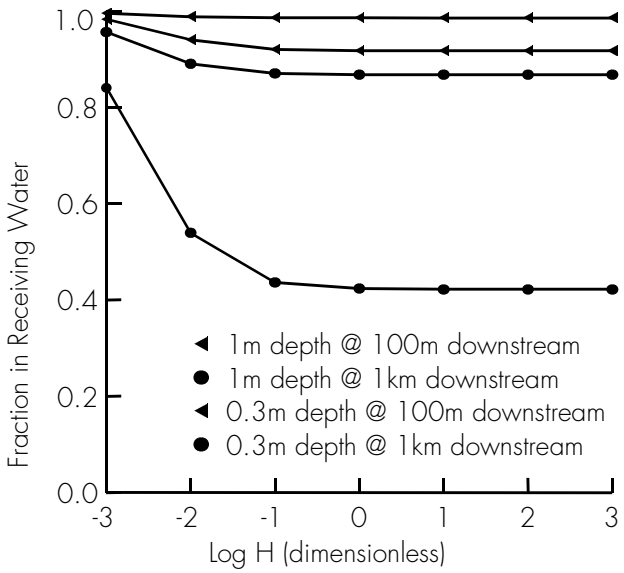
From these data it is clear that the TDG default river represents a tributary.

The default river velocity specified in the regional EUSES model can be calculated from the assumed volume and residence time of water in the unit world. Based on these values a default velocity of 0.058 m/sec is calculated. This estimate is consistent with the characteristic velocity cited by de Greef and de Nijs (1990) for a tributary so this regional estimate seems applicable to the local scenario as well. Based on the depth values reported above for the various types of receiving water a 1m depth seems reasonable for the default TGD receiving water. Given the assumed velocity and depth, the width was calculated based on flow continuity. The morphometric parameters associated with the default TGD receiving water are summarised in the above table. The default EUSES wind speed of 3 m/sec was also assumed for the calculation of the gas film transfer coefficient using equation (7).

The remaining parameter that is needed to illustrate the role of volatilisation on local PEC calculations is the assumed length of the receiving water mixing zone. Based on an analysis of STP discharges in the Netherlands, de Greef and de Nijs estimated median dilution factors at 1000 m downstream from the outfall of STP treating either entirely domestic or combined domestic plus industrial wastewater of 11.3 and 5, respectively (de Greef and de Nijs, 1990). These results suggest that a mixing zone of 1000m is therefore consistent with the TGD default dilution assumption of 10. Consequently, this value was used to assess the importance of volatilisation on local PEC calculations.

Based on the above equations and assumed parameter inputs, the fraction of a dissolved substance that is expected to remain in the receiving water is shown plotted as a function of the dimensionless Henry's constant of the substance. To indicate the sensitivity of the model to assumptions regarding receiving water depth and mixing zone length, several additional scenarios are also plotted for comparison in Figure 10.

Figure 10: Influence on local PEC calculations of volatilisation in receiving water



These results indicate that for the generic case (depth=1m; mixing zone =1000m) volatilisation does not have a significant impact on reducing the local receiving water PEC (i.e. about 13% of the substance is lost due to volatilisation). As expected, if a shorter mixing zone is assumed, the role of volatilisation is even less important. In accordance with equation (5), for highly volatile chemicals, the volatilisation transfer coefficient is determined by the magnitude of the liquid film resistance (i.e. the liquid mass transfer coefficient, K_L). This coefficient depends primarily on the receiving water depth but is independent of the substance's Henry's Constant consistent with equation (6) and the results shown in Figure 10. This figure also demonstrates the pronounced effect on the local PEC if a shallower receiving water depth of 0.3 meter is assumed. However, in an extreme situation the local PEC would be reduced by a factor ca. 40%, i.e. 2.5x.

The other aspect which has already been mentioned, is that the vapour pressure and Henry's Law Constant are usually measured experimentally under conditions that are not necessarily representative of the natural environment throughout the EU (Fiedler and Lau, 1998). Since volatilisation is sensitive to temperature variations, it may exhibit diurnal as well as seasonal and/or geographic trends. The temperature dependency of H has been investigated for several substances e.g. α- and γ-hexachlorocyclohexane (Kucklick *et al*, 1991).

In addition to temperature, the wind speed also influences volatilisation in natural waters or outdoor treatment facilities. Results of wind tunnel experiments suggest that under field conditions, mass transfer coefficients will be lower than those measured in the laboratory (Mackay and Yeun, 1983). In addition, the presence of dissolved chemicals such as electrolytes, solvents, detergents, and dissolved organic matter affect the activity of a chemical in aqueous solution and, thus, their apparent solubility and vapour pressure. Despite the above complexities, these factors are not expected to affect significantly the local PEC assessment.

6.3 Recommendations

According to EU or OECD guidelines it is stipulated that aquatic toxicity tests should be conducted and that the test concentration should be maintained within 80% of the measured concentration, for example by the use of flow-through conditions. However, it should also be recognised that forcibly maintaining a specific concentration of a highly volatile substance for measuring the 'intrinsic' toxicity to aquatic organisms is not only difficult to achieve, but also of debatable value for assessing the risk of highly volatile substances to the aquatic compartment. Furthermore, as indicated above, a sealed system may, under specific conditions, impose physiological constraints which may adversely affect the PNEC. According to Thomas (1982) the volatilisation process is significant in all waters if H of a substance is > 1 $Pa.m^3$/mole. The recommendation is therefore that for these highly volatile substances with a Henry's Law Constant > 1.0, flow-through systems or validated sealed systems with demonstrably no effect on the test organisms should be used and that the requirement to maintain > 80% of nominal concentrations should be relaxed to 50%.

A decision tree is given in Figure 11 illustrating the process for deriving a PEC/PNEC ratio for volatile substances. If the volatility of the substances precludes the derivation of an aquatic PNEC then an assessment of the air compartment may be considered necessary. Even if it is possible to conduct aquatic-based effect tests in sealed systems, for volatile substances that have used patterns involving limited contact with water, effect assessment may still be logically better focused on the air compartment.

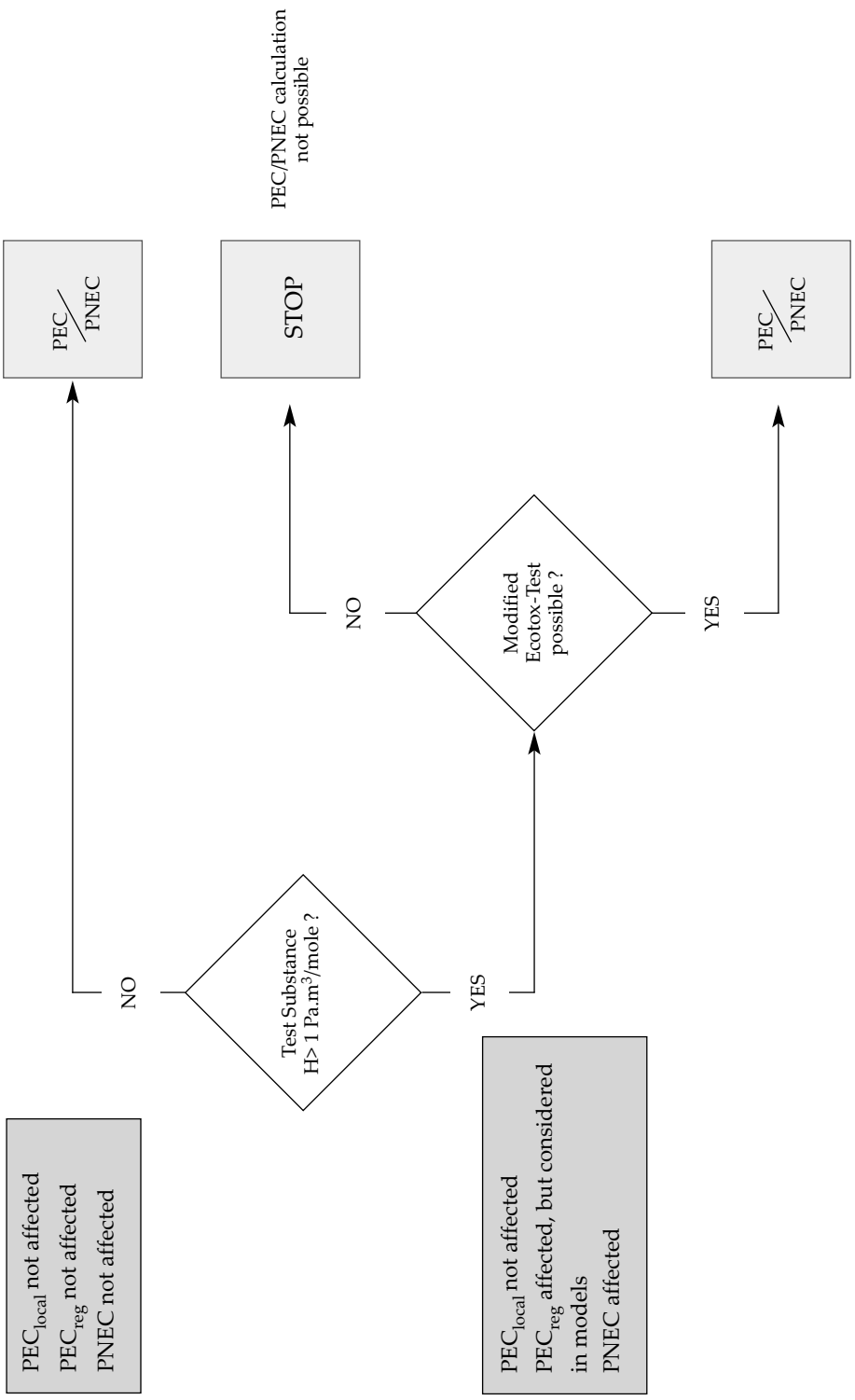


Figure 11: Decision chart as guidance on the environmental risk assessment of volatile substances for the aquatic compartment

7. METALS

Environmental risk assessment of metals is difficult because metals are characterised by a set of properties that require additional specific considerations. These unique properties are:

- The natural occurrence of metals as natural elements in all environmental compartments;
- the essentiality of some of the metals and the conditioning of organisms to natural background;
- the changes in bioavailability of metals, in the short and the long term, in the environmental compartments;
- the speciation - different ionic states - of metals in the environment.

Classical ecotoxicity tests do not consider essentiality for certain trace elements, nor the adaptation of organisms to the level of nutrients available. Further, dependent on the environmental conditions, different chemical species of a metal may be formed in the course of the test. These species may be more or less toxic than the metal species considered. This will have its implications on the derivation of the PNEC.

With regard to the PEC, exposure to metals in the environment is related to a) the natural cycling through different compartments and b) anthropogenic inputs. As EU risk assessments are related to anthropogenic activities, a proper quantification of both exposure sources is needed to prevent over or under estimation of the anthropogenic sources. Bioavailability and speciation can be different depending on the environmental conditions. This will have its implications on both modelling and monitoring data for the derivation of the PEC.

7.1 Property description - natural metal background

Metals are inherent constituents of the earth's crust. As a result of natural processes (e.g. wind and water erosion, abrasion of rocks, volcanic activity). They are cycled through the biosphere and, as such, are present at natural (background) concentrations in most environmental compartments (Thornton, 1996).

There is no such thing as a 'standard or single background concentration'. Due to local or regional differences in geochemistry, metal background concentrations can vary considerably at different sites and scales. Other geochemical factors, such as the age and leaching rate of the parent rock material, the metal complexation capacity of soils, seasonal factors (e.g. differences in river flow rate over the seasons) and also biological factors (e.g. concentration by and sedimentation of organisms, biomass input in autumn), metal concentrations in soils, sediments and surface waters may range considerably in place and time. Examples of this background variation are given below for the essential element zinc, but as a rule apply also to the other metals. A local or regional environmental compartment (water, soil, and sediment) is thus not characterised by a single background concentration, but by a background concentration range for each metal.

Background concentrations for zinc in surface waters are given in Table 9 (after Van Assche *et al*, 1996). It follows from this table that the background levels of zinc in marine waters differ by orders of magnitude from those in freshwater. Furthermore, for the freshwater compartment specifically, marked differences among geographic regions are observed. Table 9 shows that the background range of zinc in the North European lowland (covering the plains in France, Belgium, Netherlands, Germany, Denmark up to Poland) is between 5-40 µg/l (Zuurdeeg *et al*, 1992). It is emphasised that within this range, distinct waters show marked differences, e.g. the natural zinc level of the Rhine is estimated to be 5-10 µg/l (Zuurdeeg and Vriend, 1999), while the natural background of the Meuse is close to 30 µg/l (Zuurdeeg, 1980). The ranges reported in Table 9 also include the seasonal variation observed in most rivers: Zuurdeeg *et al* (1992) measured a variation of the zinc levels in the Sûre (Ardennes; considered natural levels) between 8 and 25 µg/l over a 1-year period. In Sweden, the seasonal variation on 3 rivers was included in the range 1 to 30 µg/l (Landner and Lindeström, 1998).

Table 9: Natural background zinc levels in surface waters (for references see text)

Surface water	µg zinc dissolved /l	µg zinc total / l
Marine		
Open ocean		<0.002-0.1
Coastal seas		0.5-1
Freshwater		
North American Great Lakes	0.03-0.3	
Amazon area, N-American Rocky Mountain area	0.02-0.25	
EU lowland area		5-40
Southern Ostrobothnia area (SF)	150-640	

On a local scale, elevated background levels in waters, soils and sediments that are due to naturally-occurring, metal-enriched parent rock material, are usually recognised and taken into account. It is noted that such enrichments occur rather frequently, as was shown in a recent study on mineral waters in Europe (Zuurdeeg and Vriend, 1999). However, when risk assessment is carried out at the regional scale, environments with different background levels can be included in the same legislative exercise, e.g. the EC "Existing Substances" risk assessment.

The natural background of metals, and in particular, the significant differences that can be observed in the same environmental compartment (e.g. freshwater) make direct application of the current risk assessment methodology difficult at PEC, as well as PNEC, levels.

7.2 Environmental risk assessment

7.2.1 Environmental exposure assessment

Determination of the natural background

Natural background levels are not always easy to quantify, due to the widespread influence of anthropogenic metal inputs to the environment. Several approaches for the estimation of the natural background have been employed and are currently under evaluation, e.g. derivation from geological maps, sampling in pristine areas, measurements on natural sources (water), analysis of older sediment.

Recommendation: Natural background levels and their variation in space and time for any specific metal should be assessed. A stepwise approach aiming to increase the accuracy of the estimation is recommended. Initial estimations can be made using geological maps. Such maps will allow on a large scale the identification of regions with high and low natural levels of metals. For a further refinement and for the estimation of the seasonal variation, monitoring data in time in pristine areas should be used. If no such data are available, monitoring data should be used with care, as a fraction of the concentration measured is of natural origin.

Interpretation of monitoring data

Ambient monitoring data will inevitably reflect the concentration of both the natural metal background, and the concentration added by anthropogenic activities. While risk is related to the total exposure concentration, quantification of the exposure due to the natural background concentration is needed. Indeed, when PEC/PNEC ratios greater than one are found, the contribution of both the natural background and the anthropogenic related concentration need to be known, in order to decide upon further actions to be taken to reduce the risks.

Recommendation: Background concentrations are usually estimated for a specific river or water catchment area, and the anthropogenic related concentrations are assessed by subtracting the natural background concentration from the total measured concentration. However, due to spatial and seasonal differences in bio-geochemical cycling rates (e.g. in surface waters), natural background levels measured at the local level should not be extrapolated to the regional scale. Similarly, if data are available, comparisons between ambient and background concentrations should preferably be done for the same water system. An alternative approach would be to model the anthropogenic exposure directly from an analysis of all anthropogenic (point and diffuse) sources (see section 7.2.3). In the absence of suitable data, a probabilistic approach should be used to estimate anthropogenic exposure levels (ICME, 2000).

The TGD further recommends the use of 90-percentile monitoring data. To estimate the 90-percentile of the anthropogenically related concentration there are two options:

- For each individual monitoring value, subtract the natural background concentration related to the moment and location/region of the monitoring value.
- If such data are not available, the 90-percentile of the natural background concentration of a location/region should be calculated/estimated and subtracted from the 90-percentile of the relevant 90-percentile of the monitoring data.

Identification and quantification of natural metal sources

In a modelling approach, the PEC anthropogenic (or PEC_{added}, see section 7.2.3.) can be calculated from an analysis of all emissions related to anthropogenic activities. Care must be taken however to ensure that 'so-called' anthropogenic inputs are truly man-made inputs. For example, zinc in household emissions originates to a greater extent from the natural zinc in foodstuff, and only partly from zinc contained in household products. Therefore, where the correct estimation of the natural background is an issue, the results from modelling should always be checked against monitoring data.

Recommendation: Where modelling is used for the PEC determination, potential natural metal inputs should be identified and incorporated into the model.

Defining the natural background range for an area to be assessed

For a local risk assessment, the anthropogenic inputs to the system exposure can be determined in a relatively straightforward way by using monitoring data to determine the natural background concentration range. For a regional risk assessment however, areas with strongly differing background ranges (Table 9) may be within the regional boundary. The fundamental question arising in such cases is whether or not all background conditions should be pooled together to provide one 'regional background', or areas with different backgrounds assessed separately. The overall aim is to reduce the variability and uncertainty in the data. Currently there are a number of questions for which guidance within the TGD is lacking, such as where, within the region, the area should be subdivided, or how to deal with interfaces between two areas with different background ranges.

This point is also important for setting the PNEC (see section 7.2.2) and is thus fundamental to risk assessment.

Recommendation: Areas/regions with similar ranges of background concentrations will need to be defined in the EU, aiming thereby to minimise the variability within a region and to maximise variability between regions. The issue of different backgrounds can be addressed by the metalloregion concept (see section 7.2.3), but the approach may be chosen on political, rather than on scientific considerations.

7.2.2 Environmental effects assessment

Conditioning of organisms to natural background - essentiality

Due to the ubiquitous presence of metals in the natural environment, organisms have become conditioned during the course of evolution to the natural background concentration and have developed the capacity to cope with natural variations, due for example to seasonal changes or fluctuations in river flow rates. For this reason, exposure of organisms to the natural background level reflects the theoretical lower limit of the PNEC, i.e. a concentration which, from an evolutionary perspective, does not present a risk to the survival of the species.

This theory is applicable for all metals. For the essential trace elements, however, this conditioning to natural background is even more crucial and has important proven consequences for risk assessment. Because of its importance, the relevancy for the PNEC of the conditioning of organisms to natural background will be discussed mainly from a perspective of essential elements (EEs). The possible parallelism with non-essential metals will be discussed later.

Essentiality

EEs are required by all organisms to grow and develop well. In nature, this requirement is satisfied by the natural (bio-available) background of the EEs. To keep the internal EE concentrations at required levels, homeostatic mechanisms have been developed throughout all taxonomic groups, including man.

As such, a 'window of essentiality' can be observed for each organism and for each EE, i.e. a concentration range within which the requirements of the organism for a given EE are satisfied. Since the source of this EE is the natural background, this window of essentiality is necessarily situated around the bio-available natural background range of the EE in that environment. The width of the window is defined by the organism's homeostatic capacity. Within the window of essentiality, the organism can regulate its internal EE concentration and experiences no stress (van Tilborg and Van Assche 1998).

However, when the external bio-available concentration falls outside the homeostatic regulation capacity, the organism is subject to stress from the EE, either from deficiency, or from toxicity. As a result of this stress, adaptation towards different sensitivity is observed; the organism's sensitivity will shift to lower EE levels as a result of EE deficiency, or it will become less sensitive to the EE as a result of toxic stress.

It was recently observed in laboratory experiments with zinc on standard test organisms (algae, Daphnids), that this shift in sensitivity can occur rapidly. For example, acute ecotoxicity values of algae increased by a factor of more then 30 when increasing the background concentration of zinc from 0.3 to 18 μg/l. Chronic ecotoxicity values for the same algae increased by a factor of more then 25 when increasing the background concentration from 0.3 to 1.4 μg/l. With Daphnids, it was also shown that it was readily reversible (LISEC, 1998; Muyssen and Janssen, 2000). Correspondingly, differences in sensitivity have been observed between organisms from the same species (spongae, Daphnids) that had been cultivated under conditions of different zinc levels in the field (Richelle *et al*, 1996; Muyssen and Janssen, 2000).

From these observations in the laboratory and in nature, it can be concluded that the sensitivity of organisms to EEs in an ecotoxicity test is determined to a large extent by the bio-available EE level that the organism experienced before testing. For risk assessment, this conclusion has the important consequence that an ecotoxicity result can only be relevant (or has the least uncertainty) for PNEC determination for a given area, if it was obtained under similar bio-available EE-conditions (concentration in the culture solutions before testing and in the test solutions) of that environment. Organisms originating from an environment with a bio-available EE background range different from the one assessed may indeed show a sensitivity (either higher or lower) that is not relevant for the environment assessed.

Recommendation: For PNEC determination, if different sensitivities are observed at different background concentrations, ecotoxicity data should be grouped according to the background concentration range of the culture medium. These ranges could be linked to the background concentration ranges of the EU regions defined (section 7.2.1). For each of the regions a PNEC should be derived using the relevant ecotoxicity data. Only ecotoxicity data obtained with organisms cultured under the EE concentration range of the environment under study should be used, as these have the least uncertainty.

In risk assessments, this phenomenon of conditioning to different backgrounds is usually well recognised in cases where an organism originates from an environment with elevated EE levels, e.g. in mineralised areas. However, conditioning also occurs towards very low concentrations e.g. for zinc in the N. American Great Lakes (Table 9). Such data should thus only be used to derive a PNEC for areas with a low background concentration range and not for areas with a higher background concentration range. Although frequently overlooked in laboratory studies, this may account for inter-laboratory differences. This point is discussed further below.

Conditioning of test organisms to low metal levels in the laboratory

In natural waters, the natural background of metals reflects the lower concentration range for any specific metal in that specific environment. In the laboratory, however, organisms are often cultured in artificial media, made up from distilled water, to which elements have been added. In such situation, even EEs are not always added; no metals at all are added to many standard test media, and EEs are considered to be present in the food source. For many organisms (e.g. Daphnids) this is not the natural situation (Keating *et al*, 1989). Even when EEs are added, they may be added at concentrations that are low compared to the natural EE background in the real environment e.g. the OECD 201 algae test protocol prescribes addition of 1.3 µg/l zinc, while the zinc background in the EU lowland system ranges between 5 and 40 µg/l (Table 9). Moreover as non-essential elements, as a rule, are not added to culture solutions, their concentration in artificial solutions are always lower than in natural waters.

In addition to the above, metal depletion phenomena can occur e.g. in laboratory batch cultures. Due to this depletion, the metal concentrations in the culture solution can become very low; this is rarely observed under natural conditions.

Organisms used for laboratory ecotoxicity testing are frequently cultured in artificial media for long periods of time. Due to the low or near-zero concentration of metals in the culture media, the organisms will adapt to lower concentrations than they would experience in the field. Thus they may become more sensitive to exposure to metals, as compared to the natural sensitivity of these organisms in their native environment. This phenomenon has been recognised only recently (Van Assche *et al*, 1998), and may be especially relevant to standard tests such as algae or Daphnids. This rationale does not apply to terrestrial ecotoxicity testing using natural soils where usually some background level of natural elements is present. It should however be considered if soil ecotoxicity testing is conducted using artificial substrates.

Recommendation: to avoid adaptation of laboratory test organisms to metal concentration outside the bio-available natural background range, artificial culture media for the culturing of test organisms in the laboratory should contain the bio-available metal (essential or non-essential) background levels observed in the real environment under investigation. To ensure that the culture medium is well balanced for all micronutrients, culturing in their own natural waters (at background level) is preferred.

7.2.3 Risk characterisation

There are two methods currently under discussion to address the natural background concentration: the 'added risk' approach and the 'metalloregion' approach.

The "added risk" approach

To provide a quantitative solution to the background issue, the 'added risk' approach has recently been proposed for assessing the risks related to metals. The added risk considers only exposure related to anthropogenic sources (PEC_{added}) and the effects related to added metal concentration ($PNEC_{added}$) in the ecotoxicity tests.

Recommendation: Although this approach is a step forward in dealing with the natural background on a quantitative basis, it is clear that all the more fundamental scientific issues listed above remain relevant and need to be considered in risk assessments. Therefore in its current application, the added risk approach does not provide an accurate estimate of the risk.

The 'metalloregion' concept

To integrate all background-related issues, the 'metalloregion' concept has recently been discussed (ICME, 2000). According to this concept, risk assessment should be based on data that are relevant only for the environment under assessment, with particular emphasis on natural background metal levels and physico-chemical conditions in the field and in the laboratory.

Recommendation: In practice, the metalloregion concept requires first an assessment of the physico-chemical conditions (i.e. natural background range and factors influencing bio-availability) relevant for the area under study in the risk assessment, with the problems that this may pose, (see section 7.2.1) and the possibility that more than one metalloregion needs to be distinguished in the area. Subsequently, a careful analysis should be made of the metal concentrations and conditions under which ecotoxicity data used for PNEC setting were obtained (origin of organisms, culture conditions before testing, test metal concentrations). Only ecotoxicity data obtained under conditions, relevant for the environment under study (when available) should subsequently be used to reduce the uncertainty of the risk assessment.

7.3 Property description - metal bio-availability/bio-geochemical cycling

Metals are cycled through the environment due to natural geochemical processes, and due to anthropogenic activities. As such, metals originally present in unavailable forms in the metal ores (e.g. CuS), are transformed into other chemical forms, and are cycled through the receiving ecosystem. The released metals are incorporated into the natural bio-geochemical cycles. Such bio-geochemical cycles do not distinguish anthropogenic from natural metal species and allow for the transformation of a specific metal form into other metal forms of lower/higher availability. The availability of the metal (originating from the natural background and the anthropogenically added metal) will hence depend on the physico-chemistry of the receiving environment, as well as on the reaction kinetics of the bio-geochemical processes.

7.3.1 The importance of bioavailability for metal toxicity

For many metals, only a small fraction of the metal present in the environment is available for biological uptake and hence for potential toxicity. This is already partially recognised by the fact that, for example, in the aquatic environment, only the dissolved metal fraction is accounted for in the exposure assessment. However, with copper for example, a recent study performed in the UK indicated that in British surface waters, greater than 99% of the dissolved copper was complexed by dissolved organic matter and hence unavailable for uptake (Comber, 2000). Similar observations were reported for copper by other authors, in other regions (e.g. Allen *et al*, 1999; Di Toro *et al*, 2000). On the contrary, in most artificial media, very small or no quantities of complexing agents are present. As a consequence, the availability and hence ecotoxicity of copper in artificial ecotoxicity test media is usually much higher than that observed in natural waters. This difference in metal availability between respectively natural surface waters (used for the determination of the background level and the PEC) and artificial media (used for PNEC determinations) therefore does not allow for a relevant risk evaluation and may result in PNECs below the natural background. For appropriate risk evaluation one main discussion point remaining is how to consider the variability in bioavailability among the natural surface waters as this may again vary with the physico-chemical characteristics of the surface waters investigated.

7.3.2 Ageing effects

Metal availability in the environment changes with time and hence depends on the reaction kinetics of the bio-geochemical processes.

Experimental data have shown that the results from ecotoxicity tests performed on freshly spiked media (especially sediments and soils) will be different from those obtained from ecotoxicity tests performed in 'aged' media (of similar physico-chemical characteristics). Therefore, to allow comparison of field monitoring data with ecotoxicity data at the same level of bioavailability, account needs to be taken of the reaction kinetics between the metals and the receiving environment.

7.4 Recommendations

For risk assessment purposes it is recommended that ecotoxicity tests should be performed under physico-chemical conditions similar to those of the environment under investigation (cf. background relevancy requirement explained under 7.2.2). This approach will ensure that the bioavailability of the exposure concentration [the naturally occurring metal concentration (the background) and the anthropogenic exposure concentration (PEC_{added})] and the effect concentration (PNEC) both have the same basis, typical for the environment under investigation. Alternatively, it may be advised to use speciation and/or bioavailability models (e.g. the biotic ligand model for copper (Di Toro *et al*, 2000), to further refine and extend the exposure and effects data through model extrapolations of existing literature data. To enable a proper refinement of the exposure assessment using monitoring data, besides the measurement of the metal, the physico-chemical parameters influencing the speciation/bio-availability of a metal in the environmental compartment under consideration also need to be measured.

With regard to the issue of ageing, short to medium term equilibration of the metal-spiked ecotoxicity media should be allowed for before the introduction of the test organisms to the exposure media. The equilibration times needed for the different compartments (water, soil, sediment) should be based on the knowledge of the reaction kinetics of the metal in the media (Ma *et al*, 1999). For soils and sediments, it is suggested to have no significant changes in the pore water concentrations.

7.5 Property description - multi-ionic states

Certain natural elements, some metals and metalloids, exist in the environment in a number of valency states. They are known as 'transition elements'; the transition metals are found in the centre of the periodic table in groups IB to VIIIB and form cations of +1, +2 and +3 oxidation state. (Raspor, 1991).

Examples: $Fe^{2+/3+}$; $Co^{2+/3+}$; $Cr^{3+/6+}$, $Cu^{1+/2+}$

Also metalloids can be found in different oxidation states.

Examples: $As^{-3/+3/+5}$; $Sb^{-3/+3/+4/+5}$; $Se^{-2/+4/+6}$

Transition elements can transform from one valency or redox state into another through oxido-reduction reactions.

Oxido-reduction refers to a reaction between two entities (oxidant and reductant) with a net exchange of electrons:

$$Ox\ 1 + Red\ 1 = Red\ 2 + Ox\ 2$$

Example: $Cr^{3+} + Cu^{1+} = Cr^{2+} + Cu^{2+}$

Parameters influencing the type, rate and extent of the reaction are the valency state of the element, the redox potential and pH of the medium, and the presence of reductants/oxidants.

Example:

Chromium exists in the environment in a number of valency states of which Cr^{6+} and Cr^{3+} are the most stable under prevailing environmental conditions. Under aerobic conditions and especially at higher pH, Cr^{6+} is rather stable, but under anaerobic conditions found in the environment, Cr^{6+} can be reduced to Cr^{3+}. The reaction is favoured by the presence of reductants as organic matter and Fe^{2+}, and in acidic conditions. Under such conditions, a complete reduction of Cr^{6+} to Cr^{3+} can occur in a few hours.

Cr^{3+}, stable in anaerobic conditions, can be oxidised to Cr^{6+} in aerobic conditions in the presence of a strong oxidising agent as MnO_2. The latter reaction is however more limited in rate and extent.

Speciation:

In the environment, different species of an element in a specific redox state can be found. The predominant forms are dependent on the oxidising nature of the entity and on the pH.

For example, Cr^{6+} is a strong oxidising agent and exists only as chromate or dichromate.

At environmental pHs the species of Cr^{6+} which can be found in solution are CrO_4^{2-}, $HCrO_4^-$ and $Cr_2O_7^{2-}$.

The predominant forms of Cr^{3+} present in solution range from Cr^{3+} at very low pH, to $Cr(OH)^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_3$ and $Cr(OH)^{4-}$ at very high pH.

Eh-pH diagrams can be constructed to identify the dominant form of an element likely to be found in a system at a given redox potential and pH.

The solubility of the different species differs and the solubility will determine the amount that can be found in solution and hence the bioavailablity and toxicity.

7.6 Environmental risk assessment

Multi-ionic elements, when released into the environment, can change their redox state. The reaction depends on the environmental conditions. In general, different redox states of the same element have different toxic properties and their fate and distribution in the environment will differ.

7.6.1 Environmental effects assessment

Under the prevailing environmental conditions, most of the Cr^{6+} when released into the terrestrial environment transforms rather quickly into Cr^{3+}. The absorption of Cr^{3+} on particulate matter is generally much higher than that of Cr^{6+}. In addition, Cr^{3+} is less toxic than Cr^{6+}. As mentioned above, multi-ionic substances can change their valency state during testing, and the different states have different toxic properties. The problems associated with testing of multi-ionic elements are analogous with those of unstable substances, namely, maintenance of 80% of the initial test concentration. Current OECD/EU ecotoxicity testing guidelines do not give specific guidelines on the testing of multi-ionic elements.

The OECD guidance document on aquatic toxicity testing of difficult substances (OECD, 2000a) recommends a preliminary stability study in water under conditions equivalent to those applied in the toxicity test. If significant losses due to oxido-reduction reactions occur, the exposure conditions should be modified to reduce the losses to a minimum. This can be achieved by adjusting the redox conditions and pH to minimise losses, but within the optimal range for ecotoxicity testing. Further losses can be reduced by the use of an adequate exposure regime (semi-static or flow-through) if appropriate for the test organism. When transformation occurs very rapidly, it may be impossible to keep the exposure concentration constant.

If the test substance is stable under the prevailing test conditions, toxicity can be attributed to this compound. When however the test conditions are such that the substance reduces or oxidises during the course of the test, an accurate assessment of the toxic concentration of the test substance might be difficult. In addition, if the redox product also exhibits toxic properties, the effect might be attributed to both the test compound and the redox product. It will therefore be important to know under which conditions the test compound will transform, and at what rate.

As is the case for unstable substances, the main challenge with regard to risk assessment of multi-ionic substances, is to relate toxicity, in quantitative terms, to the different redox products generated in the course of the test.

7.6.2 Environmental exposure assessment

In the current TGD, transformation due to oxido-reduction is not considered at the different scales of PEC calculation. Ignoring the redox behaviour of the test substance under environmental conditions will lead to an overestimation of the environmental exposure concentration. In addition, if the redox product is formed quite rapidly and it possess significant ecotoxic properties, its exposure concentration also needs to be assessed.

Monitoring data

Difficulties can exist in the proper interpretation of monitoring data. Field measurements are usually based on total concentrations, and it might be difficult to differentiate between the different species present in the environment. When significant differences in ecotoxicity exist between the different prevailing redox states in the framework of the risk assessment, it is important to quantify them.

Calculated exposures

Dissolved local concentrations are assessed on the basis of total concentrations and adsorption coefficients. The estimation methods given in the TGD for determining adsorption coefficients for soil, sediment and suspended matter are not applicable to metals in general. The TGD recommends using measured Kp values instead.

For multi-ionic substances the K_p values of the different redox states, which occur in the environment, will have to be determined. It might however be difficult to make the distinction between the adsorptive behaviour of the different redox states of the test compound, especially in circumstances under which the test compound transforms rapidly.

The determination of adsorption coefficients for Cr^{6+} for suspended matter and sediments is difficult, owing to its reduction to Cr^{3+}.

In addition, as the K_p value depends on the environmental conditions (pH, redox conditions), measurements should be made over a range of conditions (-at which the substance is found to be stable)- in order to extract a representative value.

Example:

Cr^{6+} added to the soil will remain mobile under neutral to alkaline conditions. Under acidic conditions, Cr^{6+} will adsorb to the soil matrix. While Cr^{3+}, when added to the soil, binds strongly to particulate matter. Adsorption will increase with increasing pH.

7.7 Recommendations

When assessing the risks related to the release into the environment of a multi-ionic element, fate and distribution of that element needs to be taken into account, especially if the substance transforms into a state which exhibits a different (more or less) toxicity to the environment.

It will therefore be important to commence by:

- Identifying the transformation (redox) products, which can be found under prevailing environmental conditions when the substance is released. These can be identified from Eh-pH diagrams;
- characterising the fate and distribution (rate and extent of redox reactions, adsorption/desorption, precipitation/dissolution) of the test substance and its redox products for each environmental compartment (soil, water, sediment, sewage treatment plant). As these are usually influenced by the pH, the characterisation should be made at acid, neutral and alkaline conditions.

If a substance is transformed rapidly, and maintenance of the test concentration is not possible within appropriate limits, the redox rate or half-life of the priority substance in the test system may serve to inform decisions on the further testing and risk assessment (test substance versus redox product). The current TGD uses a threshold of 12 hours (DT_{50}). For substances with a DT_{50}< 12 hours, the effects are likely to be attributed to the redox products rather than to the test substance. For such substances testing and subsequent risk assessment should be conducted on the redox product.

For substances for which the test concentration can be kept stable (within 80% of initial test concentration) ecotoxicity tests can be used directly for the risk assessment.

For substances with an intermediate redox rate, it should be decided, on a case-by-case basis, whether the potential redox product is to be included in the risk assessment. Factors which need to be evaluated are the toxicity of the redox product and its fate and distribution under the environmental conditions.

It should be noted that the redox reaction rate is dependent upon the pH conditions of the environmental compartment. If important differences exist between acid, neutral or alkaline conditions, an evaluation should be made at different pH's.

There may be substances for which the DT_{50} is less than 12 hours under a specific set of conditions and greater than 12 hours under another set of conditions. For such substances, an assessment is needed of both the redox product and the priority substances, each related to the environmental conditions in which they exist.

If accurate measurement of the different redox states in the environment is possible, such monitoring should be used for the characterisation of the exposure concentration.

However, if no differentiation can be made between the different redox states, calculation of the environmental exposure concentration is recommended.

The redox reaction can be interpreted as a form of abiotic degration, as it leads to the removal of a compound from the environment. The rate of the redox reaction and the DT_{50} of the multi-ionic element should be included in the assessment of the overall degradation rate constant. Again, as this reaction will depend upon the environmental conditions (especially pH and Eh), the assessment should cover different relevant environmental conditions.

Assessment of the K_p value

If a substance undergoes rapid transformation, (DT_{50} < 12 hours), the K_p value of the redox product should be measured.

For substances for which the test concentration can be kept stable (within 80% of initial test concentration) the K_p value of the priority substance needs to be measured.

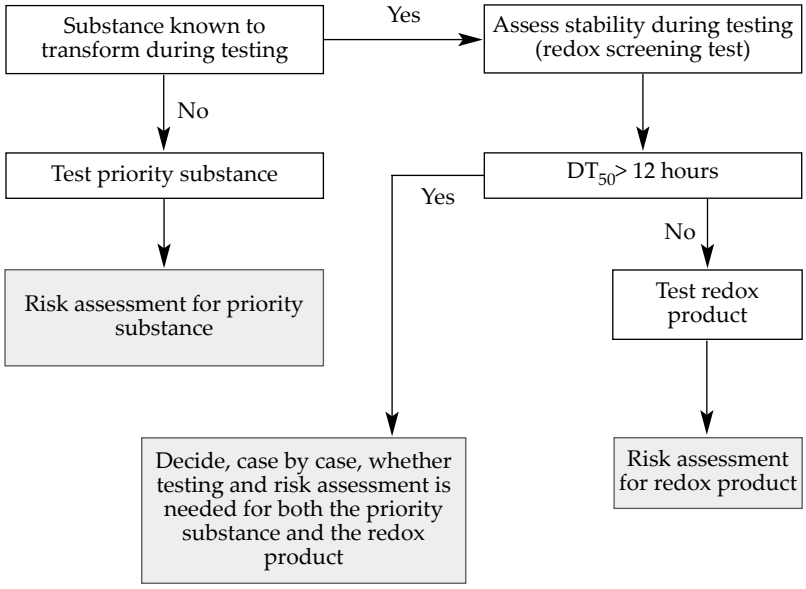
For substances with an intermediate redox rate, it should be decided, on a case-by-case basis, whether the potential redox product is to be included in the risk assessment. Factors which need to be evaluated are the toxicity of the redox product, and its fate and distribution under the environmental conditions.

As the K_p value depends on the environmental conditions (pH, redox conditions), measurements should be made over a range of conditions at which the substance is stable, in order to determine a representative value.

There may be substances which transform rapidly under a specific set of conditions, and which remain stable under a different set of conditions. For such substances, both the Kp value of the priority substance and the redox product need to be measured, each under the conditions in which they exist.

A proposed scheme incorporating the above recommendations is given in Figure 12.

Figure 12: Proposed scheme for ecotoxicity testing of multi-ionic substances



7.8 Property description - transformation to organometallic compounds

Certain metals and metalloids when released into the environment can be transformed into organometallic compounds due to biotic and abiotic processes. An important process is biomethylation of certain metals, which generally occurs by sulphate reducing micro-organisms in sediment and soil. The methylated metals or metalloids can be more or less toxic than the metal or metalloid. For example mercury and tin can be methylated and both methylmercury and methyltin are more toxic than mercury and tin. On the other hand, methylation of arsenic and selenium contributes to their detoxification.

Recommendation:

When assessing the risks related to the release of metals and metalloids, known to transform into organometallic compounds into the environment, fate and distribution of the element needs to be taken into account, especially if the substance transforms into a form that exhibits a different (more or less) toxicity to the environment.

It will therefore be important to start by:

- Identifying the organometallic compounds that can be found under prevailing environmental conditions, when the substance is released;
- characterising the fate and distribution (rate and extent of transformation reactions, adsorption/desorption, precipitation/dissolution) of the test substance, and its organometallic transformation products, for each environmental compartment (soil, water, sediment, sewage treatment plant).

If a substance is transformed rapidly, and maintenance of the test concentration is not possible within appropriate limits, the transformation rate or half-life of the priority substance in the test system may serve to inform decisions on the further testing and risk assessment (test substance versus organometallic compound). The current TGD uses a threshold of 12 hours (DT_{50}). For substances with a DT_{50}< 12 hours, the effects are likely to be attributed to the organometallic compound rather than the test substance.

For such substances, testing and subsequent risk assessment should be conducted for the organometallic compound.

For substances for which the test concentration can be kept stable (within 80% of initial test concentration), ecotoxicity tests can be used directly for the risk assessment.

For substances with an intermediate transformation rate, it should be decided on a case-by-case basis whether the organometallic compound is to be included in the risk assessment. Factors that need to be evaluated are the toxicity of the organometallic compound, and its fate and distribution under the environmental conditions.

There may be substances for which the DT_{50} is lower than 12 hours under a specific set of conditions, and higher than 12 hours under another set of conditions. For such substances, an assessment is needed of both the organometallic compound and the priority substances, each related to the environmental conditions in which they exist.

If accurate measurement is possible of both the metal/metalloid and the formed organometallic compounds in the environment, such monitoring should be used for the characterisation of the exposure concentration.

However, if no differentiation can be made between the forms of the substance, calculation of the environmental exposure concentration is recommended.

GLOSSARY

Primary biodegradation
The structural change (transformation) of a chemical substance by microorganisms resulting in the loss of chemical identity.

Ultimate aerobic biodegradation
The breakdown of a chemical substance by microorganisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralisation) and the production of new biomass and organic microbial biosynthesis products.

Bioaccumulation
The net result of uptake, distribution and elimination of a substance due to all routes of exposure.

Bioavailability
The ability of a substance to interact with the biosystem of an organism. Systemic bioavailability will depend on the chemical or physical reactivity of the substance and its ability to be absorbed through the gastrointestinal tract, respiratory surface or skin. It may be locally bioavailable at all these sites.*

Bioconcentration
The net result of uptake, distribution and elimination of a substance from water.

Bioaccumulation Factor (BAF)
The ratio of the steady-state concentration of a substance in an organism due to all routes of exposure vs. the concentration of the substance in water.

Bioconcentration Factor (BCF)
The ratio of the steady-state concentration of a substance in an organism due to water-borne exposure vs. the concentration of the substance in water.

Biomagnification
The accumulation and transfer of substances via the food web (e.g. algae - invertebrate - fish - mammal) due to ingestion, resulting in an increase of the internal concentration in organisms at the succeeding trophic levels.

CMC
Critical Micelle Concentrations (see Römpp, 2000).

Degradation rate constant
A first order or pseudo first order kinetic rate constant, k (d^{-1}), which indicates the rate of degradation processes. For a batch experiment, k is estimated from the initial part of the degradation curve obtained after the end of the lag phase.

Desorption
Reverse process of sorption, means release from the particulate to water; not always an equilibrium process but in some cases very slow, e.g. when ion exchange is required or when released from intraparticle micropores.

Dissolved organic carbon (DOC)
That part of the organic carbon in a sample of water which cannot be removed by specified phase separation, for example by centrifugation at 40000 ms^{-2} for 15 min, or by membrane filtration using membranes with pores of 0.2 μm - 0.45 μm diameter.

EC_{50} Value (median lethal concentration)
A statistically derived concentration which, over a defined period of exposure, is expected to cause a specified toxic effect in 50% of the test population.

Environmental Compartments
Subdivisions of the environment which may be considered as separate boxes, and which are in contact with each other. A simple model would separate the environment into air, water, and soil, with biota, sediment (bottom and suspended), layering of water bodies, and many other refinements being allowed if data to support their inclusion are available. Concept from Mackay (1991).

Hemimicelles
Coating of particulate surfaces with micelles at sub CMC which results in reversing of the surface charge (see Schwarzenbach, 1993).

PEC
Predicted Environmental Concentration. The concentration of a chemical in the environment, predicted on the basis of available information on certain of its properties, its use and discharge patterns and the quantities involved. *

PEC_{local}
In the EU TGD, the PEC predicted for the vicinity of a point source e.g. a production or formulation site, or a wastewater treatment plant.

$PEC_{regional}$
In the EU TGD, the PEC averaged over a standard European region of 200km x 200km, with twice the average European population density and production capacity.

PNEC
Predicted No Effect Concentration: environmental concentration that is regarded as a level below which the balance of probability is that an unacceptable effect will not occur.

Secondary Poisoning
The product of trophic transfer and toxicity.

Sorption
The process in which chemicals become associated with solid phases (either adsorption onto a two-dimensional surface or absorption into a three-dimentional matrix).

* From Van Leeuwen and Hermens, 1996

APPENDIX I: 4TH EU PRIORITY LIST OF CHEMICALS FOR RISK ASSESSMENT

Chemicals for Risk Assessment under Regulation 793/93/EC

'Difficult' property associated with the substance

	CAS No	Substance name	Rapporteur	Natural occurence	Ionic state	Sorptivity	Surface active compounds	Unstable / reactive substances	Volatility	Water solubility
1	77-47-4	hexachlorocyclopentadiene	NL							
2	79-94-7	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol	UK							
3	88-72-2	2-nitrotoluene	E							
4	98-54-4	4-tert.butylphenol	N							
5	98-73-7	4-tert.butylbenzoic acid	D							
6	107-98-2	1-methoxypropan-2-ol	F							
7	108-65-6	1-methoxypropan-2-ol acetate	F							
8	111-76-2	2-butoxyethanol	F							
9	112-07-2	2-butoxyethanol acetate	F							
10	112-90-3	(Z)-octadec-9-enylamine	D							
11	121-14-2	2,4-dinitrotoluene	E							
12	124-30-1	octadecylamine	D							
13	994-05-8	2-methoxy-2-methylbutane	FIN							
14	1222-05-5	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran	NL							
15	1309-64-4	diantimony trioxide	S							
16	1310-73-2	sodium hydroxide	P							
17	1330-43-4	disodium tetraborate, anhydrous	A							
18	1506-02-1	1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexa-methyl-2-naphthyl)ethan-1-one	NL							
19	3333-67-3	nickel carbonate	DK							
20	7718-54-9	nickel dichloride	DK							
21	7784-18-1	aluminium fluoride	NL							
22	7789-75-5	calcium fluoride	NL							
23	10043-35-3	boric acid, crude natural (<= 85% boric acid H_3BO_3 dry weight)	A							
24	11113-50-1	boric acid	A							
25	13138-45-9	nickel dinitrate	DK							
26	13674-87-8	tris(2-chloro-1-(chloromethyl)ethyl phosphate	IRL/UK							
27	26523-78-4	tris(nonylphenyl) phosphite	F							
28	38051-10-4	2,2-bis(chloromethyl)trimethylene bis(bis(2-chloroethyl) phosphate	IRL/UK							
29	61788-45-2	amines, hydrogenated tallow alkyl	D							
30	61788-46-3	amines, coco alkyl	D							

APPENDIX II: USE OF FISH HALF-LIFE IN HAZARD AND BIOACCUMULATION ASSESSMENT

For PWSS that do not undergo appreciable biotransformation, past research indicates that the half-life is related to the log K_{ow} of the substance and the lipid content and size of the organism (Thomann, 1989). Empirical relationships for a given fish species can be used to provide a log K_{ow} cut-off, below which standard chronic tests would be sufficient for achieving steady-state conditions. For example, Fisk *et al* (1998) reports the following relationship between the half-life of 18 recalcitrant organochlorine compounds in trout (mean weight and lipid content of ca. 3 grams and 3%, respectively) and log K_{ow}:

$$\log T_{50} = -3.7 + 1.5 * \log K_{ow} - 0.1 * (\log K_{ow})^2 \quad (1)$$

Based on this equation, non-metabolisable substances with log K_{ow} below ca. 4.7 are predicted to have a T_{50} < 14 days. Therefore, the results of standard chronic tests with trout with similar size/lipid content, should not be confounded by non-steady state concerns for any substance with a log K_{ow} below this cut-off. Smaller fish species (e.g. fathead minnow, zebrafish, Japanese medaka), are expected to attain equilibrium even sooner.

However, this approach may significantly overestimate the T_{50} of many commercially important industrial chemicals since biotransformation is ignored. Biotransformation will reduce the time required for achieving steady-state in aquatic toxicity tests, and limit the potential for foodchain biomagnification.

One indicator of the potential role of biotransformation is the ready biodegradability classification of the substance. In principle, substances that can be readily or inherently biodegraded by microorganisms are also likely to be biotransformed extensively by fish. For such substances, experimental determination of the half-life in fish is therefore recommended as a key input into a tiered framework for PWSS assessments.

Often, the most convenient and cost-effective approach for determining the half-life of PWSS in fish is via a dietary test. The simplest experimental design is to feed spiked food at a fixed ration over a specified period of time (e.g. one to four weeks depending on expected half-life). At the end of the exposure period a sample of exposed fish is analysed for the parent test substance (time=0 of the depuration phase). The remaining fish are transferred to clean diet and sequentially sampled and analysed over time so that a depuration curve can be established. From these data the half-life, dietary assimilation efficiency and biomagnification factor (BMF), defined as the steady-state ratio of the concentration in fish to that in the diet, can be readily derived. Since the diet is expected to be a significant route of exposure of PWSS to fish (Thomann, 1989), dietary bioaccumulation tests may in fact be more relevant than bioconcentration studies that rely on aqueous exposure. Further, dietary bioaccumulation tests are practically much easier to conduct since higher and constant exposure concentrations of PWSS can be administered via the diet than via water. As a result, concentrations in fish tissue are expected to be less difficult to quantify analytically. Further research that quantifies the relationship between *in vitro* test results (e.g. cell cultures) that indicate

biotransformation capability with half-lives obtained using *in vivo* tests, could offer a cost-effective future tool that could help reduce testing costs and minimise vertebrate testing (Sijm *et al*, 1997). Selection of a 15-day half-life cut-off in fish, not only ensures that a standard chronic partial life cycle test for fish is sufficiently long to attain steady-state but also ensures that biomagnification of the substance does not occur. Mathematically, the BMF is given by (Fisk *et al*, 1998):

$$BMF = C_{fish} / C_{diet} = E^* I / K = 1.44 * E * I * T_{50} \quad (2)$$

Where:

E = Assimilation efficiency (g substance assimilated/g substance ingested)
I = Ingestion rate (g food/g wet fish /day)
K = First-order elimination rate (1/day)
and
$K = 0.693/ T_{50}$

For a 1 gram fish with a typical ingestion rate of 0.03 g food/g wet fish/day, and assuming the maximum possible assimilation efficiency (i.e. E=1), the maximum BMF for a substances with a half-life < 15 days is below 0.6. Since the BMF is below one, such substances are not expected to biomagnify in the aquatic foodchain.

The results obtained from a dietary bioaccumulation test may also provide additional information helpful for the risk assessment of PWSS. For example, the bioconcentration factor (BCF), which expresses the steady-state concentration ratio in fish to that in water, is usually estimated assuming first-order kinetics:

$$BCF = k_u / K \quad (3)$$

Where:

k_u = uptake clearance (ml/g_{wet}/day)

Previous research indicates that the uptake clearance in fish is relatively constant between log K_{ow} of 3 to 6 but varies as a function of weight (W) in grams wet as follows (Sijm and Hermens, 2000):

$$k_u = 550\ W^{-0.27} \quad (4)$$

For nonionic organic chemicals with log K_{ow} above 6, the uptake clearance has been shown to decline so the above equation provides a conservative estimate if applied to more hydrophobic substances. If the uptake clearance estimate obtained from equation 4, is combined with the results of an experimentally derived elimination rate obtained in a dietary bioaccumulation test, equation (3) can be used to provide a conservative BCF estimate for a PWSS. To illustrate this calculation, the following example is provided.

A dietary bioaccumulation test with 2,2,4,4,6,6-pentamethylheptane (PMH) was recently performed with fathead minnow (weight = 0.5 grams), yielding a first order elimination rate of 0.23 d-1 (unpublished data, Exxon Biomedical Sciences). Using equation (4) the uptake clearance for this size fish is estimated to be 660 ml/g/day. Application of equation 3 therefore yields a BCF estimate of 2888 ml/g_{wet}. This estimate is in excellent agreement with a recent bioconcentration study performed with fathead minnow by Tolls and van Dijk (in press), who report a BCF in the range of 880 to 3500 ml/g wet for this substance. These experimentally derived BCF estimates are more than an order of magnitude lower than the predicted BCF of 45182 that is estimated from log K_{ow} (6.0) based on the recommended QSAR by Geyer *et al*, 2000 (assuming a fish lipid content of 5%). This QSAR was developed for organic chemicals that are poorly metabolised in fish, based on a detailed critical review of available BCF literature.

Dietary bioaccumulation tests can also be used in conjunction with mode-of-action-based internal effect concentrations to provide a rationale for selecting appropriate doses in a dietary toxicity test. Based on rearrangement of equation (1), the dietary concentration that corresponds to a narcosis threshold can be determined. For example, the internal effect concentration reported to elicit chronic effects via a nonpolar narcotic mechanism is > 0.1 mmol/kg_{wet} (McCarthy and Mackay, 1993). Based on test previously discussed above for PMH, a dietary assimilation efficiency of 0.12 was determined. Given a molecular weight of 158 g/mol, and assuming a daily food ratio of 0.03 g_{food}/g_{wet}/d, a dietary concentration of 1,000 ppm is predicted to correspond to an internal threshold for chronic narcosis. Logically, this concentration might be selected for investigation in a "limit" test. If chronic effects were not observed at this elevated concentration, more specific modes of toxic action would appear unlikely.

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ECETOC PUBLISHED REPORTS

Monographs

No.	Title
No. 1	Good Laboratory Practice
No. 2	A Contribution to Strategy for Identification and Control of Occupational Carcinogens
No. 3	Risk Assessment of Occupational Chemical Carcinogens
No. 4	Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No. 6	Acute Toxicity Tests, LD_{50} (LC50) Determinations and Alternatives
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary)
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No. 11	Eye Irritation Testing
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity)
No. 13	DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment
No. 14	Skin Sensitisation Testing
No. 15	Skin Irritation
No. 16	Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals
No. 19	Respiratory Allergy
No. 20	Percutaneous Absorption
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation
No. 22	Evaluation of Chemicals for Oculotoxicity
No. 23	Receptor Mediated Mechanisms in Chemical Carcinogenesis
No. 24	Risk Assessment for Carcinogens
No. 25	Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents
No. 26	Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances
No. 27	Aneuploidy
No. 28	Threshold-Mediated Mutagens - Mutation Research Special Issue
No. 29	Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment
No. 30	Genetic Susceptibility to Environmental Toxicants
No. 31	Guidance on Evaluation of Reproductive Toxicity Data
No. 32	Use of Human Data in Hazard Classification for Irritation and Sensitisation

Technical Reports

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man
No. 6	Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2
No. 7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8	Biodegradation Testing: An Assessment of the Present Status
No. 9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11	Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5
No. 12	The Phototransformation of Chemicals in Water: Results of a Ring-Test
No. 13	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment
No. 14	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health
No. 15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16	A Review of Recent Literature on the Toxicology of Benzene
No. 17	The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4
No. 18	Harmonisation of Ready Biodegradability Tests
No. 19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20	Biodegradation Tests for Poorly-Soluble Compounds
No. 21	Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment
No. 22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24	The EEC 6th Amendment: Prolonged Fish Toxicity Tests
No. 25	Evaluation of Fish Tainting
No. 26	The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride
No. 27	Nitrate and Drinking Water
No. 28	Evaluation of Anaerobic Biodegradation
No. 29	Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns
No. 30	Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available)
No. 31	The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment
No. 32	Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data
No. 33	Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34	Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man
No. 35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36	Biomonitoring of Industrial Effluents
No. 37	Tetrachlorethylene: Assessment of Human Carcinogenic Hazard
No. 38	A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39	Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No. 40	Hazard Assessment of Chemical Contaminants in Soil

No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43 Emergency Exposure Indices for Industrial Chemicals
No. 44 Biodegradation Kinetics
No. 45 Nickel, Cobalt and Chromium in Consumoducts: Allergic Contact Dermatitis
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models
No. 51 Environmental Hazard Assessment of Substances
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment
No. 55 Pulmonary Toxicity of Polyalkylene Glycols
No. 56 Aquatic Toxicity Data Evaluation
No. 57 Polypropylene Production and Colorectal Cancer
No. 58 Assessment of Non-Occupational Exposure to Chemicals
No. 59 Testing for Worker Protection
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard
No. 61 Environmental Exposure Assessment
No. 62 Ammonia Emissions to Air in Western Europe
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man
No. 65 Formaldehyde and Human Cancer Risks
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank
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No. 69 Toxicology of Man-Made Organic Fibres
No. 70 Chronic Neurotoxicity of Solvents
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members)
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No. 80 Aquatic Toxicity of Mixtures
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No. 82 Risk Assessment in Marine Environments
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union
No. 84 Scientific Principles for Soil Hazard Assessment of Substances
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies
No.86 Derivation of Assessment Factors for Human Health Risk Assessment
No.87 Contact Sensitisation: Classification According to Potency

Joint Assessment of Commodity Chemicals (JACC) Reports

No.	Title
No. 1	Melamine
No. 2	1,4-Dioxane
No. 3	Methyl Ethyl Ketone
No. 4	Methylene Chloride
No. 5	Vinylidene Chloride
No. 6	Xylenes
No. 7	Ethylbenzene
No. 8	Methyl Isobutyl Ketone
No. 9	Chlorodifluoromethane
No. 10	Isophorone
No. 11	1,2-Dichloro-1,1-Difluoroethane (HFA-132b)
No. 12	1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124)
No. 13	1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123)
No. 14	1-Chloro-2,2,2-Trifluoromethane (HFA-133a)
No. 15	1-Fluoro 1,1-Dichloroethane (HFA-141B)
No. 16	Dichlorofluoromethane (HCFC-21)
No. 17	1-Chloro-1,1-Difluoroethane (HFA-142b)
No. 18	Vinyl Acetate
No. 19	Dicyclopentadiene (CAS: 77-73-6)
No. 20	Tris-/Bis-/Mono-(2 ethylhexyl) Phosphate
No. 21	Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3)
No. 22	Hydrogen Peroxide (CAS: 7722-84-1)
No. 23	Polycarboxylate Polymers as Used in Detergents
No. 24	Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25	1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0)
No. 26	Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27	n-Butyl Acrylate (CAS No. 141-32-2)
No. 28	Ethyl Acrylate (CAS No. 140-88-5)
No. 29	1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30	Methyl Methacrylate (CAS No. 80-62-6)
No. 31	1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32	Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33	1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34	Acrylic Acid (CAS No. 79-10-7)
No. 35	Methacrylic Acid (CAS No. 79-41-4)
No. 36	n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37	Methyl Acrylate (CAS No. 96-33-3)
No. 38	Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39	Tetrachloroethylene (CAS No. 127-18-4)
No. 40	Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
No. 9	Styrene Criteria Document
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
No. 11	Ecotoxicology of some Inorganic Borates
No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents
No. 14	n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
No. 15	Examination of a Proposed Skin Notation Strategy
No. 16	GREAT-ER User Manual

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development
No. 33	Environmental Oestrogens: A Compendium of Test Methods
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
No. 37	EC Classification of Eye Irritancy
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction