Risk Assessment in Marine Environments

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SUMMARY

Within Europe, risk assessments for new and existing substances for the freshwater aquatic environment are based on the standardised procedures embodied in the Technical Guidance Document (TGD) and the EUSES model. These enable PECs to be determined for generic environments covering a range of spatial scales, when suitable measured data or site-specific models are not available or not applicable. Methods to address risk assessment procedures for marine environments are currently being developed and it is desirable to define marine assessment schemes which parallel and complement this freshwater approach.

The extension of the well-established principles of the risk assessment framework elaborated in the TGD (i.e. exposure assessment, hazard identification, risk characterisation, risk characterisation revision/reiteration, risk reduction, risk acceptance) should provide a consistent approach which may be readily implemented and accepted and which can be used to address issues surrounding the impact of anthropogenic emissions to the marine environment. Additional concerns associated with the marine environment, i.e. secondary poisoning of marine predators, need to be accommodated within this framework. To avoid confusion, in all cases a PEC/PNEC ratio of ≥1 should be adopted as an indicator of unacceptable risk. Any precautionary allowances for uncertainty should be applied to either the PEC or PNEC, as appropriate.

In certain instances, some or all of a marine risk assessment will be site specific (relating to a known geographical area) and in such cases monitoring data representative of that area, or site specific models, may be available that can be used to define the distribution of a chemical resulting from a single or from multiple emission sources. When relevant monitoring data are not available or when a site specific model is not appropriate, the assessment scheme is based on the generic model used in the TGD to represent the freshwater and terrestrial environment of Europe, but identifying when and how this model can be modified to address the marine environment. Although a number of different mathematical models exist to predict the behaviour and fate of chemicals in the marine environment, for both specific and generic scenarios, these cannot be adopted or refined until the underlying assumptions have been examined more thoroughly.

As in the TGD, the spatial scales proposed are (marine) local, regional and continental, but these are conceptually nested within a further "global" scale, representing the world's oceans, although the proposed risk assessment techniques are restricted to the three smaller scales. Generic local scenarios, comparable with those described in the existing TGD, are defined for the marine environment. A conceptual marine region is proposed as the estuarine and coastal sea area that receives the river water flow and atmospheric load emanating from the terrestrial/freshwater "default" region defined in the TGD, although it may be beneficial to consider two or more different regional scenarios. The dimensions of the continental scale will depend on the size selected for the regions within it; an example is given which is similar in size and characteristics to the North Sea. The report demonstrates how the marine regional and continental scales can be modelled by adjusting the parameters of the current EUSES model.

The modelling approaches used in risk assessment rely on the accuracy of key physico-chemical data. To assess the fate and effects of organic solutes in marine waters, it is important to consider the apparent increase in hydrophobicity of the substance, or its tendency to partition out of the aqueous phase, beyond that which applies in fresh water. However, the data suggest that, for nonpolar organic substances, the magnitude of difference in solubility and partition coefficients between sea water and fresh water is relatively small and will generally be of little significance for elucidating environmental risk.

Persistence needs to be addressed when estimating the presence of a chemical in the marine environment. In principle, half-life criteria should be used to determine the rate of degradation in the environment and both biotic and abiotic degradation pathways should be taken into account. It is difficult to assess degradation in the marine environment on the basis of existing laboratory tests, however, it is reasonable to conclude that biodegradation rates in marine environments, especially in estuarine and coastal regions with higher nutrient levels and sediment disturbance, can be as great as in fresh water. The same translation of rate constants from standard test data used for freshwater assessments should be employed for the marine regional model.

In the absence of half-life values for biodegradation, positive ready biodegradation test results should be used with care to provide rate constants (from the TGD) for use in the marine assessment. When data from specific inherent tests are available, a less conservative approach should be considered, and the data should be taken as evidence of marine biodegradation. A strategy for marine biodegradation testing is proposed.

Although further research is required in a number of areas, a framework is proposed, for marine risk assessment, using existing tools and knowledge, that allows exposure (PECs) to be defined at the local, regional and continental scale.

Bioconcentration from water into marine organisms is only likely to differ significantly from that in fresh water when the form or speciation of the substance is directly affected by the ionic composition of sea water. For neutral, hydrophobic organics, bioconcentration measurements or predictions based on freshwater data will provide reasonable estimates of marine bioconcentration potential.

As for bioconcentration, the biomagnification potential in marine food chains may differ significantly from that in fresh water when the chemical speciation of the substance is directly affected by the chemistry of sea water. It is also possible that the greater complexity of marine food chains, and the more variable lipid content of marine organisms, requires special consideration in risk assessment, compared with the existing EU process for fresh water. To allow for such uncertainty, modifications are proposed to the current method for determining the exposure (PEC) for birds and mammals feeding on fish at the local-regional level and, at the regional-continental level, for larger mammals preying on these fish-eating birds and mammals. Whenever possible the estimated PEC values in marine organisms and their predators should be supported by existing measurements in biota.

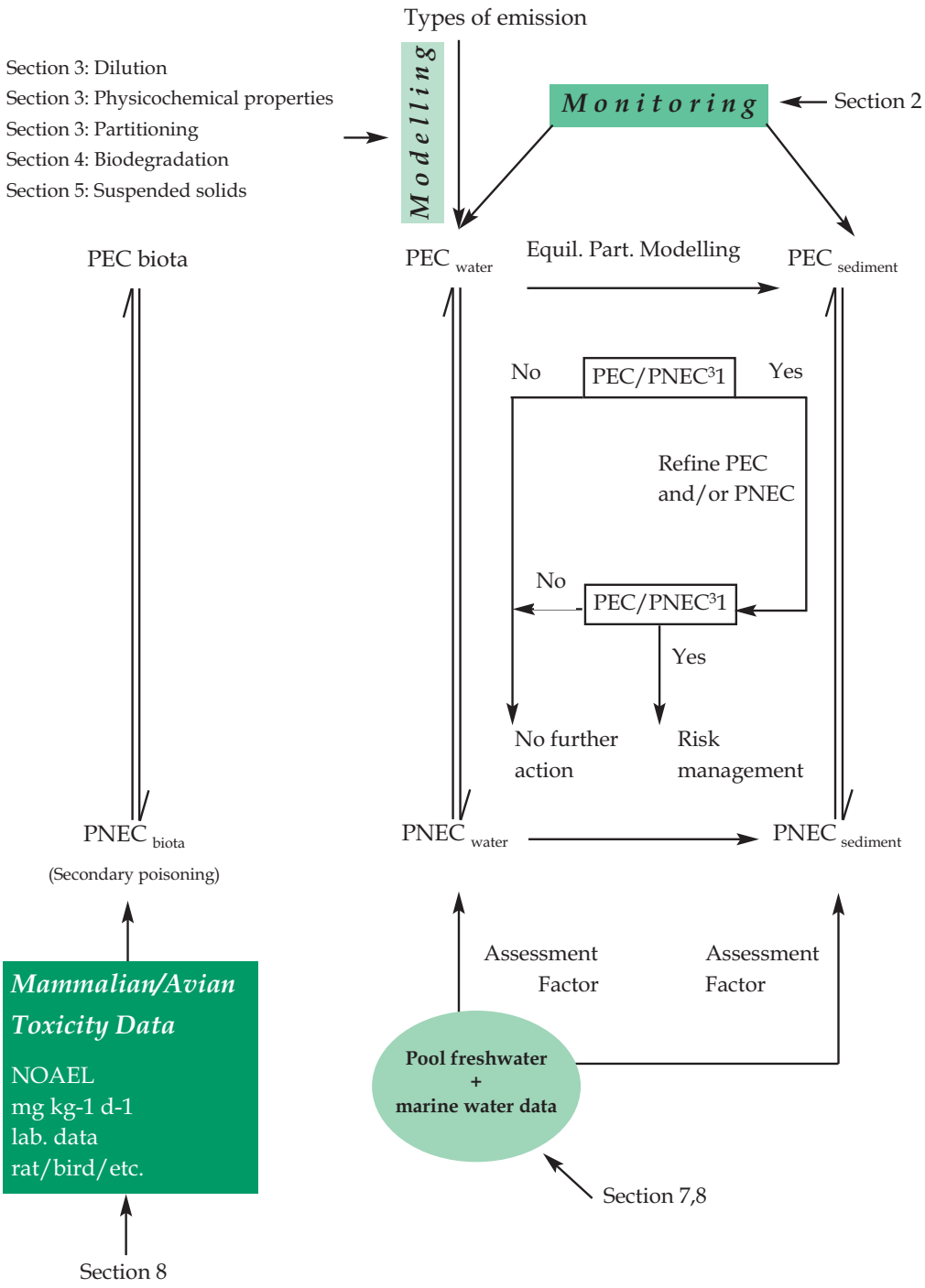
The ecotoxicity database relating to the effects of chemical substances on marine and estuarine organisms is comparatively limited, especially for organic compounds. Although it is desirable that the ecotoxicity database for such species is extended, the data reviewed and current marine risk assessment practice suggest a reasonable correlation between the ecotoxicological responses of freshwater and saltwater biota - at least for the classical aquatic taxa (i.e. fish, crustacea, algae). There does not appear to be any marked difference in sensitivity between freshwater and saltwater biota that systematically applies across all three trophic levels considered. Where evaluated, differences between trophic levels within each medium were generally as significant or even more marked. Such variation is implicitly assumed in the use of assessment factors in current risk assessment practice. Overall, the use of freshwater acute effects data in lieu of, or in addition to, saltwater effects data for risk assessment purposes is not undermined by the empirical data reviewed in this report. The use of pooled freshwater and seawater data is therefore recommended. Under such circumstances, PNEC values should be derived from the most sensitive result regardless of the medium.

Within the marine environment (although not necessarily exclusively) there is a need to consider the impact of the unknown long-term effects of bioaccumulation and biomagnification in complex food chains which include larger marine species of mammal or birds (i.e. high trophic level species). In order to characterise the risk to biota, it is necessary to obtain an estimate of the concentration of the chemical to which the organisms are exposed, considering all potential routes of exposure. The exposure concentration may be measured by analytical monitoring or predicted by modelling, or a combination of both. The ways in which monitoring data and different modelling techniques can be used to describe the distribution of a chemical depend on the spatial scale considered. For example, preliminary release from a point source necessitates a local assessment in the immediate proximity of the point of discharge, whilst further distribution and fate is dealt with at the regional scale. A similar differentiation may also be applied to the marine environment, and a multi-stage/scale approach is proposed for the risk assessment of chemicals in marine ecosystems:

- at the local scale, concentrations of contaminants from point sources will generally be greatest in close proximity to the discharge and the assessment of risk within the water and sediment compartments will therefore be necessary;
- secondary poisoning effects are considered based on the local-regional mean exposure level, for birds and mammals feeding on aquatic biota;
- dependent upon the properties of the chemical substance under assessment, it may also be necessary to determine risk at a regional scale in particular when a local risk has been established or when residual concentrations from multiple emissions may combine. It is then necessary to establish the extent of potential impact. Under such circumstances the risk will be assessed in water and sediment compartments;
- the secondary poisoning effects on higher tier mammalian predators (e.g. bears eating seals) are considered based on the regional-continental mean exposure level.

At continental scale, risk can be characterised as for the regional scale, as appropriate for substances which are more persistent and capable of being bioaccumulated. In Figure 1, a PEC-PNEC comparison scheme illustrates the way the proposed risk assessment should be made, at the different scales, and indicates the relevant section in the report.

Figure 1: Summary of proposed approach to marine risk assessment



1. INTRODUCTION

The need to protect the marine environment as a vital resource was recognised at the Earth Summit in Rio de Janeiro in 1992, when governments from around the world adopted Agenda 21, a programme for sustainable worldwide development. A section was devoted to protection of the oceans and seas, expressing particular concern regarding pollutants which exhibit toxicity, persistence and bioaccumulation (UN, 1992). It called on governments to take priority action to control such substances and to promote risk and environmental impact assessment to ensure an acceptable level of environmental quality.

An evaluation of the risk that the manufacture, formulation, transportation, storage, use and disposal of a chemical might cause harm to the flora and fauna of the surrounding environment has been an integral part of responsible product management for many years. However, the recent rapid development of the techniques and methodologies has allowed such risk to be quantified more reliably, and a quantitative assessment of environmental risk, for new and existing chemical substances, has been incorporated into various national and international regulatory schemes. In the European Union (EU), the procedures to be employed are described in a Technical Guidance Document (TGD) which attempts to address the entire range of industrial and chemical categories, and estimates risk at a local, regional and continental level (EC, 1996). However, in its initial form, the TGD assumptions for the aquatic compartment relate only to fresh water, not the marine environment.

The need to extend the scope of the EU risk assessment process to include the marine environment has been recognised (ECB, 1999a) and work has commenced to consider how this can be achieved. In addition to the overall aim of the existing process, to protect all species within the given ecosystem compartment, three additional concerns have been identified for the marine environment (ECB, 1999b):

- concern for the impact on large marine species (e.g. marine mammals);
- concern for remote areas of the ocean remaining untouched by human activity;
- concern for the unknown potential long-term effects of accumulation of chemicals in parts of the environment which are practically difficult to reverse.

A similar initiative is being undertaken by the Oslo-Paris Commissions for the Protection of the Marine Environment of the North-East Atlantic (OSPAR), arising from the 4th North Sea Conference held in Esbjerg in 1995, at which ministers agreed in their final declaration "to prevent pollution of the North Sea by continuously reducing discharges, emissions and losses of hazardous substances, thereby moving towards the target of their cessation within one generation (25 years)….". In 1998, OSPAR adopted a strategy to control the discharge of hazardous substances (OSPAR, 1998a) and a working group was established to develop a "dynamic selection and prioritisation mechanism for hazardous substances" (DYNAMEC, see Section 2.3.1.1). Although the OSPAR strategy emphasises the need for controls based on the intrinsic hazardous properties of a chemical,

the criteria to be used in this selection and prioritisation mechanism should include "that the substances….show strong indications of risk for the marine environment". Thus, the strategy and the terms of reference given to DYNAMEC (OSPAR, 1998b) recognise that an assessment of risk is necessary to focus effort, and any actions leading to control measures, on substances which are most likely to be causing adverse environmental effects. The second meeting of DYNAMEC identified a number of issues that needed development and for which freshwater risk assessment schemes might need considerable modification, including the estimation of emissions and exposure concentrations, and the estimation of biodegradation rates taking into account lower temperatures, lower substrate concentrations and lower microbial biomass (OSPAR, 1999a).

Although a number of schemes have been developed for risk assessment in relation to specific marine scenarios, in particular the discharges from off-shore oil installations (e.g. Chemical Hazard Assessment and Risk Management of off-shore exploration and production chemicals, (CHARM) Karman *et al*, 1996 and Johnsen *et al*, 2000), there is a lack of more generally applicable models. This, and the relative paucity of data on the occurrence and effects of chemicals in the marine environment compared with fresh water, has led some to suggest that the task of risk assessment is too difficult, or will take too long to achieve. However, the alternative, controls based on intrinsic hazard alone, also has a number of disadvantages in practice. As exemplified by the OSPAR situation, when faced with the need to consider the entire universe of chemicals, there is a need to prioritise, to concentrate limited resources on the chemicals which are most likely to be already causing, or are approaching levels likely to cause, harm; whether this is judged using a simple or complex process, it is risk assessment. Furthermore, if one chemical is phased out, there is almost inevitably a need to identify a substitute; there is little value in substitution by a less hazardous substance unless it also can be shown to present less risk of causing harm.

The aims of this report are to identify the extent to which existing knowledge and techniques can be applied to assessment of risks to the marine environment arising from chemical substances and to propose modifications or, where appropriate, further research, to address areas of uncertainty. In particular, this report considers the applicability to the marine environment of the principles and practices employed for the risk assessment of new and existing substances in Europe (EC, 1996). In common with this existing procedure, the emphasis is on non-ionisable organic substances because, at present, the environmental behaviour of these is more reliably predictable from their physico-chemical properties, especially the octanol-water partition coefficient. Whilst the same principles of risk assessment can be applied to inorganic and ionisable organic substances, there is a greater need with these for specific data, in particular to describe their partioning and distribution in the marine environment.

The Terms of Reference for the Task Force were as follows:

1. identify how risk assessment of chemicals for marine environments should differ from established procedures for the freshwater environment, particularly in terms of:

   i) physico-chemical properties of the chemical;
   ii) sources, transport, partitioning and dilution;
   iii) degradation processes and kinetics;
   iv) bioaccumulation and body burdens;
   v) bioavailability and toxicity.

2. propose a scheme for marine risk assessment incorporating relevant aspects of existing risk assessment procedures or specifying appropriate extrapolations and modifications.

2. PRINCIPLES OF RISK ASSESSMENT AND EXISTING PRACTICES

2.1 Principles of risk assessment

The risk assessment of a chemical in the environment is based on some form of comparison between the levels to which the organisms in a particular compartment are exposed, and the maximum levels which the organisms can tolerate without suffering significant (any, or some specified level of) adverse effects. In practice, the exposure level is either derived from analytical monitoring or predicted from models of varying complexity, using data on emissions, dilution, fate, transport etc. When based on monitoring data, exposure is often referred to as the "monitored environmental concentration" (MEC); however, there is usually a need to select or calculate representative values for the different scenarios of interest, and the term "predicted environmental concentration" (PEC) is used here, unless otherwise specified, to include values derived from both monitoring and modelling. Similarly, the true "no-effect" level cannot be determined directly, and must be estimated based on available toxicity data, often with factors applied to account for uncertainty and precaution; hence the term "predicted no-effect concentration" (PNEC) is frequently used.

Risk assessment is generally defined as the entire process of assessing and predicting exposure and toxicity (effects, and their incidence and severity as a function of concentration), followed by the comparison of PEC and PNEC which is generally termed risk characterisation. The TGD (EC, 1996) defines risk characterisation as the "estimation of the incidence and severity of the adverse effects likely to occur ... due to actual or predicted exposure to a substance, and may include risk estimation, i.e. the quantification of that likelihood". The TGD (and other schemes) characterise risk using the PEC/PNEC ratio (termed risk characterisation ratio, RCR). Although this does not directly quantify the "incidence and severity" of effects, it provides a simple indicator for the likelihood of effects occurring.

2.2 Existing EU practice

The European Union System for the Evaluation of Substances (EUSES) was developed in the EC for quantitative assessment of the risks posed by new and existing chemical substances to man and the environment. EUSES is based on the Dutch USES model (RIVM, VROM, WVC, 1994), and is harmonised with the EU Technical Guidance Documents (EC, 1996).

The environmental assessment considers all stages of the lifecycle of a substance (production, processing, transport, storage, formulation, uses and disposal) to determine emissions to the atmosphere, water and soil. PECs are derived for local (where applicable), regional and continental scenarios. Using PNECs derived for sewage treatment microorganisms and aquatic, sediment-dwelling (benthic) and terrestrial organisms, risk is characterised (as PEC/PNEC ratio or RCR) for these and for top predators in the food chain (birds and mammals feeding on fish and worms) at the local and regional scale.

The local scale considers risks in the vicinity of point source emissions to a generic environment. Although site-specific data may be used where these are available (for example, when European production, or a particular application, is known to be limited to only a few, well-characterised locations) generic, "standard environment" conditions are defined as default assumptions. For example, for emissions to water, the waste is assumed first to enter a sewage treatment plant (STP), of defined proportions, with potential removal by biodegradation, volatilisation and adsorption onto sludge. The resulting effluent is then assumed to mix with river water, with a decrease in the aqueous concentration by sorption (if applicable) onto the natural suspended particulate matter (with generic characteristics, 15 mg l^{-1} with 10% organic carbon) and by dilution; the default dilution factor is 10. Although in reality this dilution factor would be achieved at varying distances downstream of the discharge, depending on local circumstances, there is no generic assumption of a particular distance from a point source; the dilution factor defines the point at which the local risk is evaluated. After the STP, no further allowance is made for degradation or volatilisation in calculation of the aquatic PEC. Local concentrations are calculated from the daily emission rate, based on a fraction of the substance used and the number of days per year that emissions are expected to occur. The local PEC for sediment (generic characteristics, including 5% organic carbon) is obtained by equilibrium partitioning with the aqueous PEC (but without further reducing the aquatic concentration). The PEC_{local} for agricultural soil is derived from generic assumptions of sewage sludge application. Regional PECs (below) provide the background levels to be added to the local predictions to provide the final local PECs for the risk characterisation.

The derivation of regional PECs is based on a Mackay-type level III multi-media model, SimpleBox Version 2.0 (Brandes *et al*, 1996). The regional environment can be visualised as a highly industrialised area, since the assumptions are that 10% of the total EC emissions occur within an area of 200 by 200 km (approximately 1% of the EU area), with 20 million inhabitants. The area is assumed to have an atmospheric mixing height of 1000 m and comprise 3% by area of water (depth 3 m), with an effective sediment (mixing) depth of 30 mm. The characteristics of the environmental components (e.g. sediment, soil, suspended particulates) are the same as for the local scenario. The media (air, water, sediment and three soil types) are represented by "boxes"; flows into and out of each box are modelled by mass balance equations, which are solved simultaneously to compute concentrations. The media are assumed to be homogeneous and well mixed, and the emission rates to be constant. Removal by inter-media transport and degradation are assumed to follow first order kinetics, with removal rates proportional to concentration. It is assumed that (non-equilibrium) steady state has been achieved and the output concentrations should be interpreted as spatially and temporally averaged values. The regional model is nested within a continental model, having the dimensions of Europe (3.52×10^6 km^2) and receiving the total estimated emissions but with otherwise similar characteristics, which provides the background concentrations for the regional model.

EUSES is a comprehensive and well-established procedure for risk assessment for the freshwater and terrestrial environments that has been applied to a large number of substances, in particular those identified as priority existing substances under (EC) 1488/94. This report will focus particularly on this model when considering the extent to which the principles and practices can be adopted, extended or modified for assessing risks to the marine environment.

2.3 Approaches to marine hazard, classification and risk assessment

2.3.1 OSPAR

2.3.1.1 DYNAMEC

The OSPAR Commission established a working group with the remit to develop a mechanism (DYNAMEC) to select and prioritise hazardous substances for action leading to programmes and measures. This is to implement the strategy described in the OSPAR 1998 Ministers statement ("Sintra Statement"), which has the ill-defined objective of "achieving concentrations.... close to zero for man-made synthetic substances". The timeframe envisaged is to produce a list for priority action by 2000; to draw up programmes and measures for that list by 2003; and to move towards the target of cessation of emissions by 2020. A first list of 12 substances was approved and submitted to the OSPAR Commission in June 2000.

DYNAMEC has been based on a 3-step process:

- starting from the "universe of chemicals" the first step provides an initial selection of approximately 400 substances of possible concern - based on hazardous properties, in particular persistence, bioaccumulation and toxicity associated with threshold values;
- ranking of the substances using a scoring algorithm which combines an exposure index, based on either monitoring or modelling, with a hazard index;
- selection of substances for priority action from the top of the ranking list. This selection has been made after an expert review of the data quality.

The OSPAR strategy refers to the use of "risk characterisation and risk assessment techniques" as one of the actions following final selection to determine the extent of the "problem", although it appears possible that this may be used principally to determine which measures are to be taken rather than to decide whether measures are necessary. The DYNAMEC working group is in the process of developing an approach to the assessment of risk in the marine environment. This is being undertaken in conjunction with the EC, with the aim of establishing a common approach (OSPAR, 1999b) that will satisfy the needs of both the OSPAR strategy and the EC's extension of the TGD to include the marine environment in risk assessment of new and existing substances (see Section 2.3.3).

2.3.1.2 EACs

Marine ecotoxicological assessment criteria (EACs) for metals, polychlorinated biphenyls (PCBs), organochlorine pesticides and polynuclear aromatic hydrocarbons (PAHs) have been developed under the aegis of ASMO OSPAR (OSPAR, 1998c). Such EACs are intended as effects benchmarks against which the results of environmental monitoring can be assessed in an attempt to identify possible areas of concern and/or risk. The principle of the procedure for deriving EACs is based on the derivation of an extrapolated concentration, in turn based on ecotoxicological information. Dependent upon the extent of the data set, varying extrapolation factors are applied to the lowest NOEC (no observed effect concentration) or $L(E)C_{50}$ (lethal or effect concentration) value from the available ecotoxicological database.

2.3.1.3 Off-shore chemicals and produced water

The OSPAR Harmonised Mandatory Control System (HMCS) for the use and reduction of discharges of off-shore chemicals utilises the principles of hazard assessment as the basis for classification of chemicals added to the various exploration and production related off-shore processes. OSPAR does not require the application of a defined hazard assessment scheme, but proposes a ranking approach based on bioaccumulation, biodegradation and toxicity tests, the Harmonised Off-shore Chemicals Notification Format (HOCNF).

The CHARM model (Karman *et al*, 1996) was developed as a common tool for all North Sea operators to serve this purpose. This model applies toxicity, bioaccumulation and biodegradation of the chemical as a ranking fundament (the HOCNF principle). CHARM is employed by the North Sea countries as a basis for the regulation of off-shore chemical discharges. However, the interpretation and utilisation of the CHARM results varies between the countries. In 1999 OSPAR initiated a process to harmonise the national regulation policy on off-shore chemicals. This work was completed in 2000.

In the Norwegian approach to off-shore chemical management, weight is given especially to biodegradation and bioaccumulation, while toxicity is of less importance. Based on the biodegradability and bioaccumulation potential, the chemicals are classified in the following categories:

A: Can be discharged;
B: May be discharged upon permission given by the authorities. Should be phased out or replaced;
C: Cannot be discharged. (In specific cases where no substitute chemicals are available, discharge for limited periods may be allowed).

The UK approach also ranks off-shore chemicals based on CHARM results, but more weight is assigned to acute toxicity of the chemical. Classification of chemicals is a two-step system, where the chemical first is classified into one of five groups, A-E, where A represents the most toxic chemicals (aquatic toxicity < 1 ppm, sediment toxicity < 10 ppm). The chemical's final position in the grouping system is then decided by adjusting the chemical up or down one or two groups based on the biodegradation and bioaccumulation potential (CEFAS, 1999).

The CHARM or hazard ranking approach at present only applies to the chemicals added to the different processes off-shore (e.g. drilling, separation, well stimulation, etc) to serve special technical purposes. The major part of the discharge of organic compounds from the off-shore activity is related to regular discharges of compounds of natural origin released from the reservoirs together with oil and gas (produced water). These discharges are only regulated by the maximum allowed level of 40 mg l^{-1} of oil in water.

Norwegian authorities and oil companies operating in the Norwegian sector of the North Sea are now developing a produced water management system based on the Dose-related Risk and Effect Assessment Model (DREAM) system, integrated with Environmental Impact Assessment studies and environmental monitoring programmes. Based on this marine risk assessment system, an Environmental Impact Factor (EIF) is determined for each discharge point (platform), and on a regional scale. The EIF allows comparison of different discharges and provides the operators with the possibility of quantifying the impact of different discharged chemicals and thus, identifying and prioritising measures to reduce such discharges. The EIF also covers man-added chemicals, even though the present OSPAR hazard ranking system is still applied as the management system for these compounds (Johnsen *et al*, 2000).

2.3.2 BUA

Beratergremium für Altstoffe (BUA) has considered the special properties of the marine environment, such as low chemical concentrations, low biodegradation rates, low temperatures and high salinity, which necessitate the development of a specific marine risk assessment procedure. The determination of realistic PECs, the estimation of relevant long-term exposures in consideration of substance-related intrinsic properties, and the question of whether data from freshwater organisms are suitable for judging the risks to marine organism populations are considered to be of particular importance.

Because the concentration of anthropogenic substances in the open sea is generally very low, usual risk assessments based on PEC/PNEC considerations are often limited to areas with local emissions from e.g. river mouths, oil platforms, harbours and coastal production sites. To assess regional exposure, distribution and enrichment processes are of particular importance.

Consequently the BUA excludes chemicals which are readily biodegradable (according to OECD 301) or which can be mineralised under marine conditions and have no significant bioaccumulation potential from regional risk assessment on the basis of specified exclusion criteria (BUA, 1999). Furthermore, relevant inputs emitted into the hydrosphere and returning to the sea via the atmosphere, are only to be expected from semi-volatile substances.

BUA maintain that, at present, there are only a few standardised and validated ecotoxicological test methods for the marine environment, and the number of reliable and comparable data on marine organisms is limited. On the other hand a huge database on effects on limnic organisms exists, which can give valuable references to mode of action and target organisms. Hence the BUA concept foresees, after applying additional assessment factors, the use of freshwater data as a substitute for a first assessment of local risks in the marine environment. In the course of refining risk assessment at the local scale and with respect to regional pollution of marine waters, data from studies on marine organisms are considered to be mandatory. In order to comply with the aim of protection of "marine populations - marine ecosystems", population-relevant chronic data are indispensable.

BUA propose that a local marine risk assessment should be carried out according to the TGD based on PEC/PNEC ratios. Likewise, for a regional assessment, a PEC/PNEC comparison based on effect data of marine organisms and higher safety factors is proposed. For evaluation of risks resulting from long-term exposure to bioaccumulating substances, BUA believes this approach, which does not consider any bioaccumulation and persistency, to be unsuitable. In these cases BUA consider it is advisable to follow with a long-term risk assessment, even if PEC/PNEC < 1, to estimate the long-term development of the concentration and, in the event of expected increase in toxic and accumulating substances, to initiate reduction of emissions or substitution. To evaluate the expected long-term development in concentration trends in discharges to the marine environment resulting from changes in economic importance, trend analyses from monitoring studies, the knowledge of mineralisation processes and their time course should be used.

2.3.3 European Chemicals Bureau

The European Chemicals Bureau (ECB) has provided some guidance on the development of risk assessment methodology for the marine environment based on a three-step approach (ECB, 1998). Step 1, which applies to the local scale (point source emissions) is the most critical because of the highest risk. Step 2 applies to the regional scale and is considered applicable only to high levels of diffuse emissions of substances which are not readily degradable but which pass Step 1. Step 3 applies to a global assessment and is limited to persistent, bioaccumulative and toxic substances (PBTs) to meet the OSPAR and HELCOM concerns. This approach is being progressed in conjunction with OSPAR (Section 2.3.1.1) with the aim of developing a common approach (ECB, 1999b).

2.4 *Existing models for the marine environment*

Marine environmental models consist of mathematical tools to describe the various processes implicated in the emission, transport and fate of contaminants in the marine environment in order to predict environmental concentrations of contaminants. Models are often necessary because analytical measurements are costly and it is not always feasible to monitor large marine areas, although validation of such models by analytical measurements is always desirable.

In 1999, the Health and Safety Executive (HSE) in the UK, and Conseil Européen de l'Industrie des Peintures, des Encres d'imprimerie et des Couleurs d'Art (CEPE) working on modelling of antifoulants, have performed extensive reviews of existing marine models. Both concluded that none of the existing models was suitable to perform marine risk assessments.

A summary of a selection of the existing marine models is given in Table 1. The majority of these are designed for particular marine situations (such as off-shore platforms and antifouling paints) and are generally not applicable to modelling of all emissions in the production and use of different chemical substances. However, two of the models are designed for marine risk assessments which parallel the existing European risk assessment process described in the TGD and modelled by EUSES (see Section 2.2). These are described below.

- USES 2.0 is a system for the evaluation of substances and is a decision support tool. The system is based on a standardised environment and takes a *realistic worst case* approach using average values for all parameters and variables. Estimated concentrations can be overwritten by monitoring data;
- "Scremotox" is based on a multiple box system describing the North Sea either wholly or partially modelling the exchange between boxes both via air and water.

Both merit further investigation and their potential application in determining PECs is discussed in more detail in Section 6. Information on general distribution models is given in Section 3.3.

2.5 *Monitoring*

Environmental monitoring has recently been reviewed by ECETOC (1999a). In addition there are numerous procedures and guidelines on the design and execution of environmental monitoring programmes (for example, US EPA, 1982; UN/ECE, 1996a,b; Berg, 1982; HMSO, 1986; WRc, 1989; Groot and Villars, 1995; ECETOC, 1999a; Holt *et al*, 2000;) which should be referred to for greater detail.

The use of living organisms ("biota") as sampling devices for environmental monitoring of chemicals with a potential for uptake and accumulation in tissue has also been reviewed by ECETOC (1999a).

Data generated by monitoring programmes are being used increasingly to evaluate the environmental impact of chemicals. To allow an adequate evaluation of the results of monitoring programmes for use in risk assessment, information is needed on sampling characteristics, detection limits, number of samples and site description is needed. The use of the appropriate statistics to represent and summarise the data is important to take into account the space and time variability of the concentration. At a local scale, the TGDs suggest that the 90^{th} percentile of concentration data could be considered as a reasonable worst case approximation to PEC_{local}. At the regional scale, full justification should be given for any measured data suggested to be representative of a $PEC_{regional}$.

To use measured data for regional risk assessment, it is necessary to relate the characteristics of a monitoring site to the distribution of site characteristics in Europe. Recently, a methodology has been proposed to statistically combine measured data from different locations and thus derive a distribution of concentrations representative of a region. This approach has been used to assess the risk to the marine environment of several chlorinated organic substances (De Rooij *et al*, 1998a; 1998b; 1998c; Boutonnet *et al*, 1998).

2.6 Conclusions

There is a need to develop risk assessment strategies for the marine environment which compare quantitatively the exposure levels (PECs) of organisms in the different environmental compartments of water, sediment and biota (as food for top predators) with the concentrations predicted to cause no adverse effect (PNECs), making allowance for uncertainty in the PEC and PNEC estimates.

Within Europe, for the freshwater environment, this is achieved for new and existing substances by the standardised procedures embodied in the TGD and the EUSES model. These enable PECs to be determined for generic environments covering a range of spatial scales, when suitable monitoring data or site-specific models are not available or not applicable. It is desirable to define marine assessment schemes which parallel and complement this freshwater approach.

This need is being addressed by a number of different organisations that have identified various issues and uncertainties which may necessitate minor, or more substantial, modifications to be made to the freshwater procedures. Although a number of different mathematical models exist to predict the behaviour and fate of chemicals in the marine environment, for both specific and generic scenarios, these cannot be adopted or refined until the underlying assumptions have been examined more thoroughly.

This report aims to contribute to the development of a marine risk assessment procedure by a detailed examination of the scientific knowledge (and uncertainty) concerning these issues and where possible by proposing a way forward.

Table 1: Summary of existing models

Model	Reference	Scale	Description
Antifouling paint model	Johnson and Luttik (1999)	local	Assesses PEC of antifoulant active substances released from antifouling paints in marinas and harbours. The model uses the PEC/PNEC approach. PEC is calculated from emission fluxes (ship coating leaching rate) into a marina/harbour water volume by taking into account dilution, biotic and abiotic degradation and adsorption to particles (sediments). It is specific to paint emissions.
MAM PEC model	Delft Hydraulics - IVM (1999)	local and regional	The "Marine Antifoulant Model" predicts environmental concentrations of antifouling products in five generalized "typical" marine environments (open sea, shipping lane, estuary, commercial harbour, yachting marina). The model takes into account emission factors (e.g. leaching rates, shipping intensities, residence times, ship hull underwater surface areas), compound-related properties and processes (e.g. K_d, K_{ow}, K_{oc}, volatilization, speciation, hydrolysis, photolysis, bacterial degradation), and properties and processes related to the specific environment (e.g. currents, tides, salinity, DOC, suspended matter load). Default QSAR approaches to estimate missing data are included. It is specific to paint emissions.
CHARM	Karma et al (1996)	local	That "Chemical Hazard Assessment and Risk Management" model assesses the local scale the risks (water, sediment and biota) from the use and discharge of off-shore exploration and production chemicals. The model is specific to platform discharges and does not apply to persistent and bioaccumulable substances.
PROTEUS	BMT Marine Information Systems (1999).	local and regional	"Pollution Risk Off-shore Technical EvalUation Systems" assesses dispersion and behaviour of drilling and production discharges from off-shore oil and gas platforms. The model supports the following risk assessment methods: PEC/PNEC, critical body residues, whole effluent toxicity scheme. It is specific to platform discharges.
DREAM	Johnsen et al, 2000	local and regional	"Dose related Risk and Effect Assessment Model". This model assesses the environmental risks from water discharges into the ocean. Plumes of discharged chemicals are modelled in a three-dimensional time dependent concentration field (PEC). Risk can be determined in several ways: 1) as RCR comparing the modelled PEC with PNEC (TGD method), 2) as time variable RCR based on the modelled PEC and exposure dependent PNEC and 3) based on body burden and critical body burden ratios. Present version of DREAM does not include the latter, which is still to be developed. DREAM offers the possibility of risk assessment of mixtures, total risk is the sum of the risk of each sub-group of compounds. Risk is presented as three-dimensional, time variable risk maps.

Table 1: Summary of existing models (continued)

Model	Reference	Scale	Description
ECOFATE		local and regional	EcoFate is for conducting ecosystem-based environmental and ecological risk assessments of chemical emissions by point and non-point sources in freshwater and marine aquatic ecosystems, including lakes, rivers and marine inlets. It is designed to assess the cumulative impact of chemical inputs in terms of contaminant concentrations in water, sediment and biota of an entire ecosystem and to interpret these concentrations in terms of exceedance of environmental criteria and standards, potential for toxic effects in biota of the ecosystem and risks to human beings exposed to contaminated fish products or contaminated water. (http://www.rem.sfu.ca/ecofate/ecofate.html)
CORMIX	US EPA (1996)	local	The Cornell Mixing Zone Expert System (CORMIX) may be used for the analysis, prediction, and design of aqueous toxic or conventional pollutant discharges into diverse waterbodies. Its major emphasis is on the prediction of plume geometry and dilution characteristics within a receiving water's initial mixing zone so that compliance with regulatory constraints may be judged. The system also predicts discharge plume behaviour at larger distances and in all types of receiving water bodies, including streams, rivers, lakes, reservoirs, estuaries, and coastal waters.
LERAM	US EPA (1995)	local	LERAM is a bioenergetics ecosystem effects model that predicts the effects and risk probabilities for chemicals and other stressors on a littoral ecosystem.
PLUMES	US EPA (1994)	local	PLUMES is intended for use with plumes discharged to marine and some freshwater bodies. Both buoyant and dense plumes, single sources, and many diffuser outfall configurations can be modelled
SCREMOTOX	Baart and Boon (1998)	local and regional	North Sea specific, taking into account transport and retention within river systems, estuaries and North Sea mixing zones, using a grid comprising 3915 computational elements (DELWAQ). In addition to PECs based on estimated emissions from EC production and use figures, the methodology allows indirect emissions from the atmosphere and direct marine emissions to be modelled and monitoring data to be included. Provides ranking of substances based on PEC/PNEC.
EUSES	EUSES (1996)	local and regional	EU System for the Evaluation of Substances (EUSES 1.0) is a decision support tool. The system is based on a standardised environment and takes a realistic worst case approach using average values for all parameters and variables. The system is flexible and allows the parameters and variables to be changed as appropriate. Estimated concentrations can be overwritten by monitoring data.

Table 1: Summary of existing models (continued)

Model	Reference	Scale	Description
USES 2.0	RIVM, VROM, WVC (1998)	local, regional continental and global	Allows calculations according to EUSES but also option to include full implementation of Simplebox 2.0, with five nested spatial scales with regional, continental and global scales consisting of three parts, representing Arctic, moderate and tropical geographic zones. The regional and continental scales contain both freshwater and marine aquatic compartments.
EXAMS	US EPA (1997A)	local and regional	EXAMSII is an interactive modeling system that allows a user to specify and store the properties of chemicals and ecosystems, modify either via simple commands, and conduct rapid evaluations and error analyses of the probable aquatic fate of synthetic organic chemicals. EXAMS combines chemical loadings, transport, and transformation into a set of differential equations using the law of conservation of mass as an accounting principle. It accounts for all the chemical mass entering and leaving a system as the algebraic sum of external loadings, transport processes that export the compound from the system, and transformation processes within the system that convert the chemical to daughter products. The programme produces output tables and simple graphics describing chemical exposure, fate, and persistence.
MERMAID	Varskog (2000)	local	The model offers all the most common PEC/PNEC methods for risk assessment in the marine environment. Included in MERMAID is the PEARLS-database containing toxicity information for about 3,000 substances for 1,000 different aquatic species collected from 3,500 different literature references.

3. PHYSICO-CHEMICAL PROPERTIES AND DISTRIBUTION MODELLING IN MARINE ENVIRONMENTS

3.1 The marine environment

3.1.1 Marine areas

The marine environment cannot be characterised as one single homogeneous compartment. There are different sub-areas where substances are discharged from different sources where different organisms are affected and where the environmental conditions vary considerably. Estuarine and coastal waters and, locally, salt marshes, are the most important zones since the organisms are subjected to the highest concentrations of materials, support the highest densities of marine organisms and are particularly important as spawning grounds for many species. In contrast, the open ocean supports far fewer marine species. This is not to say, however, that these three nominal zones are mutually exclusive; movement of migratory species between the zones will take place.

Estuaries, at the interface of freshwater and marine environments, exhibit a salinity gradient between the river and sea, may be stratified vertically and large areas may dry out every tidal cycle. The water composition reflects that of the river upstream of and, in the tidal zone, the coastal water. Sedimentation rates are high and therefore sediments are often an important niche in the estuarine environment. The high ratio of sediment/muds to overlying water is an important parameter in determining the fate of incoming chemicals. In these compartments, anaerobic fermentative biodegradation, followed by oxidation by sulphate-reducing bacteria, are important metabolic pathways.

Coastal marine waters differ substantially from the open ocean in salinity and temperature as well as in concentrations of inorganic nutrients and organic substrates due to terrestrial discharges. This depends on the proximity to an estuary and the depth of the coastal shelf.

The open oceans, such as the North Atlantic Ocean, due to their vast areas, great depth and efficient water circulation, are still relatively unaffected by human activities compared with coastal areas and enclosed or semi-enclosed seas (e.g. the Mediterranean and the Baltic). The smaller, shallower seas can be classified into those that are nearly completely enclosed by landmasses and those that have a large water exchange with the oceans.

3.1.2 Natural physico-chemical conditions of the marine environment

Sea water, compared with fresh water, is characterised by a high ionic strength and a relatively constant inorganic composition, with generally a smaller temperature range and a more constant pH (typically 7.8 - 8.2). However, the general nature of the enclosed and semi-enclosed seas is essentially dependent on whether or not the fresh water lost through evaporation is more or less than the amount of freshwater input from precipitation and direct runoff from the land. For example, in the Mediterranean, water loss by evaporation exceeds the water input from runoff and precipitation, resulting in its characteristic high salinity (38.5‰).

Another important physical feature is the retention or turnover time within the seas. This is more relevant to the enclosed and semi-enclosed seas (e.g. the Mediterranean, retention time 80 years, and the Baltic, retention time 30 years, rather than the more open "oceanic" seas (e.g. the Barents and Norwegian seas and the North Atlantic Ocean). Retention time has a direct influence on how contaminants are retained or accumulated in the marine ecosystem.

The effect of some important environmental factors on physico-chemical properties and partitioning of organic compounds are discussed in this section. These are parameters which can directly affect bioavailability.

3.1.3 Water solubility

For most organic chemicals, solubility in water is reported at a defined temperature in distilled or deionised water. However, the presence of electrolytes modifies the water structure and changes the solubility. The presence of dissolved inorganic salts present in natural waters generally decreases the solubility of solutes (salting-out). In sea water, the solubilities of neutral nonpolar compounds are generally lower by about 10-50% when compared with pure water, depending on the type of compound considered (Schwarzenbach *et al*, 1993). In a recent review, a simple correlation for the salting-out factor in sea water as a function of organic solute molar volume was suggested, which typically results in a reduction in solubility by a factor of 1.36 (Xie *et al*, 1997). The introduction of a polar substituent into a molecule generally decreases the salting-out effect, due to a decrease in the hydrophobic surface area, and favourable interactions of the polar group with the ions present in the water (Schwarzenbach *et al*, 1993). For ionising substances, the pH dependence of solubility in water should be known, especially when the ambient pH (approx. 8.0 for sea water) is relatively close to the pKa value.

In the temperature range of natural waters (i.e. ≈0-35°C), the aqueous solubilities of organic liquids vary with temperature only by a factor of about two or less (Schwarzenbach *et al*, 1993).

3.1.4 Partitioning parameters

Octanol-water partition coefficient, K_{ow}

As with solubility, K_{ow} is affected by the presence of electrolytes; in addition, for dissociating chemicals it is a function of pH. However, there appears to be little information in the open literature on the effects of salts on the K_{ow} of organic compounds. Since the solubility in sea water will be lower than in fresh water for most nonpolar organic solutes, partitioning from water to lipids and therefore the equilibrium bioconcentration factors for fish and other aquatic or marine organisms are likely to increase from fresh water to sea water. Variation in K_{ow} is primarily attributable to variation in activity coefficient in the aqueous phase (Miller *et al*, 1985); thus, since at moderate salt concentrations (e.g. in sea water), the effect of salinity on aqueous solubility is usually less than a factor of 2, the K_{ow} will probably increase by the same factor. Since

the introduction of a polar group decreases the salting-out effect, the effect of salinity on the K_{ow} of more polar organic compounds will be expected to be less. However, for ionised substances, the effects of ion pair formation shifting the equilibrium towards the non-ionised form should be considered (Esser and Mosser, 1982).

Organic carbon-water partition coefficient, K_{oc}

The organic carbon-water partition coefficient (K_{oc}) is used at several stages in the risk assessment to obtain the solids-water partition coefficient in the various compartments. For organic, nonpolar substances, K_{oc} can be estimated from K_{ow}. Since the hydrophobicity of most organic solutes is increased in sea water, this implies an increased partitioning from aqueous solutions into organic carbon.

Henry's law constant

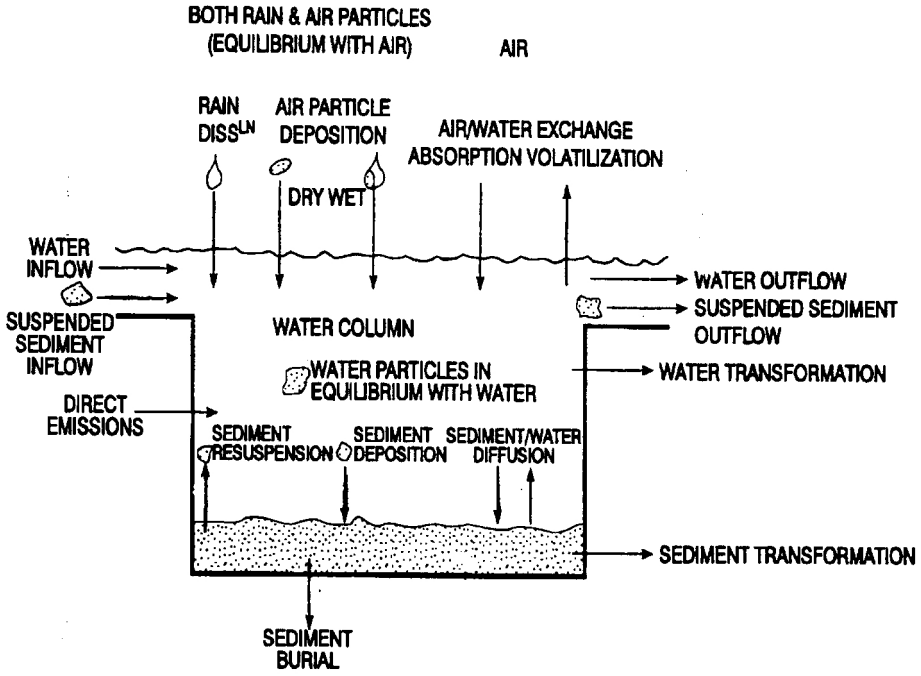
The residence time of chemicals in soil and water is, among other factors, determined by the volatility of the substances, i.e. the tendency to evaporate into the air compartment. Partitioning and transport of substances between environmental media are thus affected by vapour pressure. However, the volatilisation of chemicals from water is described by Henry's law constant (H), which is defined as the ratio of solute partial pressure (in Pa) in the air to the equilibrium water concentration (in mol m^{-3}). Thus, it is a function of both vapour pressure and solubility. As discussed above, seawater solubilities are usually smaller, but within a factor of 2 of, distilled water values. It is expected that air-water ratios determined for sea water will be generally enhanced to the same degree (Schwarzenbach *et al*, 1993).

3.2 Distribution modelling

Substances and possible degradation products in a marine ecosystem distribute between water, sediment and air compartments and possibly to the living organisms. Distribution and partitioning depend on physico-chemical properties, bioaccumulation factors and degradation mechanisms.

Distribution modelling uses the physico-chemical parameters described above to produce a quantitative water-air-sediment interface as shown in Figure 2 below:

Figure 2: Distribution modelling: physico-chemical parameters



Modelling consists of transcribing in the form of mathematical equations, the various processes implicated in the transport and fate of contaminants, for subsequent calculation of the mass balances at a given point within an environmental compartment. Four types of mathematical models may be distinguished:

- upstream models designed primarily to estimate raw inputs to the sea via sewage treatment plants, rivers, or from a diffuse source;
- modelling of estuarine processes influencing the transformation of inputs in coastal areas;
- modelling of transport and fate of contaminants on a regional scale;
- models designed to establish correlations between contamination levels and biological effects.

The OSPAR (ASMO) working group on modelling and fate of contaminants at sea has completed a review of existing modelling tools and listed a series of questions that models should be expected to answer:

- what are the contaminant inputs in a geographically delimited area?
- what are the fluxes and main transport pathways of contaminants in the marine environment?
- in which compartments do persistent contaminants accumulate?
- how much time is necessary before regulatory measures implemented to reduce inputs can be observed to have positive impacts on the contamination of the environment?

3.2.1 Estuary and delta behavioural models

Estuary input data are governed by riverine contaminant concentrations and direct local emissions from STPs and production plants. They can be either measured or modelled.

Contaminant behaviour in estuaries can be modelled in several ways:

- multivariate models;
- physical process models;
- biological, chemical and biochemical process models.

Multivariate models simulate the transport of a number of items present in the water in dissolved or particulate form and cover a specific geographical area. These models may be either:

1. Hydrodynamic and fine particle transport models, either 1D, 2D horizontal or vertical and/or 3D, describing the movements of water masses and of suspended particles (including exchanges with sediment) during time periods that may exceed one year. These models solve hydrodynamics equations based on bathymetric data, and may incorporate forced parameters such as tide, swell or wind. When applied to dissolved, conservative contaminants, hydrodynamic models provide a way, once inputs are known, to calculate the dilution of discharges and the concentration levels at all points within the domain under study (isoconcentration curves);

2. Fate models derived by integrating physico-chemical data on substance behaviour, such as partition coefficients between suspended particles and the dissolved phase, or degradation rate. Because of a lack of dynamic consideration for the processes which control the fate of contaminants, due to the physico-chemical conditions of the marine environment, these models do not always provide an adequate prediction of the behaviour of chemical substances.

Physical process models are used to simulate specific phenomena (e.g. flocculation/deflocculation of sediment particles in relation to turbulence) and are not always integrated into the transport models.

Biological, chemical and biochemical process models are related to such features of an estuary as the biological barriers of osmoregulation imposed on biota in estuaries by salinity gradients. These models can be used to simulate the distribution of contaminants within the biota at different trophic levels.

They consider biological processes in relation to forcing variables such as nutrients in the water (nitrogen, phosphorus, silica), temperature, salinity and light intensity. These mathematical models can simulate the production of phytoplankton or macroalgae, taking into account the kinetics of uptake and excretion of nutrients previously determined by laboratory trials.

The increased complexity of such models tends towards a gradual integration of other compartments, such as zooplankton and fish, and to an increased consideration of interactions between pelagic and benthic populations. Some of these models can thus simulate certain short food chains, for the dab and sea bass for example. Generally, a simplified trophic web is established from a detailed analysis of the nutrition of the target species and of intermediate species.

Modelling the bioaccumulation of chemical substances within the food webs requires, in addition to an exact knowledge of the transfer pathways (water, food, sediment), an estimate of the contaminant uptake from water and food, of the excretion rate and of the "dilution" due to growth.

An example of a chemical process model is the so-called "surface complexing" models which have been developed to account for the complexity of the estuarine environment, for metals in particular (e.g. cadmium). These models factor in the concentration of the various ligands present in the water for subsequent calculation of the concentration of the different chemical species within the contaminant under study. By coupling with the particle transport and hydrodynamic models, it is then possible to describe the behaviour and fate of cadmium in an estuary (SAM-2DH/SAM-3D/MOCO) or in a coastal area (NOSTRA-DAMUS).

This summary review of modelling tools for transport of contaminants at sea has revealed that:

- large-scale advection/dispersion hydrodynamic models are capable of accurately describing the long-term evolution of water masses in coastal and deep-sea environments. However they remain inadequate to calculate the distribution of substances within the different compartments due to the lack of a model for fine particle transport. Consequently, their application is limited to the calculation of dilution coefficients and isoconcentration curves for conservative or dissolved contaminants or for substances with simple transformation;

- no comprehensive model exists that takes into account all of the hydrodynamic, hydro-sedimentary, physico-chemical and biological processes influencing the fate of substances in the marine environment.

A number of multidisciplinary research models are currently being developed by various European research institutions and applied to pilot areas, based on an integrated approach designed to develop regional management tools.

A European project of this sort has been initiated under the framework of the European Chemical Industry Council (CEFIC) Long-range Research Initiative (LRI) in which a number of estuaries which have been examined in varying degrees of complexity will form the basis of a "virtual estuary" model which should be useful as a tool for risk assessment, simulating partitioning and fate and including a biological sub-model to predict bioaccumulation in biota.

3.3 Conclusions

The modelling approaches used in risk assessment rely on the accuracy of key physico-chemical data, most notably the water solubility, vapour pressure, log K_{ow} and log K_{oc}. To assess the fate and effects of organic solutes in marine waters, it is important to consider the apparent increase in hydrophobicity of the substance, or its tendency to partition out of the aqueous phase, beyond that which applies in fresh water. However, the bulk of experimental data on physico-chemical properties in water is generated, according to existing guidelines, using distilled or deionised water.

When the form or speciation of the chemical is directly affected by the ionic composition of sea water, as is the case for certain inorganic and ionisable organic substances, then the partition coefficients may differ significantly from those in fresh water. For a number of such substances, there may be sufficient information available to allow the relevant partition coefficient in sea water to be calculated from the freshwater data; otherwise, measurements under marine conditions may be necessary.

For non-ionisable organic substances, the decreased solubility in sea water, compared with fresh water, would be expected to result in proportional increases in the partition coefficients between water and octanol, organic carbon, and air. However, the expected decrease in solubility and resulting increase in partition coefficients would be less than a factor of 2. Considering the uncertainty in measured partition coefficients and the uncertainty associated with the frequent need to predict some or all of the partition coefficients, the differences attributable to the seawater environment are unlikely to be significant in risk assessment. Unless measured seawater data of equal reliability are available, freshwater data can be used without adjustment for the marine environment.

4. DEGRADATION

4.1 Abiotic degradation

The EU TGD gives an account of acceptable procedures to be used for the determination of hydrolysis and photolysis in fresh water and there is no reason why these should not be applied directly to the marine environment. There are however, a number of factors that should be taken into account which could modify the abiotic degradation of certain substances:

In estuaries and on the coast, turbulence is high and the suspended solids in these areas may significantly reduce photolysis by reducing light intensity. Light intensity is effectively reduced below the level necessary to support algal growth (the euphotic zone) at a suspended solids concentration of 100 mg l^{-1}. In pelagic areas where the concentration of suspended solids is lower, photolysis will occur, but most wavelengths are completely absorbed within a few metres of the sea surface.

4.2 Biotic degradation

The TGD assumes (for the local scenario) that a substance will have had the opportunity to be mineralised by biotic degradation within a wastewater treatment plant, prior to its release into the environment. At the regional scale, it is assumed that only 80 % of the wastewater emissions are treated, recognising that a proportion of effluents are emitted directly to surface water or go to sewage systems that receive no more than primary treatment. Similarly, emissions to the marine environment may or may not be treated before discharge. However, most substances that are released into rivers or estuaries from a point source such as a production site, will almost certainly have passed through a physico-chemical and/or biological treatment plant. Some substances will arrive at municipal sewage treatment plants at concentrations which vary continuously and will degrade to different extents during the treatment process leading to detectable concentrations of these substances in the estuaries and at the coast. Moreover, off-shore production sites depend on the sea for direct dilution of their effluents.

4.2.1 Factors affecting biotic degradation rates in the marine environment

Conditions in marine zones

Marine environments, defined as comprising estuarine and coastal zones, vary from eutrophic to oligotrophic and from fresh to highly saline. Away from the coast sea water is essentially oligotrophic and contains low concentrations of nitrogen and phosphorus relative to fresh water (Gschwend *et al*, 1982).

Coastal and estuarine zones may be considerably more turbulent than the open sea, leading to:

- increased eutrophication (because of the amount of nutrient originating from the freshwater environment and also because essential nutrients and organic matter are regularly stirred up into the water column);
- increased interaction between the interface of the particle surface and water so that degradation is more likely to occur;
- particle resuspension increases bioavailability of adsorbed substances and increases ambient nutrient concentration to aerobic degraders.

Shimp *et al* (1990), using a model to determine flushing time, determined that biodegradation can be an important removal mechanism in estuaries.

Salinity in both the coastal and estuarine areas varies spatially and temporally and biodegradation rate may depend on mixed populations of organisms. Bacteria may be both autochthonous (developed and acting within a specific ecosystem) or allochthonous (originating from another ecosystem) in origin, as many microorganisms are washed into the estuary or coast from a river or from wastewater treatment plants (WWTP). However, mixed populations of bacteria do not necessarily increase the rate of biodegradation due to saline toxicity to non-halophile strains.

Physico-chemical conditions

Laboratory biodegradation test results depend on factors such as the physico-chemical conditions, the duration of the latency (lag) period prior to initial degradation, the concentration of the substance to be tested, the bacterial cell density of the inoculum and the subsequent biodegradation rate. The lower cell counts of bacteria which tend to be found in seawater samples, compared with fresh water, can affect both the lag period and the final rate, leading to lower marine degradation rates in standard tests. This is used as supporting evidence that marine degradation must therefore be lower in the field. However, this evidence is insufficient as the conditions found in marine (or indeed freshwater) environments are not simulated by these screening experiments where the consortia and redox conditions necessary for efficient degradation are not recreated. While experiments in estuaries have shown that the bacterial strains found *in situ* do not have lower degradation potential than those in fresh water some literature suggests that there is a difference between biodegradation rates measured in sea/estuarine water and that in fresh water.

Pritchard *et al* (1987) collected water from brackish (5 ‰) and salt (22 ‰) water systems and compared their biodegradation potentials in microcosms using p-chlorophenol. The p-chlorophenol in the brackish water assays was degraded about three times faster than in the saltwater tests. Comparison of bacterial populations within the sediment from the microcosms demonstrated that the number of degraders present in brackish water microcosms was 5 times greater than in the saline microcosms while brackish sediment microcosms contained 1.8×10^3 this number.

Street (1984) compared seawater and freshwater biodegradation values using standard BOD_5 tests inoculated with secondary effluent and non-modified sea water. He tested readily biodegradable substances and observed that seawater cultures generally produced BOD_5 values 10-20% lower than those measured in standard BOD medium. This was not thought to be due to the low nitrogen and phosphorus content of the sea water as generally no increase in biodegradation was noted when these elements were added to the BOD medium. When performing further experiments, by increasing the salinity of BOD medium by sodium chloride addition, the same author noted that, at 35 g l^{-1} salinity, biodegradation dropped to 60-80 % of that found with standard BOD medium. However, the inoculum used originated from freshwater systems and the degradation potential of some degraders may have been inhibited by the high sodium chloride concentration. A further experiment by the same author, pre-exposing the bacterial culture to high salinity for a period of 6 months before performing a biodegradation test, did not improve the results.

Ernst (1988) observed a reduction in number of bacterial heterotrophs along a salinity gradient when measuring bacterial populations in estuaries. It is probable that the higher number of bacteria found upstream originated from sewage treatment plants as Kuehnemann and Battersby (1992) recorded 10^7 bacteria ml^{-1} released by biological treatment plants.

From these studies it seems that non-halophile bacteria originating from freshwater sources may be deactivated as they are transported into areas of high salinity. In estuarine and coastal areas biodegradation of substances from freshwater origin may still occur as non-halophile degraders are inactivated by increasing salinity concentrations and halophile degraders intercept these contaminants.

Reinheimer *et al* (1990) found that, at environmentally relevant concentrations of 4-nitrophenol there was a reduction in number of *Pseudomonas* with increasing salinity. Spain *et al* (1984) also found that 4-nitrophenol degradation was reduced at marine salinities. However, Reinheimer *et al* noted an 80-90% degradation of 2-nitrophenol in the Elbe estuary and in the North Sea while in fresh water degradation of this substance up to only 50% occurred.

Several authors working on hypersaline environments have noted that biodegradation of adapted strains is reduced progressively as salinity increases but total inhibition of hypersaline strains was observed only at salinities above 200‰ (Ward and Brock, 1978; Oren *et al*, 1992). It is generally considered that this concentration represents a biochemical barrier for degraders. However, relatively rapid biodegradation is found at salinities of 20 to 30‰ and this approximates the salinity of seawater.

Osswald *et al* (1995) found that bacteria taken from a marine aquarium filter grew best at a salinity of 33‰ while Mille *et al* (1991) found that biodegradation of Ashtart crude oil in a mixed bacterial community isolated from a marine sediment was greatest at salt concentrations of 0.4 mol l^{-1}(23.4‰) and decreased both above and below this salinity (the next highest concentration being 58.5‰).

Widdel (1985) reported that many sulphate-reducing bacteria require moderate salt concentrations for growth and that moderately halophilic forms of these bacteria can still function at salt concentrations close to saturation.

An elegant study was carried out by Kerr and Capone (1988) who sampled a series of sites following the salinity gradient of the Hudson River from 0 up to 28 ‰ salinity. They determined the rates of biodegradation of several PAHs (naphthalene, phenanthrene, anthracene) at a series of salinities over a period of time (weeks). They used slurries containing 1:1 by volume of water:sediment in order to reduce variation between replicates. These authors found that:

- there was no significant change in biodegradation of naphthalene when salinities were increased or reduced in the mainstream of the tidally dominant part of the river;
- the biodegradation rate of estuarine samples depended more upon the concentration of the substance in the sediment (the rate increasing with concentration) than on the position of the sample site;
- a strong positive correlation was found between initial lag time and mineralisation rate after lag regardless of the salinity;
- the biodegradation rate of an estuarine sample from a site just 8 km from the maximum salinity point did not change when tested in the laboratory at salinities varying from 13 to 36 ‰ but was inhibited (35%) by a lower salinity of 9‰;
- mineralisation rates of naphthalene by samples from Iona Marsh (which normally has a salinity around 2-4 ‰) were found to be inhibited compared to freshwater treatments as salinity was increased to 15 ‰.

Similarly Larson and Ventullo (1986) found no significant difference between numbers of nitrilotriacetic acid (NTA) degraders along the estuary and degradation rates were relatively insensitive to changes in salinity or DOC levels. The NTA degraders in the estuary appeared to be indigenous members of the estuarine microbial community and not wastewater associated microorganisms.

From these findings it can be postulated that bacterial strains are adapted to specific ranges of salinity and function optimally within those limits. Biodegradation rates may be inhibited by increasing or decreasing salinity outside the boundaries specific to that population. If biodegradation rates of sediments depend more upon exposure concentration than site it is possible that the low number of degraders of 4-nitrophenol found in the Elbe estuary was due to low level of the substance at that site.

Microbial populations and the importance of microbial adaptation

Sea water is reported to contain between 10^3 and 10^8 bacteria ml^{-1}(Austin, 1988), compared to 10^6 ml^{-1} (at high altitude) to 10^7 ml^{-1} (at low altitude) generally found in river water (Goulder, 1986). Widdel (1985) reported values for marine and estuarine sediment sulphate-reducing bacteria from 10^3 to 10^5 degrader colony-forming units (cfus)/cm^3 taken from a few centimetres below the oxic layer. It is the number of cfus which determine the success of the degradation test.

Freshwater screening tests are the most common source of existing information on biodegradation for most types of chemicals. These tests are based on results from so-called "non-adapted" bacteria, taken from the field, which may, in reality, have met high production volume substances more frequently than marine bacteria in the open sea and therefore may already have, at least partially, become adapted to many chemicals. It is therefore recognised that the results from these tests depend partly on chance (i.e. the presence in the inoculum used in the test of a bacterial strain capable of catabolising the test substance). Thus, while a positive result for ready biodegradation strongly suggests a short life span of the substance in fresh and sea water, a negative result cannot be taken as sufficient proof that a substance is persistent. Therefore, a substance should not be classified as non-biodegradable in the marine environment until further more relevant test methods have also provided negative results. Even a mediocre result from a ready test (failing the 10 day window, or a cut off, for example, >20% CO_2 evolution within 28 days), can be considered adequate proof that the parent substance will not persist in the environment. The exact percentage degradation which can be considered as sufficient to indicate that a substance will not be persistent will be the subject of further expert judgement.

Shimp and Young (1987) found that in two laboratory biodegradation tests using inoculum from a non-impacted estuary, benzoic acid was degraded at low substance concentrations (50 μg^{-1} determined using the radiolabeled substrate method) but no degradation was detected at a higher concentration of 20 mg l^{-1} using an OECD screening technique (OECD 301 A - die-away test adapted for marine use). This is similar to certain results observed by Nyholm *et al* (1992). When the same comparison was made using an inoculum from a high impacted estuary, biodegradation was detected using both methods.

In fact the criterion of non-adaptation, mandatorily stipulated as a validity criteria for ready biodegradation tests in fresh water, is likely to be a less useful requirement in the marine context. In the freshwater scenario it is important to test bacterial populations with no deliberate, prior exposure to the chemical under scrutiny. WWTPs, for example, are commonly subjected to episodic, relatively high input concentrations of substances. For reasons of toxicity of chemicals at high concentrations, and WWTP residence times (the number of degraders for a given chemical, the latency period to initial degradation, the kinetics of degradation and the doubling time of the competent organisms will determine the concentration of the chemical in the effluent), it is important that a reasonably conservative estimate of degradation under such circumstances be described.

In the marine environment, however, the situation is different, as episodes are less common and the highest areas of concern are those where massive dilution cannot occur or where the concentrations are the highest, notably in estuarine and coastal areas. In these areas adaptation will occur while in the open sea dilution is sufficient that bacteria will barely be exposed to all but the most persistent of chemicals.

This was illustrated by Saltzmann (1982) and Massie *et al* (1985) who studied the potential for heterotrophic microbial population to degrade hydrocarbons around the oilfields in the North Sea. They demonstrated that the total microbial population in waters and sediments contaminated with hydrocarbons did not increase, rather it was the case that the existing population adapted their metabolic activity to degrade the nutrient input. Selection pressure from the degree of contamination meant that the rate of degradation was found to increase with decreasing distance from the platforms. Bauer and Capone (1988) provided evidence to show that degradation rates, in marine sediments, of a number of PAHs were influenced by pre-exposure (2 weeks) to benzene, naphthalene and anthracene and suggested that the enhanced degradation was a result of either populations with broad substrate specificities or common biodegradation pathways.

The concept of adaptation seems to be widely accepted in the scientific community even though it does not fit in with the current philosophy of standardised "easy" biodegradability tests (Nyholm, 1991) which favour the use of indigenous inocula. The importance of a pre-adaptation phase was studied by Thouand and Block (1993) and Thouand *et al* (1996). Any new testing procedures should include a pre-adaptation step to the substance in question, as this is closer to the environmental situation and will provide far more realistic predictions of the behaviour of the substance in these areas.

The variation and extent of the biodegradation measured may also reflect intrinsic differences in carbon and energy flow of the different microbial communities. Shimp (1989) reported that both linear alkylbenzene sulphonate (LAS) and glucose mineralisation were comparable under marine and freshwater conditions and Hashimoto *et al* (1998) demonstrated similar half-lives for haloacetic acids (2-halogenated alkanoic acids) tested at 20-60 $\mu g\ l^{-1}$ in fresh water and marine waters from Tokyo Bay. Bartholomew and Pfinder (1983) reported that the estuarine communities incorporate a higher fraction of the substrate carbon into microbial biomass than freshwater communities. Crawford *et al* (1974) made similar observations for 15 amino acids and glucose and reported less than 50% of substrate carbon respired and released as CO_2 by estuarine communities.

Finally, it should be remembered that freshwater screening tests are purely aerobic as this is assumed to constitute the greater part of degradation. The role of anaerobic degradation should not however be overlooked. Whilst it is true that a number of anaerobic screening tests also exist, they are used only to a limited degree and more method development is required for some anaerobic environments. In the past few years several strains of recently discovered sulphate-reducing bacteria, often neglected in laboratory tests, have been shown to metabolise hydrogen at low partial pressures more rapidly than methanogenic bacteria and it appears the importance of these organisms in the degradation process is only now beginning to be recognised.

Temperature

Standard biodegradation screening tests are generally carried out between 20 and 24°C and up to 26°C for the MITI test. In contrast, surface sea water varies from about -1.4 to +27.5°C while at depth, temperature is virtually constant at about +2 to +3°C, only reaching the lower limits close to the poles (Rankin and Davenport, 1981). Thus the temperatures used in screening tests are not really applicable to the marine environment. But is this important when extrapolating freshwater biodegradation test data to the marine situation?

Quiroga and Sales (1989) studied the effect of temperature changes on LAS biodegradation in sea water. They performed biodegradation tests at 5, 10, 15, 20 and 25°C and found that at 5°C no degradation occurred, while increasing amounts of LAS were degraded as temperature was increased (about 30% at 10°C, 50% at 15°C and 60% at 20°C). LAS was degraded completely within 15 days when the temperature was increased to 25°C.

However, laboratory tests using sludge are of limited relevance when extrapolating degradation test results to the marine environment. Short-term studies conducted at different temperatures do not permit adaptation of bacteria.

Literature examining this aspect is limited but Widdel (1985) stated that the greatest variety of sulphate-reducing bacteria is found at moderate to low temperatures and that many anaerobes live at low temperatures (never above 10°C) all year round because of stratification. Furthermore, a field study by Gschwend *et al* (1982) does not support the temperature dependent biodegradation found in the laboratory by Quiroga and Sales (1989). Gschwend *et al* noted that alkyl benzene degradation increased only slightly during the summer and temperature would not be the only factor influencing this increase.

Rivkin *et al* (1996) examined the relationship between temperature and specific growth rates (SGR). Based on the analysis of published data, where approximately 50% of the observations were from environments <4°C they found the mean (0.39-0.42d^{-1}) and median (0.25-0.29 d^{-1}) SGR of bacteria from cold (<4°C) and warm (>4°C) were not significantly different. They concluded that the growth rates for bacteria from cold and temperate oceans are similar at their respective ambient temperatures. Arnosti *et al* (1998) carried out a series of experiments to investigate whether relative temperature responses differ between the microbial communities of temperate and permanently cold environments. They compared the temperature responses of the microbial communities at two temperate sites to the temperature responses at two permanently cold Arctic sites. Carbon turnover rates were used in order to determine whether communities in permanently cold environments function more effectively at lower temperatures than do communities which experience cold temperatures only on a seasonal basis. They concluded that temperature in the marine environment did not affect biodegradation rates as bacterial populations were fully adapted to these conditions and that half-lives as short as those from standard tests could be observed even when the temperature was close to 0ºC. Other studies support their hypothesis that carbon turnover rates in Arctic

environments are not necessarily longer than in temperate environments (Wheeler *et al*, 1996; Kostka *et al*, 1999; Hodson *et al*, 1981), while Meyer-Reil and Köster (1992) concluded that rates of enzymatic hydrolysis in pelagic sediments are regulated by organic matter concentration and not by temperature.

As 90% of the ocean is estimated at a permanent temperature between 0 and 4°C, the larger part of the marine environment would be expected to achieve carbon turnover rates close to that found in temperate regions and the necessity to consider specific persistence criteria for pelagic zones is therefore debatable.

Sediment versus water

It is estimated that a greater number of bacteria are found in fresh than marine water columns (Bartholomew and Pfinder, 1983), but the microbial density increases dramatically in the sediment, and it is suggested that the majority of estuarine bacteria are fixed to suspended matter (Goulder, 1986).

The attachment of microorganisms to particles increases the biodegradation rate compared to the rate in water columns containing low quantities of suspended solids (Pritchard *et al*, 1987; Van Veld and Spain, 1982). Biofilms are also responsible for higher biodegradation rates than those found in the water column (Osswald *et al*, 1995) and even obligate anaerobes can survive and function during periodic resuspension when attached to particles due to the presence of micro-niches which provide the necessary reducing conditions (Widdel, 1985).

Lee and Ryan (1979) found at least two-fold increases in the mineralisation rates of p-chlorophenol, 2,4,6-trichlorophenol and chlorobenzene in sea water when sediments were added with their associated microbial flora. Walker *et al* (1988) concluded that, for seven of the nine pesticides compared using estuarine water and saline water/sediment slurry systems, primary degradation was more rapid when slurries of water/sediment were used rather than water alone. In these cases however, the main reasons for increased biodegradation rate are higher bacterial population numbers and higher nutrient concentrations caused by adding natural sediment. However, increased biodegradation due to interaction between bacteria and sediment interface alone has also been recorded.

Pritchard *et al* (1987) performed shake-flask tests on sea water alone, or by adding a quantity of sediment, and observed that the biodegradation rate increased proportionally with the amount of sediment added. Their work using sterilised sediment, sand particles and glass beads, all devoid of organic matter which could otherwise supplement the nutritional requirements of the bacteria, suggests that the provision of a particle-water interface, is the overriding factor in the promotion of biodegradation, given the presence of degraders. Degradation rates were found to be directly related to exposed surface area of the particulates. Moreover, the "Most Probable Number" of p-chlorophenol-degrading bacteria from shake-flasks designed to evaluate surface effects was determined by adding a known number of degraders into a series of flasks containing sea water

alone, or water with glass beads, sterile sediment or sand. Initially (first 188 h), densities of bacteria 100 to 1000 times higher were found on all the substrates used than in water alone and degradation was higher throughout the test (453 h).

Finally, evidence from 28-day degradation tests mentioned in a review by Boethling *et al* (1995) led the authors to conclude that "limited data suggest that biodegradation tends to be faster in aerobic fresh and marine water containing sediments compared to water without sediment".

In the sediment compartment, anaerobic degradation occurs mainly just millimetres or centimetres below the sediment surface. The first few centimetres of sediments around coastal and estuarine regions are regularly suspended and redeposited allowing alternately, aerobic and anaerobic biodegradation to occur. At least for simple, non-xenobiotic compounds, the critical anaerobic degradation pathways seem to be similar for freshwater and seawater sediments (Ward and Winfrey, 1985, Steber *et al*, 1995), although it is suggested that the degraders involved in the degradation of specific substances in marine systems may not be the same as those in the freshwater environment (Rheinheimer *et al*, 1990).

Even when the degradation pathway is different, mineralisation cannot be precluded. For example Galushko *et al* (1999) reported the discovery of a new type of marine sulphate reducing bacterium capable of completely oxidising naphthalene to CO_2. The initial metabolic pathway for naphthalene was different in freshwater bacteria responsible for degradation of this compound (where the intermediate was naphthalenol) from the marine type (where the intermediate was 2-naphthoate).

Seasonal variation

Sea water normally contains lower concentrations of nitrogen and phosphorus than lowland surface waters. Both waters are subject to seasonal fluctuation of these elements (Gschwend *et al*, 1982) due to changes in temperature, light and turbulence due to storms. This last factor is responsible for increasing water column nutrient fractions and, in conjunction with increased light intensity and temperature, results in the occurrence of algal and zooplankton blooms. The rapid feeding rate of the primary consumers is thought to be responsible for the increased ammonia concentration which is noted in summer and thence its degradation producing short peaks of nitrite and nitrate in the weeks that follow. But the above factors are not the determining factors of biodegradation rate in sea water. Gschwend *et al* monitored concentrations of various volatile organic compounds over a 15-month period and observed that seasonal variation in levels of these substances was controlled by many factors such as changes in anthropogenic input from land (alkylphenol thought to be from heating oil, was more common in the winter months), petrol spills and biogenic sources (pentadecane, heptadecane and pristine) or from various sources (aldehydes from zooplankton and breakdown products of organic chemicals).

4.2.2 Existing methods for biodegradation testing

Several methods have been proposed for testing biodegradability in marine systems: Wakeham *et al* (1986), Nyholm *et al* (1992) and Reinheimer *et al* (1990) for water column tests and Spain *et al* (1984), Pritchard *et al* (1979) and Smith and Klug (1987) for sediment or sediment/seawater microcosms (eco-core tests). For a review of these publications see ECETOC (1993a). In 1992 the OECD guideline 306 "biodegradability in sea water" was adopted and the ISO/ Water quality - guideline for the determination of biodegradability in the marine environment was published in 1998.

OSPAR published results of a ring test comprising a comparison between four marine biodegradation tests: closed bottle test; marine BODIS test; marine CO_2 evolution test and marine CO_2 headspace test. It was concluded that these tests were statistically comparable in terms of the quality of results and in the ranking of biodegradability of the four test substances studied (sodium benzoate> Anco Green B> Aquambul Bll > Pentaerythritol), although inter-laboratory reproducibility was slightly superior for the closed bottle test (OECD 306) than for the other three.

Several other test guidelines have recently been developed and could possibly be modified for use in the marine environment:

- Aquatic (pelagic) compartment: ISO/DIS 14592-1 "Evaluation of the aerobic biodegradability of organic compounds at low concentrations - Part 1" (draft guideline). The ISO method has been the basis for a proposal for a new OECD test guideline "Simulation test - Aerobic transformation in surface water" (OECD, 2000a).
- Turbid aquatic/sediment dispersed compartment: ISO/DIS 14592-2 "Evaluation of the aerobic biodegradability of organic compounds at low concentrations - Part 2" (draft guideline) and OECD: "Aerobic and anaerobic transformation in aquatic sediment systems" (aerobic test - draft guideline) (OECD, 2000b).
- Anaerobic sediment compartment: OECD draft guideline "Aerobic and anaerobic transformation in aquatic sediment systems" (OECD, 2000b). Data from anaerobic screening tests conducted with digested sludge (e.g. ISO 11734) are of limited value for predicting the degradation potential in sediments. Anaerobic screening tests may be performed by using a sediment inoculum (Horowitz *et al*, 1982; Madsen *et al*, 1995), and the observed biodegradability may then be used as an indication of the potential biodegradability of the substance in anaerobic sediment.

Few data exist, however, comparing laboratory tests on aerobic or anaerobic degradation of substances in sea water with existing results from freshwater tests.

Aerobic tests using sea water

Nyholm and Kristensen (1992) published results of a ring test of the two OECD marine screening methods - the shake flask test and the closed bottle test. Both these tests have been based on modified versions of the standard freshwater tests described by the OECD.

The modifications included:

- salinity, at approximately 30 parts per thousand as nutrient enhanced natural sea water is used;
- in the seawater closed bottle test the pass rate was reduced to 40%, as BOD_5 was higher in the blank than in the freshwater version and growth more variable;
- in the case of the shake flask method the test time was increased to 60 days (this was considered necessary to take into account the increase in latency period in the marine tests).

Standard sea water was used as the inoculum in both cases.

Relatively high concentrations (mg l^{-1}) of test substance are employed in screening tests. The kinetics of degradation is generally expected to be zero-order as the test substance represents the major carbon energy source. The bacterial population is expected to increase using the substance, thereby increasing the degradation rate until the concentration of substance in the medium becomes a limiting factor.

Nyholm and Kristensen (1992) found that, in the ring test, substances which are considered very readily biodegradable in freshwater tests (e.g. aniline) will also degrade readily in the modified seawater tests. However, the biodegradation rate of other substances tested was less clearly predictable.

The authors state that a negative result from a marine test does not exclude biodegradability in sea water. Some of the reasons for this may be:

- incorporation into the biomass is higher in the case of seawater and estuarine microorganisms (Bartholomew and Pfinder, 1983; Crawford *et al*, 1974);
- in fresh water a higher proportion of substance is released as CO_2. This is likely to bias results of seawater systems as measurements will underestimate the amount of substance that has been degraded.

In seawater tests, low concentrations of test substance resulted in higher biodegradation rates. It is postulated that high concentrations of organic material in the test medium inhibit oligotrophic bacteria (Alexander, 1985).

The ratio between the concentration of test material and naturally occurring carbon is too high for co-oxidative interaction to occur in screening tests. This is supported by further work by Nyholm *et al* (1992) in which the shake flask simulation method was compared to the closed bottle and shake flask screening tests. The simulation tests were conducted at lower test substance concentration than the screening tests and here first-order degradation kinetics are expected. The carbon energy source is primarily the natural organic substrate of the test medium and the test substance is a secondary substrate. Co-metabolism is possible and the substance concentration is not high enough for an increase in microorganism population size. From the results it was observed that some simulation tests gave positive biodegradation results while the screening tests were negative.

Aerobic tests using sediment

Tests using sediment based on the same principles as above but with the addition of 500 mg l^{-1} of sediment have been described by Cripe *et al* (1987). Such tests may provide a more representative test method in the future where the equivalent of the so called "ready biodegradability" can be determined. This is currently not possible with the OECD 306 tests which have been designed to measure only the capacity of a substance to degrade in sea water.

Anaerobic tests

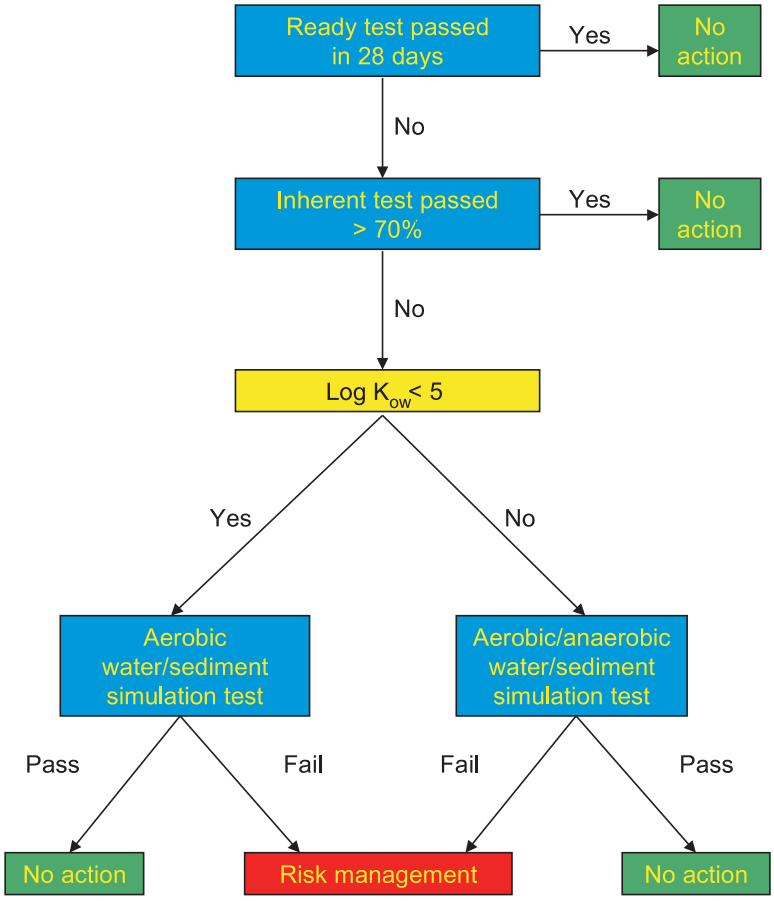
The ECETOC "Guideline for screening of chemicals for anaerobic biodegradability" (ECETOC, 1988), now ISO 11734 "Water quality - Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of biogas production" uses digested sludge from a wastewater treatment plant but has also been evaluated for use with marine drilling fluids (Steber *et al*, 1995). The authors speculate that the anaerobic test is applicable to marine anaerobic systems as the hydrolytic and acidogenic degradation pathways to form simple, broadly amenable degradation intermediates (e.g. short chain organic acids), which can be subsequently ultimately biodegraded by the various groups depending on the environmental conditions, are common to all types of anaerobic bacteria. The final stage of degradation of the remaining short-chain organic acids and other generally amenable intermediates can be brought about by methanogenic bacteria in freshwater systems, or more probably in marine systems, by denitrifying or sulphate reducing bacteria. In any case, ultimate degradation from these intermediates would be expected to occur. Thus, this test may also be suitable for determining anaerobic biodegradation in the marine environment although little is currently known about certain parameters within the test, such as biogas production in sea water.

The range of substances tested included fatty acids and fatty alcohols which underwent ultimate anaerobic degradation while other substances such as mineral oils, dialkyl ethers, linear alkylbenzenes and acetal derivatives did not show any evidence of anaerobic biodegradation. The authors support the results of these assays by citing marine sea-bed surveys of an exploration well drilled using an ester-based mud system. The cuttings pile had largely disappeared within one year, analysis of sea-bed samples showing close to background levels for the ester which was thought to have been degraded into hydrophilic intermediates allowing the cuttings pile to disperse. In a second oil-based cuttings pile, mineral oil was still detected several years after deposition and it is suggested that these substances are degraded aerobically but not anaerobically and the resulting BOD reduces oxygen concentration to anaerobic levels just beneath the surface of the cuttings pile. They conclude that this conservative laboratory test, using sludge from a municipal digester can be used to predict the general anaerobic degradation potential in the marine environment while negative results require further supporting evidence to demonstrate non-biodegradability.

4.2.3 Recommended methodology for the determination of biotic degradation in marine risk assessment

BUA (1999) postulates that there are fewer bacterial strains in the marine environment as the low concentration of organic chemicals exerts less selection pressure on the bacteria (although substrate specificity is judged to be lower allowing the bacteria to degrade a greater variety of chemicals) and therefore the capacity to biodegrade diverse substances is less than in freshwater systems. Since the experimental proof of complete mineralisation under marine conditions is extremely laborious, the BUA recommends as a first approach, performing the OECD marine biodegradation test or a modified version of the standard freshwater biodegradation tests (OECD 301B,C,D or F) to get an initial qualitative assessment of whether the chemical can be mineralised or not. If subsequent experiments using concentrations typical for the marine environment, demonstrate primary degradation within 12 months, a long-term increase in the chemical is not expected. While the ECETOC TF recognises the importance of a tiered approach to testing, it does not entirely support the views of the authors that there is a relationship between species diversity and degradation rate of organic carbon. Nor is it accepted by the scientific community that the strain variety is lower in marine ecosystems given that most marine bacterial strains have not yet been cultured in the laboratory (Evans *et al*, 2000) and so this statement cannot be justified. Based on the evidence presented in this document the scheme in Figure 3 is proposed for the assessment of persistence in the marine environment.

Figure 3: Proposed strategy for marine biodegradation testing



It is clear that, to date, there are insufficient data on biodegradation studies in sea water to recommend a specific test to be systematically performed in the risk assessment procedure as a supplement to the standard ready biodegradation tests. Even if the sediment-free test methods proposed above have been sufficiently adapted to be suitable for the determination of marine biodegradation, and have been validated with a handful of substances, the results from the less biodegradable substances are variable and further testing on more substances is necessary for comparative purposes with freshwater biodegradation results.

Some of the sediment tests take into account anaerobic degradation, and this is an important element, considering the thin layer of aerobic sediment and the fact that in estuarine and coastal regions silt is constantly being shifted from one area to another, redistributing and displacing the aerobic and anaerobic fractions. To date, no anaerobic seawater tests have been validated.

Adaptation of bacteria in tests could also enhance their extrapolation to the marine environment where the highest areas of concern are those where massive dilution cannot occur or where the concentrations are the highest, notably in estuarine and coastal areas and particularly in the sediment fraction of these zones. In these areas adaptation will occur, where possible. Any new testing procedures should include a pre-adaptation step to the substance in question as this is closer to the environmental situation and will provide far more realistic predictions of the behaviour of the substance in these areas.

ECETOC (1993a) proposed the marine eco-core test using sediment sea water mixtures. As a further indication of degradability in the environment, the use of adapted microorganisms as a supplement to this test would seem appropriate

When results from a ready biodegradation or inherent test are available, the TGD approach for freshwater biodegradation assessment uses a rate constant approach for the determination of half-lives in surface waters. The methodology is simple and places threshold levels for each type of test result. It would be desirable to extrapolate such a procedure to marine biodegradation in the short term. The use of freshwater half-lives to predict marine biodegradation rates is supported by Boethling *et al* (1995), who compared available data on degradation rates taken from BIODEG + CHEMFATE databases. The authors state that while petroleum degradation tests based on limited nitrogen and phosphorous availability have led to the general belief that biodegradation is slower in saline and brackish water, the authors' results indicate that degradation may also be faster in saline water.

Based on this information it can be expected that estimations of degradation kinetics in fresh water should be considered appropriate for use in marine risk assessment for both estuarine/coastal and off-shore biodegradation rate estimates. Employing the same half-lives for all freshwater and marine zones when only evidence from inherent and ready biodegradation tests is available also seems to be appropriate.

However, the table of half-life estimations in the current TGD document is already conservative. To illustrate this the following example is used:

A ready biodegradation test (such as the OECD 301 D, which may be performed using river water and can therefore be considered to reflect freshwater environmental bacterial concentrations and conditions to some degree) does not favour the rapid degradation of chemical substances and does not include the use of adapted inocula. In order to "pass" a ready biodegradation test pass which does not meet the 10-day window criteria, mineralisation must reach 70% within a period of 28 days. Logically, the half-life must be inferior to this time and would therefore not be equivalent to 50 days as proposed in the Danish persistence document. A maximum possible half-life for this test would be 27 days while a more realistic worst case half-life would be 25 days.

Furthermore it is considered appropriate to add a further tier based on inherent test results. Inherent tests are based on higher concentrations of inocula which do not really equate to environmental conditions but more to wastewater treatment plants. However, the Zahn-Wellens test (OECD 302B)and the MITI II (OECD 302C) are little more than ready tests with higher bacterial concentrations (OECD, 1998). The test substance is challenged by a higher microbial inoculum and thus there is a greater probability that competent organisms are present (while allowing the microbial population time to adapt to the substance in certain cases). These factors would increase the chance of a positive result, reduce the latency period and increase initial degradation rate. These tests are however only a means of demonstrating that substances which do not mineralise rapidly in ready tests are still capable of being degraded. One advantage of these tests is that they can be used to measure primary degradation (which is not the case for ready biodegradation tests). The OECD guidelines specify that a pass of 20% degradation in an inherent test can be considered as proof of primary degradation, while a result of 70% or greater can be considered proof of ultimate biodegradation. This suggests the introduction of a further persistence value before characterising a substance as "persistent". BUA recommends that primary degradation within one year in a long-term test would be sufficient to consider that the substance is not persistent in the environment. Based on this suggestion it would appear that a half-life of one year could be considered as a reasonable worst case for a substance achieving a 20% pass in an inherent test.

As a supplementary step, when no further data have been located, the extent of biodegradation in these former zones should be determined preferably by modelling estuaries and then coastal areas (a process potentially taking many days even for soluble substances) before using the open sea half-lives.

4.3 Conclusions

From the previous sections it appears that:

- non-halophile degraders may not be capable of biodegradation in saline environments;
- bacteria generally grow optimally at the salinities in which they are found in the environment;
- there is no evidence of a reduction in mineralisation due to biodegradation as salinity increases;
- freshwater data, ready or inherent, can be considered acceptable to determine the disappearance rate of the substance, in the absence of specific marine biodegradation tests if they meet certain endpoint criteria.
- sediment resuspension has been shown to increase biodegradation rate of degradable substances;
- when addressing persistence in the marine environment, biodegradation in the aqueous phase and in sediments under both anaerobic and aerobic conditions may need to be considered;
- for degradable substances, biodegradation rate in the marine environment is positively correlated with concentration of that substance found in the sediment;

- the role of adaptation to test substance and influence of substrate concentration should be further investigated and considered as an essential addition to future methodology to determine half-life in pelagic waters;
- low temperature does not necessarily impair the capacity of marine bacteria to degrade organic matter and should not be used as a criteria influencing persistence.

From the discussions and the data presented, it is reasonable to conclude that biodegradation rates in marine environments, especially in estuarine and coastal regions with higher nutrient levels and sediment disturbance, can be as great as in fresh water. Considering that the existing rate constants used in the TGD regional model are relatively conservative, the same translation of rate constants from standard test data can be employed for the marine regional model.

5. PEC DETERMINATION

5.1 Introduction

In order to characterise the risk to biota, it is necessary to obtain an estimate of the concentration of the chemical to which the organisms are exposed, considering all potential routes of exposure. The exposure concentration can be measured by analytical monitoring or predicted by modelling, or a combination of both. As described in Section 2.1, the term PEC is used in this report, for convenience, to describe the exposure concentration derived by either monitoring or modelling. The ways in which monitoring data and different modelling techniques can be used to describe the distribution of a chemical are described in Section 3. This section considers the different environmental compartments and spatial scales for which a PEC is required for risk characterisation, and proposes how PECs can be derived, in particular for generic estimates when site-specific data are unavailable or not appropriate.

5.2 Environmental compartments

For the marine environment, the relevant environmental compartments (as potential routes of exposure) are as follows:

- water - exposure of aquatic organisms across respiratory and other permeable surfaces;
- sediment - exposure of sediment dwelling (benthic) organisms by ingestion of, or direct contact with, sediment particles;
- biota - exposure of higher trophic levels via the food chain (secondary poisoning), by predation on organisms that have been exposed via the water, sediment or predation on other organisms.

Another potential route of exposure for marine birds and mammals is by inhalation of the chemical in the air they breathe. However, except for certain special cases, this route of exposure is unlikely to be the most significant (Roberts and McGarrity, 1985) and the risk is not routinely assessed (other than for human occupational exposure) in the existing EU procedure (EC, 1996). The principles of such an assessment for marine species would be no different from that for terrestrial birds and mammals. Therefore, although certain models to describe the partitioning of the substance and to predict inputs to the marine environment may generate a PEC_{air}, this report does not consider the direct risk of inhalation by air-breathing animals.

The soil compartment and soil fauna are not relevant to marine ecosystems. Although salt-marshes are inhabited by higher plants, with a route of exposure which parallels that of terrestrial plants, this can be considered as part of the sediment compartment. The risk to microorganisms in sewage treatment (prior to effluent discharge to the environment) is already addressed in the EU TGD (EC, 1996).

5.3 Spatial scales

5.3.1 Local scale

As a general rule, for aqueous emissions from point sources, concentrations of contaminants are greatest in the immediate vicinity of the source. This local concentration (PEC_{local}) must be measured or calculated for each relevant stage in the lifecycle of a substance (e.g. production, processing, formulation, use, and disposal) using either site-specific data, if available, or generic assumptions. Typically (e.g. EC, 1996), the local PECs are determined by applying a predicted or measured dilution of the effluent at a certain, short distance from a single source, with an allowance for partitioning between aqueous and solid phases. Generally when PEC_{local} is calculated, there is no allowance for degradation in the receiving water because of the short time interval from discharge. Proposed definitions of the local scale for the marine environment are given in Section 5.4.

5.3.2 Regional scale

Depending on the properties of the substance, the scale of emissions and the results of the local risk assessment, there is often a need to determine the PEC over a wider spatial scale. If there is predicted to be a local risk, it may be necessary to determine how far the impact extends into the surrounding region. Furthermore, although concentrations will tend to decrease with distance from a source, due to dilution, partitioning and degradation, the residual concentrations from one source may combine with those from other point and diffuse sources and cumulate over time. Depending on the total emissions and the degradability of the substance, this may result in a significant "background" concentration over a considerable area. This background concentration is termed $PEC_{regional}$ and proposed definitions for the marine environment are given in Section 5.5. If PEC_{local} is calculated rather than measured, this background concentration must be taken into account, by adding $PEC_{regional}$ to C_{local} (the initial estimate of PEC_{local}), before calculating the local risk.

Although the regional scale may vary in dimensions, depending on the needs of the risk assessment, it is generally a real or generic marine area that may be at particular risk for various reasons, such as:

- the area is subject to high or multiple emissions, e.g. the waters close to a heavily industrialised land area, or an off-shore oil/gas field;
- the physical characteristics (tidal flow, depth, residence time etc) of the area result in a low capacity for dilution and dispersion;
- the ecosystems are considered particularly important or vulnerable.

Thus, the regional assessment tends to consider a particular problem area or a generic "reasonable worst case" scenario, subject to multiple sources but with sufficiently large spatial and temporal scales to allow any degradation and partitioning to occur (further details are given in Appendix A).

5.3.3 Continental scale

It is unlikely to be necessary to estimate the risk to an area larger than the region unless there is found to be a significant regional risk, which would only be expected for substances that were relatively persistent and toxic. However, if a regional risk is identified for such substances, it may be necessary to consider their impact on a larger sea area. Furthermore, just as the $PEC_{regional}$ provides a background concentration for predicting the final PEC_{local}, so a background concentration may be required by models to predict the final $PEC_{regional}$. For consistency with the EU TGD, the term $PEC_{continental}$ is used here for this larger scale.

Conceptually, it is convenient to consider the continental scale as a sea area within which a number of the (real or generic) regional areas are located, in the same way that the region contains multiple point sources. The possible dimensions of the continental scale and definitions of $PEC_{continental}$ for the marine environment are discussed in Section 5.6 and Appendix A.

5.3.4 Global scale

The continental sea areas (as described above) exchange water with the considerably larger, oceanic water bodies. Man-made substances that possess the extreme properties necessary to become oceanic or global contaminants are termed Persistent Organic Pollutants (POPs), which are the subject of international programmes of identification and control (UNEP, 1995, 1997; UN/ECE, 1996b). Marine risk assessment of POPs at the global scale is beyond the scope of this report. As a concept in certain box models (e.g. Simplebox version 2.0; Brandes *et al*, 1996), the continental area is nested within the world's oceans, and the concentration at this global scale (PEC_{global}) is calculated as the background concentration necessary to predict $PEC_{regional}$. However, PEC_{global} will be negligible for the majority of substances and its derivation is not described in this report.

5.4 Derivation of PEC_{local}

Various types of point source emissions to marine waters may require calculation of PEC_{local}. These can be conveniently classified as follows:

- land based emissions - from the production, formulation, processing or use of the substance;
- off-shore platforms - emissions from oil and gas exploration and production (E and P);
- river outflows - from river catchment areas subject to multiple point and diffuse emissions, constituting a point source input to the marine environment;
- other direct emissions - e.g. harbours, marinas and fish-farms.

In all these cases, site-specific data may be available which may define the emissions and the dilution in the immediate vicinity or may define PEC_{local} directly from monitoring data. However, if these data are not available for all, or a representative number, of the point sources, it is necessary to develop generic scenarios, comparable with those described in the existing TGD for the freshwater environment. The following sections refer particularly to such generic PEC_{local} estimates (for water and sediment), although they could also be used as guidance in defining site-specific PECs. PECs in biota related to secondary poisoning are discussed in Section 5.7.

5.4.1 PEC_{local} for land-based emissions

As in fresh water, aqueous emissions to estuarine and coastal waters may, or may not, be subject to biological treatment processes (municipal or on-site) before discharge to the environment and the dilution provided by the receiving water can vary considerably. Generally, the available dilution in the marine environment is very much greater than the TGD default assumption for rivers (x10 dilution). However, the generic local scenario should aim to represent the "reasonable worst case" (EC, 1996). This could be envisaged to be the upper reaches of an estuary, approaching the tidal limit, where the potential dilution was not significantly greater than that which would be available if the discharge were situated a short distance upstream, above the tidal limit. As a "reasonable worst case", the risk assessment will address organisms living there permanently. However, many species will experience a lower average exposure, due to their movement or migration out of, or through, the area.

A default marine local scenario can be proposed which is essentially the same as the generic local scenario described in the TGD with regard to emission estimation, but with a modification to the default characteristics of the receiving environment. No adjustment is required to the following TGD assumptions that estimate the concentration entering the environment:

- emission estimates based on industry and use categories according to the A and B tables of the TGD or, where available, emission scenario documents;
- emissions to water are via a sewage treatment plant (STP) before release to the environment, with standard STP flow rates and removal potential, as described in the TGD;
- the default assumption is a 10-fold dilution of the effluent from the STP.

In addition to dilution, potential sorption of the substance to suspended particulate material must be taken into account, when determining the PEC_{local} for water. For fresh water, the TGD assumes a suspended particulates concentration of 15 mg l^{-1} with an organic carbon content of 10%. However, the upper reaches of an estuary, as described above as the conceptual worst case, are characterised by much higher levels of suspended material, typically up to 100 mg l^{-1} at the junction of the estuary and river up to 10,000 mg l^{-1} in the area of maximum turbidity (IFREMER, 2000). Although some marine discharges may be direct to coastal waters with a lower suspended solids content, these

would also, typically, have much greater than 10-fold dilution. Therefore, the upper estuarine area, with a higher level of suspended solids (25 mg l^{-1}) but with the same carbon content as the TGD default, is proposed to maintain the worst case concept. Thus:

- allowance (calculated from K_{ow} or K_{oc}) for partitioning to 25 mg l^{-1} of suspended particulates ($SUSP_{water}$), with an organic carbon content of 10%, to give the local dissolved concentration due to the point source discharge, termed C_{local};
- the final $PEC_{local\ seawater}$ is determined by adding the relevant $PEC_{regional\ seawater}$ (Section 5.5), if significant, to the C_{local}.

The final stage is to predict PEC_{local} for sediment ($PEC_{local\ sediment}$) by calculating partition to bottom sediments whilst maintaining $PEC_{local\ seawater}$ unchanged. For fresh water, the TGD assumes a sediment with standard physical characteristics and an organic carbon content of 10%. Conceptually, this is based on the carbon content of the suspended particulates (see above) and it is reasonable to assume the same carbon content for the upper estuarine environment. It should be noted that the TGD assumes a lower organic carbon level (5%) in the final "bedded" sediment when estimating the $PNEC_{sediment}$ (see Sections 10.4.4 and 10.4.5).

Thus, for the marine environment:

- $PEC_{local\ sediment}$ is predicted from $PEC_{local\ water}$ according to TGD assumptions.

It should be noted that the regional PEC for sediment is not added to derive $PEC_{local\ sediment}$, since $PEC_{local\ water}$ already includes $PEC_{regional\ water}$.

5.4.2 PEC_{local} for off-shore platforms

A number of dilution and dispersion models have been developed and validated for many of the exploration and production platforms in the North Sea and other marine areas, as a consequence of Environmental Impact Assessment (EIA) studies. These provide data on concentration fields surrounding platforms, for both water and sediment. The modelling approach assumes that sufficient physico-chemical data and relevant biodegradation rates are available for the particular chemical, and also that discharge volumes and discharge concentrations of target chemicals are known. However, EIAs for off-shore fields focus on both man-made and naturally occurring compounds originating from the reservoir (e.g petroleum-related hydrocarbons and other organic compounds, metals, salts and radionuclides). Existing modelling or monitoring data for representatives of these groups of chemicals can provide the basis for estimating local PECs of other substances with similar physico-chemical properties.

Based on these site-specific studies, C_{local} can be defined as the concentration, achieved as a result of dilution and partitioning to suspended particulates, at a distance of 500 m from the platform. Although high phytoplankton levels can often result in total suspended solids concentrations similar to fresh water in such areas, these are typically lower, and a generic default of 2 mg l^{-1} is proposed. Thus, a generic model can be proposed for platforms for which monitoring or modelling data are not available:

- a dilution factor of 1,000 is applied to the discharge concentration, typically achieved within 500 m of the platform;
- C_{local} is calculated by allowance (calculated from K_{ow} or K_{oc}) for partitioning to 2 mg l^{-1} of suspended particulates, with an organic carbon content of 10%;
- the final $PEC_{local\ seawater}$ is determined by adding the relevant $PEC_{regional\ seawater}$, if significant, to the C_{local}.

As discussed in Section 5.5, the $PEC_{regional\ seawater}$ for this purpose may need to take into account the discharges from a cluster of platforms with overlapping concentration fields.

Because of the relatively large depth of water around platforms, the $PEC_{local\ seawater}$ cannot be assumed to represent the concentration above the local sediment. Furthermore, the discharge of cuttings and other solids may affect the transport of chemicals to the bottom sediments. Sediment biomonitoring, monitoring for specific substances, or site-specific models are available for many platforms; if not available for the chemical of interest, data for chemicals with similar physico-chemical properties and emission characteristics can be used to derive $PEC_{local\ sediment}$ (or generic defaults).

5.4.3 PEC_{local} for river outflows

The concentration of a substance in outflowing river water provides the basis for a local PEC where it meets the marine environment, at the tidal limit of an estuary. Generally, this riverine output will be more important as an input to the marine regional model (see Section 5.5), but on occasions it may be appropriate to assess the local risk. In the absence of sufficient, representative monitoring data, the outflowing river water concentration can be estimated using models such as GREAT-ER (ECETOC, 1999b) or the existing EUSES (TGD) model; in the latter case, the $PEC_{regional\ water}$ is the appropriate output.

Although in some cases there will be immediate and significant dilution at the point where the river meets the sea, frequently the dilution is small over large parts of the tidal cycle, particularly in estuaries with extended, narrow, upper reaches, as evidenced

by the low salinity in such areas. In such a situation, it is reasonable to assume a 50 % dilution of the fresh water with sea water, and an allowance for partitioning to the higher level of suspended solids which characterise an estuary (see Section 5.4.1). Thus:

- $PEC_{local\ seawater}$ is calculated as the mean of $PEC_{regional\ freshwater}$ and $PEC_{regional\ seawater}$ with allowance for partitioning of the resulting concentration to 25 mg l^{-1} of suspended particulates with an organic carbon content of 10%.

Then:

- $PEC_{local\ sediment}$ is predicted from $PEC_{local\ seawater}$ according to TGD assumptions.

5.4.4 PEC_{local} for other direct emissions

This category encompasses the various emission point sources that are uniquely marine and therefore relevant emission estimates or scenarios are not provided in the current TGD. Although harbours, marinas and some fish farms could be considered land-based, in most cases it would be inappropriate to use the assumptions given in Section 5.4.1, because the emissions are not via an STP and the dilution available would typically be larger. Emission scenarios could be developed based on any available site-specific or generic data (e.g. the MAM PEC model for antifoulant paints, see Section 2.3); however, it is beyond the scope of this report to develop detailed approaches.

5.5 Derivation of $PEC_{regional}$

As defined in Section 5.3.2, the regional PEC integrates all point source and diffuse emissions to a specific or conceptual (generic) marine area that has a spatial and temporal scale large enough to reflect the various biotic and abiotic processes which govern the ultimate fate and distribution of a chemical. However, within this definition, the dimensions of the region may vary considerably, depending on the needs of the risk assessment. It is self-evident that if the assessment relates to a known particular marine region (site-specific) then the dimensions are defined largely by its geography and hydrography, and cannot be described precisely in this report. However, for a generic region, the dimensions relate only to a typical "area of concern" and a conceptual model for this is proposed.

For both site-specific and generic regions, the PECs may be derived from monitoring, modelling, or a combination of both. If monitoring data of sufficient quantity and quality exist for the substance (ECETOC, 1999a) these can be used directly to generate the necessary $PEC_{regional}$ values, which are generally preferable to modelling predictions. However, this will require selection of the data, to ensure that only values relevant to, and representative of, the specific or generic region are included, and to exclude, for example, values from samples taken close to point sources. There is also generally a need to reduce the data, by statistical analysis, to one or a few summary values (such as means or percentiles) before risk characterisation. In the absence of adequate

monitoring data, the PECs must be predicted by modelling and the approaches that can be employed (for water and sediment) are described in the following sections. PECs in biota related to secondary poisoning are discussed in Section 5.7.

5.5.1 $PEC_{regional}$ using site-specific models

Distribution and fate models have been developed for a number of estuaries and coastal regions in Europe and elsewhere and, where they exist and have been validated, they can provide valuable information on PECs. It should be noted that these will usually provide concentration "contours" for the modelled region, rather than a single value for each compartment. This potentially enables risk to be evaluated in terms of the "geographical extent of the impact" rather than a "risk/no risk" statement for the area as a whole. The recent model "Scremotox" (Baart and Boon, 1998; Section 2.4) for the southern North Sea is of particular interest since it includes ten of the major European river estuaries and impact zones, and the surrounding North Sea, and allows average values to be obtained for user-defined "regions" within the overall area. Further evaluation of this model may also allow better definition of generic European regions.

Site-specific regional models have also been developed for a number of exploration and production fields, to integrate the impacts from a number of adjacent platforms. In 1999, the Norwegian authorities launched a modelling-based marine environmental monitoring programme for the off-shore oil and gas production activities on a local and regional level (SFT, 1999). This programme is based on the development and application of fate modelling and risk assessment of produced water discharges, where dilution models are validated by field measurements of a number of discharged compounds (Johnsen *et al*, 1998; Utvik *et al*, 1999; Durell *et al*, 2000). The validated modelling results are then applied in a risk assessment based on the DREAM model, and the discharge prognoses from the regional environmental impact assessment performed for the area (Johnsen *et al*, 2000).

5.5.2 $PEC_{regional}$ using generic "box" models

- EUSES

In the European context, it is logical to consider how marine regions can be defined and modelled in a way that parallels, and in the future can be integrated with, the existing EUSES model which implements the TGD procedures. As described in Section 2.2, the existing terrestrial/freshwater region is 40,000 km^2 (approximately 1% of the EU area) but is deemed to receive 10% of the total European emissions of the substance, conceptually based on the heavily industrialised area around a major river catchment. PECs are estimated using a Mackay-type level III box model (SimpleBox Version 2.0). It is therefore reasonable to envisage (and model) the marine region as the estuarine and coastal sea area that receives the river water flow and atmospheric load emanating from this conceptual terrestrial/freshwater "default" region.

One of the principal difficulties in describing such a marine "box" is to select the volume of water it contains (in practice the area and depth), since this is clearly important in setting the available dilution. However, the water residence time, which is a function of the flow of water from the river(s) plus the exchange of water with the larger (continental) sea area within which it is nested, also has a major impact on the resulting PECs. The difficulty of selecting the dimensions of the marine region could perhaps be reduced by defining two (or more) sizes, to assess the degree to which the PECs are affected by the spatial scale, which would vary depending on the properties of the substance.

EUSES in its present form can be used to model a marine region, by resetting the existing default characteristics. Appendix A discusses in detail how this can be done and proposes possible dimensions and characteristics for the region, for further development and refinement. The region proposed in Appendix A is relatively small (approximately 0.1% of the North Sea volume) but with a relatively long residence time (96 days). This probably tends very much towards the worst case situation, but more work is needed to assess the relative realism of these parameters.

The inputs to this marine region (assuming there are no uniquely marine emissions, such as off-shore platforms) are derived by running EUSES in its standard form, to obtain:

- the input from rivers (freshwater $PEC_{regional}$ x flow rate of 6.9×10^7 m^3 d^{-1});
- the input from the atmosphere, assuming that 50% of the airflow through the terrestrial region enters the marine region (regional PEC_{air} x 0.5 x 1.85×10^{13} m^3 d^{-1}).

Thus, this marine region receives the residual inputs of a chemical resulting from 10% of total EU emissions being subjected to potential cumulation, degradation and partitioning in the terrestrial/freshwater environment. It should be noted that this does not allow for the fact that a proportion of the emissions, which are assumed under current practice to enter the freshwater/terrestrial region, will in reality enter the marine environment directly, and will not be subjected to partitioning and degradation in the freshwater/terrestrial region. This aspect could be incorporated, but would require a generic default for the proportion of marine emissions to be developed; this proportion could be added to the marine inputs but must also be subtracted from the freshwater/terrestrial inputs.

This scenario would also require modification for substances with major emissions which would not be included in the normal terrestrial model, such as the emissions which are "uniquely" marine, and may be direct to the sea without sewage treatment, as described in Sections 5.4.2 and 5.4.4. This would be particularly the case for off-shore discharges, e.g. from platforms, which might require different regional size and features.

• USES 2.0

USES 2.0 is a development of the USES model which was also the basis for EUSES. USES 2.0 incorporates an optional marine compartment at the regional and continental scale, and includes a global scale to provide background concentrations. The total regional area is 4×10^4 km^2 (the same as in standard EUSES) and the sea water area is 50% of the total, at a depth of 25m, resulting in a marine volume of 5×10^{11} m^3, approximately 20-fold larger than that proposed in Appendix A. However, as with EUSES, the box dimensions and characteristics can be reset from the default values. It would therefore be possible to employ USES 2.0 with the values changed to those proposed in Appendix A, with the advantage that the freshwater and marine assessment would be performed simultaneously rather than sequentially; this possibility merits further investigation.

5.5.3 $PEC_{regional}$ using generic estuary distribution models

Research is currently being initiated as part of the CEFIC LRI to develop a "virtual estuary" model, which incorporates the complex factors controlling the transport and fate of chemicals in estuaries. The objective is a validated model which could be used to determine chemical distribution in any kind of theoretical European estuary and with any substance. This should enable the geographical extent of any impact to be assessed, as described above for a site-specific model, but applicable to a generic scenario.

5.6 Derivation of $PEC_{continental}$

The principles by which PECs may be determined at the continental scale are essentially similar to those described above for the regional scale, and will not be reiterated. The dimensions of the continental scale will depend on the size selected for the regions within it, and for site-specific assessments may also depend on the availability of monitoring data or relevant models.

For the generic box model approach, Appendix A describes proposed modifications to the existing EUSES defaults, to give a continental scale similar in size and characteristics to the North Sea, but these could be varied to more closely represent other large sea areas. USES 2.0 (see Section 5.5.2) incorporates a considerably larger marine continental volume but this can be reset by the user to different dimensions.

5.7 PECs for secondary poisoning (PEC_{biota})

For a substance with indications of bioaccumulation potential and certain toxicity classifications based on mammalian data, the TGD estimates the concentration of a chemical substance in earthworms ($PEC_{earthworm}$) and fish (PEC_{fish}) to define the exposure of terrestrial birds (eating worms) and fish-eating birds and mammals, respectively. As discussed in Section 7, although marine invertebrates (e.g. mussels, clams, worms) may also be eaten by marine birds and mammals, the potential for biomagnification along

the food chain for highly bioaccumulative substances suggests that a diet which is assumed to consist only of fish will represent a worst case situation, unless there are specific data to indicate otherwise. However, the current procedure to estimate PEC_{fish}, by multiplying PEC_{aqua} by the fish bioconcentration factor (measured or predicted from K_{ow}) does not allow for possible biomagnification (Section 7). It has been proposed (ECETOC, 1995) that for substances with a log K_{ow} between 5 and 8, only the PEC_{fish} is calculated using an additional factor, as follows:

$PEC_{fish} = PEC_{aqua\ (local\text{-}regional)} \times BCF_{fish} \times$ biomagnification factor (BMFactor)

where: $PEC_{aqua\ (local\text{-}regional)}$ is the mean of the $PEC_{aqua\ local}$ and the $PEC_{aqua\ regional}$, based on the assumption (as described in the TGD) that the piscivorous animals feed for 50% of the time in the PEC_{local} zone and 50% of the time in the $PEC_{regional}$ zone.

The BMFactor should be related to the log K_{ow} (or measured BCF) in a progressive manner, such that at log K_{ow} 5 the factor is 1 (no adjustment) and at log K_{ow} 8 it is at a maximum. The maximum log K_{ow} value of 8 is used to reflect the fact that the bioaccumulation potential of such highly hydrophobic substances declines rapidly above this value (ECETOC, 1995). Based on the food chain models reviewed by ECETOC (1995; Section 7) and considering that, for water-breathing organisms, the evidence for biomagnification is limited, the maximum BMFactor proposed is 4. A simple method of defining intermediate adjustments would be:

BMFactor = log K_{ow} - 4 (applied only if log K_{ow} is 5 to 8)

If the BCF_{fish} is measured, rather than estimated from K_{ow}, then the BMFactor could be derived from the BCF and applied when BCF was greater than 5,000 (log BCF x 3.7) using a similar formula (BMFactor = log BCF - 2.7, with maximum value of 4).

Section 7.3.3 also concludes that a further tier of secondary poisoning is relevant to the marine environment, to assess the risk to mammals preying on other predatory mammals and birds (exemplified as polar bears eating seals). This second tier is considered to be relevant only to more remote, particularly arctic, environments, and therefore it is proposed that this risk is only assessed at the regional-continental level. This can be achieved by applying a further adjustment factor to allow for possible biomagnification between the fish and the seal, to calculate the PEC_{seal} as the exposure estimate for its predators. Thus:

$PEC_{seal} = PEC_{fish\ (regional\text{-}continental)} \times$ BMFactor

where: $PEC_{fish\ (regional\text{-}continental)} = PEC_{aqua\ (regional\text{-}continental)} \times BCF_{fish} \times$ Biomagnification adjustment

It is proposed that (in the absence of specific data relevant to the chemical of interest) the BMFactor is the same for both the first and second tier calculations. Therefore:

$PEC_{seal} = PEC_{aqua\ (regional\text{-}continental)} \times BCF_{fish} \times (BMFactor)^2$

where: $PEC_{aqua\ (regional\text{-}continental)}$ is the mean of the $PEC_{aqua\ regional}$ and the $PEC_{aqua\ continental}$.

Worked Example:

Substance with log K_{ow} = 6, $PEC_{aqua\ local}$ = 1 µg l^{-1}, $PEC_{aqua\ regional}$ = 0.1 µg l^{-1}, $PEC_{aqua\ continental}$ = 0.05 µg l^{-1}.
BCF calculated from K_{ow} = 25,000

$PEC_{fish} = PEC_{aqua\ (local\text{-}regional)} \times BCF_{fish} \times (\log K_{ow} - 4)$
$= 0.5(1+0.1) \times 25{,}000 \times (6-4)$
$= 0.55 \times 25{,}000 \times 2$
$= 27{,}500\ \mu g\ kg^{-1}$

$PEC_{seal} = PEC_{aqua\ (regional\text{-}continental)} \times BCF_{fish} \times (\log K_{ow} - 4)^2$
$= 0.5(0.1+0.05) \times 25{,}000 \times (6-4)^2$
$= 0.075 \times 25{,}000 \times 4$
$= 7{,}500\ \mu g\ kg^{-1}$

5.8 Conclusions

Although further research is required in a number of areas, a framework is proposed using existing tools and knowledge, that allows exposure (PECs) to be defined at the local, regional and continental scale, for marine risk assessment. A summary of the proposed system to calculate generic PECs is given in Section 10.

6. BIOACCUMULATION IN MARINE RISK ASSESSMENT

6.1 Introduction

Bioaccumulation is defined as the net result of uptake, distribution and elimination of a substance in an organism. It includes all routes of exposure, which are principally those across respiratory surfaces and by ingestion of food, water and other material (see also Section 5.7). For aquatic (water breathing) organisms, accumulation from water across the gills (or other respiratory surfaces) is termed bioconcentration; this is the major route of exposure for many substances and is more commonly measured in laboratory studies than bioaccumulation from food. For air-breathing animals, the inhalation route is included, but dietary exposure is generally considered to be more important. Biomagnification is defined as accumulation and transfer of substances via the food web, in particular to express an increase in the concentrations within organisms at successive trophic levels.

ECETOC (1995) reviewed in detail the role of bioaccumulation in aquatic environmental risk assessment. The principal conclusions are summarised as follows:

- bioaccumulation of a substance into an organism is not an adverse effect or hazard in itself;
- bioaccumulation may lead to a body burden which may cause toxic effects on the organism due to water and/or dietary exposure, or may be toxic to its predators;
- biomagnification is less widespread than commonly believed, having been demonstrated only for a limited number of substances;
- the potential of a substance to bioaccumulate is related primarily to its lipophilicity;
- if measured values are not available, the octanol-water partition coefficient (K_{ow}) is useful as a predictor of bioconcentration for organic, nonpolar substances, although for other substances this may be less reliable;
- metabolism (biotransformation) of the substance may increase the elimination rate and therefore decrease the bioconcentration factor;
- bioaccumulative substances which are relatively persistent and toxic, show negligible metabolism and with log K_{ow} between 5 and 8 may represent a concern when widespread in the environment. A trend of declining bioaccumulation is observed as log K_{ow} exceeds 8 and molecular weight exceeds approximately 700;
- toxicity effects data for bioaccumulative substances should be evaluated in relation to the time necessary for bioaccumulation to achieve equilibrium (in practice, 95% of steady state). If steady state is achieved during the course of the experimental exposure then bioaccumulation is accounted for within the effects endpoint and within any PNEC derived from such data.

This report considers aspects of bioaccumulation that may be different from those in freshwater environments or particular to marine risk assessment. These can be summarised by the following questions:

- is the potential for bioconcentration of substances from water different due to the composition and properties of seawater or the physiology of marine organisms ?
- is the potential for dietary bioaccumulation and biomagnification different due to the physiology of marine organisms or particular features of marine food chains ?

In the existing EU TGD approach, bioaccumulation potential is used to determine the exposure of top predators in the food chain, such as fish-eating birds and mammals. However, it is also relevant to the probability that the toxicity of chemicals will differ between marine and freshwater organisms (Section 8). If the kinetics of uptake and loss of chemicals is different, then the toxicity (as measured by the external exposure level) is likely to be different, even if the inherent (internal) sensitivity of marine and freshwater organisms is the same.

6.2 Bioconcentration from seawater

6.2.1 Physico-chemical properties of seawater

The ionic strength, composition, pH and temperature of sea water, compared with fresh water, have implications for the bioconcentration potential of chemical substances. Although the water solubility of hydrophobic, nonpolar organics is lower in sea water, and the octanol-water partition coefficient (K_{ow}) is higher, the differences are small (Section 3) and the effects on bioconcentration would be expected to be of a similar magnitude. However, for ionisable substances, the different pH and ionic strength of seawater may have a more significant impact, if the equilibrium is shifted towards the non-ionised form. The non-ionised form is assumed to be bioavailable whilst the ionised form is assumed not to be able to penetrate cell membranes at a significant rate (Pärt, 1989). Saarikoski *et al* (1986) showed that the rate of uptake of phenols by *Poecilia reticulata* was independent of pH provided that the pH was below the pKa; above the pKa, uptake declined markedly. Pärt (1989) showed a similar effect with a number of ionisable substances, using perfused gill techniques in fresh water, but concluded that the decline in uptake rate above pKa was somewhat less than expected from the declining non-ionised fraction, which he attributed to a possible local depression of pH at the gill surface. In principle, the relative constancy of seawater pH should decrease the uncertainty in predicting the bioconcentration potential of ionisable substances.

More pronounced effects of salinity on bioconcentration potential are observed for metals and organo-metallic compounds, related to speciation changes. In general, metal concentrations in biota decrease with increasing salinity (Hawker, 1989). For example, cadmium accumulation by marine molluscs decreased 400% with a salinity increase from 20 to 30‰ attributed to declining free cadmium ion concentrations, possibly linked with increasing calcium concentration (Jackim *et al*, 1977). Laughlin *et al* (1986) found that the K_{ow} of tributyltin varied with salinity, with a minimum at 25 ‰, attributable to speciation changes.

Uptake rates of substances into fish increase with increase in temperature, due to increasing respiration and gill ventilation rates (ECETOC, 1995). Passive elimination would be expected to be affected to a lesser degree, and thus bioconcentration factors would tend to increase with temperature; however, metabolic transformation rates may also increase (Davies and Dobbs, 1984). However, for the purposes of risk assessment, it is not necessary to assume a systematic difference in temperature between sea water and fresh water, although for any given region the temperature range will generally be lower in the marine environment.

6.2.2 Comparative data and biotic factors

Davies and Dobbs (1984) compiled data for five hydrophobic organics for which BCFs had been determined for both marine and freshwater fish. For four substances, the BCF in fresh water was greater than that in sea water, by factors of 4 to 49. The exception was the bioconcentration of a-hexachlorohexane by *Lebistes reticulatus* in fresh water and after acclimation to sea water, for which the seawater BCFs were approximately three times higher than those in fresh water (Canton *et al*, 1978). This was attributed to the additional uptake through the osmoregulatory drinking of sea water. However, in a similar study by Tachikawa *et al* (1991), using pentachlorophenol with seawater-acclimated *Oryzias latipes*, the seawater BCF was a factor of 4.5 lower than the freshwater value.

Zaroogian *et al* (1985a) used published bioconcentration data, for two species of marine fish and two species of marine mollusc, and found significant linear relationships for each species between BCF and log K_{ow}. They showed that similar models derived from data for freshwater fish (*Pimephales promelas*) were not significantly different and could be used to predict the BCF for the marine species, within one order of magnitude for 71% of the chemicals, covering a log K_{ow} range of 1.6 to 6.5. Measured BCF values for the marine species were within one order of magnitude of those measured for *Pimephales promelas* for 63% of the chemicals. They concluded that the same mechanisms of bioconcentration operate in both freshwater and marine species.

Zaroogian *et al* (1985a) pointed out that the variation in the measured BCF between different test species can be attributed to various factors, but can be reduced considerably if normalised to the lipid content of the animal and not to body weight. The lipid content of the gill tissue has been shown to increase during acclimation of freshwater fish to sea water, but this was expected to have little or no effect on uptake rate for chemicals of log K_{ow} >1 (Pärt, 1989). However, the lipid content of fish is also a function of their age/size, and laboratory determinations have tended to employ relatively small animals. Thomann and Connolly (1984) found that the lipid content of lake trout increased from 7% in the 3-5 years age group to 16% at 7-10 years, which would tend to increase the BCF on a body weight basis. Increasing body weight in fish also results in a decreasing uptake rate, associated with decreased metabolic, gill ventilation and oxygen consumption rates as a function of body weight (Murphy, 1971), although elimination rates may also change. Fisk *et al* (1998) demonstrated that elimination half-lives in rainbow trout were longer (elimination lower) by a factor of approximately 10 for 900 g fish compared with

10g fish, for substances with log K_{ow} 6 to 7. Although, for water-breathing fauna, the general size of marine species is not very different from the equivalent freshwater species, the upper limit of the range tends to be greater (Section 6.3.1) and the lipid content, under certain circumstances, is higher (Section 6.3.2). These factors need to be considered, particularly when assessing the risk to predatory fish.

ECETOC (1995) details how metabolism (biotransformation) of chemicals can have a profound effect on bioconcentration. Rates of xenobiotic biotransformation vary between phyla and species and are a function of general metabolic rate, but there is no evidence to indicate a different biotransformation capacity in marine organisms.

6.3 Dietary bioaccumulation and biomagnification

ECETOC (1995) reviewed in detail the literature on food web transport of chemicals in the aquatic environment and the evidence for, and models to predict, biomagnification, that may be defined as a successive increase in the concentrations in biota with increasing trophic level. The review included both marine and freshwater laboratory and field data; the overall conclusions can be summarised as follows:

Although many substances have been shown to be transferred by ingestion of food, the majority of substances do not biomagnify (biomagnification factor =1). Both data and models suggest that ingestion only contributes significantly to bioaccumulation for substances with log K_{ow} greater than 5. Certain models (Thomann, 1989; Clark *et al*, 1990; Barber *et al*, 1991) predict a contribution from biomagnification of up to one order of magnitude at the highest trophic levels, for highly lipophilic substances that are resistant to metabolism.

The lipid content of the predator, relative to its prey, has a strong influence on the biomagnification potential of lipophilic substances.

Connell (1990) examined the theoretical potential for biomagnification and the observations from laboratory and field investigations. Various theoretical approaches suggested that within aquatic (water breathing) food chains, if equilibrium is reached, the biomagnification factor will be approximately unity when concentrations are expressed in terms of lipid weight, and that the bioaccumulation factor (lipid based) will be the essentially the same as the bioconcentration factor (from water), regardless of whether food or water is the actual source. However, he noted that whilst a considerable number of field and experimental data supported these conclusions, there were also data that did not. He also found that the evidence for biomagnification by air-breathing predators was more convincing, especially for birds.

Thus, despite a considerable degree of uncertainty, biomagnification may be significant for a limited number of highly lipophilic substances. The following sections consider the degree to which the potential for biomagnification may be different in the marine environment, compared with fresh water. As discussed for bioconcentration (Section 6.2), the behaviour of certain groups of chemical (such as inorganic and ionisable organic

substances) may be affected considerably by the high ionic strength of sea water, and the same may apply to bioaccumulation from food. Similarly, the relatively minor effects of sea water on the solubility and partitioning of hydrophobic, neutral organics, which were concluded to have no significant impact on bioconcentration, would also be expected to make little difference to the kinetics of biomagnification. Therefore, the following considers only whether the structure and function of marine food chains give rise to a different biomagnification potential.

6.3.1 Food chain structure and complexity

The Arctic Monitoring and Assessment Programme (AMAP) has presented a comparison of freshwater and marine ecosystems with respect to characteristics that are of importance for bioaccumulation and biomagnification (AMAP, 1998). This concluded that studies in both temperate and Arctic ecosystems have shown that the accumulation and metabolism of organic contaminants in fauna is dependent on the length of the food chain.

In both freshwater and marine ecosystems, there are five basic trophic levels, with primary producers (predominantly plants) at the base, primary consumers (herbivores) that include zooplankton, detrital feeders (e.g. benthic insect larvae and crustacea), secondary consumers (carnivorous invertebrates, fish, birds and mammals) and degraders (e.g. fungi and bacteria). The trophic level of some species may change with their life stage. However, within the secondary consumer level there may be multiple steps in the food chain, each of which may provide an opportunity for biomagnification. As a general rule, the predator is larger than the prey, and therefore the greater the range of size of secondary consumer, the greater is the potential for longer food chains. Marine systems tend to contain carnivorous invertebrates that are considerably larger than are typical of fresh water (e.g. crabs, lobsters and squid) that are in turn prey to large fish, and piscivorous fish that are generally larger than found in fresh water (e.g. sharks and tuna). Thus, within the aquatic (water breathing) organisms, there exists a potential for more links in the marine food chain, although it would be difficult to quantify average differences. However, the majority of the data for biomagnification is based on analysis of field residues in relatively simple and hypothetical food chains (e.g. phytoplankton, crustaceans, small fish and large fish) and there is little evidence to suggest that more predator:prey steps would necessarily increase overall biomagnification in the top predators. Biomagnification of air-breathing top-predators in the food chain are discussed in Section 6.3.3.

6.3.2 Lipid content

Due to the hydrophobic nature of organic contaminants, their dynamics in the food chain are closely related to the lipid content of the organisms. Especially in Arctic regions, high lipid levels are found in the organisms as an adaptation to the cold climate and the cyclic annual productivity. Long lipid-based and complex food chains may contribute to high levels of organic contaminants in the Arctic. The transfer of lipids is particularly evident in the marine food chains. The lipid content of primary producers, mainly diatoms, is high (Sargent and Henderson, 1986). Pelagic marine organisms that feed on

the phytoplankton have a strong propensity for converting phytoplankton into lipid stores (Falk-Petersen *et al*, 1981; Sargent and Falk-Petersen, 1981). The lipids produced by phytoplankton during the spring bloom in March and April are evident as high lipid levels in the zooplankton species *Calanus finmarchicus* and *Thysanoessa* spp. (krill) in June to August, and then as high lipid levels in a fish species, *Mallotus villosus* (capelin), which feed on these zooplankton in September and October. Transfer to higher levels of the food chain occurs over many years, since most species at the top of the food chain, particularly marine mammals, are long-lived (AMAP, 1998).

There are no latitudinal patterns in the lipid content of dominant fish (cod, herring and mackerel) in northern waters or in the lipid content of marine mammals (Wania, 1995). However, latitudinal differences in the types of lipid in organisms could influence the bioaccumulation of organic contaminants. Numerous latitudinal differences in lipid metabolism have been reported. For example, poikilotherms increase the ratio of polyunsaturated to saturated fatty acids in membrane lipids in response to lowered ambient temperatures (Greene and Selivonchick, 1987). Also, the lipid content of organs involved in osmoregulation has been reported to be lower at marine salinities (Henderson and Tocher, 1987).

6.3.3 Marine birds and mammals

The evidence for biomagnification is more convincing for the air-breathing top predators (Connell, 1990) which do not have the opportunity to depurate residues to water across respiratory surfaces. However, metabolic capability and rates will be higher in birds and mammals, compared to fish and invertebrates, which may reduce or reverse any tendency for biomagnification.

The existing EU practice evaluates the risk to birds feeding on earthworms and birds and mammals feeding on fish; this is referred to as secondary poisoning. It is achieved by a comparison of the concentration predicted (or derived from monitoring) in the prey (PEC) with a no-effect level by the oral route derived for the predator. Thus the risk to "freshwater" avian (e.g. heron, osprey) and mammalian predators (e.g. otters, mink) is already addressed. The issue for the marine environment is whether the exposure or sensitivity is greater for birds and mammals that feed exclusively or predominantly on marine organisms (in particular seals, dolphins, whales and bears). Although many of these air-breathing predators feed on marine fish and invertebrates (as a first tier), there is also a second "tier" to be considered, which are the larger mammals feeding on smaller mammals and birds, such as polar bears feeding on seals.

For the first tier, it is reasonable to conclude, from the preceding discussion of biomagnification, that the highest concentrations of bioaccumulative contaminants would be expected in fish rather than invertebrates, due to an uncertain but precautionary assumption of biomagnification within the aquatic food chain. Thus it is appropriate to consider fish as the sole dietary source for the first tier. The TGD procedure is to multiply the PEC_{aqua} at the local-regional scale (the mean of the local and regional values) by the bioconcentration factor. Since this does not allow for possible biomagnification, a modification is proposed (Section 5) which increases (by a factor dependent on K_{ow}) the resulting PEC_{fish} for highly bioaccumulative substances.

For the second tier ('bears eating seals') it is necessary to estimate the PEC_{seal}. As discussed in Section 5, this is considered appropriate only at the regional-continental scale. In the absence of monitoring data, this can be estimated by using an additional factor on PEC_{fish} to allow for an additional biomagnification step. The proposed method of calculation is given in Section 5, and the necessary amendments to the calculation of the $PNEC_{predator}$ are described in Section 8.

6.4 Conclusions

Bioconcentration from water into marine organisms is only likely to differ significantly from that in fresh water when the form or speciation of the substance is directly affected by the ionic composition of sea water. For neutral, hydrophobic organics, bioconcentration measurements or predictions based on freshwater data will provide reasonable estimates of marine bioconcentration potential. However, as in fresh water, bioconcentration factors may vary significantly between different species and as a function of size, metabolic capability and lipid content.

Despite a considerable degree of uncertainty, biomagnification may be significant for a limited number of highly lipophilic substances. As for bioconcentration, when the chemical speciation of the substance is directly affected by the chemistry of sea water, the biomagnification potential in marine food chains is likely to differ significantly from that in fresh water. It is also possible that the greater complexity of marine food chains, and the more variable lipid content of marine organisms, requires special consideration in risk assessment, compared with the existing EU process for fresh water. To allow for such uncertainty, modification are proposed (see Section 5) to the current method of determining the exposure (PEC) for birds and mammals feeding on fish at the local-regional level and, at the regional-continental level, for larger mammals preying on these fish eating birds and mammals.

Further research is needed to resolve the uncertainty over the likelihood, extent and significance of biomagnification.

7. ECOTOXICITY

7.1 Introduction

In contrast to the substantial ecotoxicity database for freshwater biota, data relating to the effects of chemical substances upon marine and estuarine organisms are comparatively limited, especially for organic compounds (Solbé *et al*, 1993). This is because there are fewer standard test methods for saltwater species and the fact that, up to now, aquatic risk assessments have traditionally focused on freshwaters. Although it is desirable that the ecotoxicity database for such species is extended, a current requirement to conduct risk assessments for some substances in the marine environment may necessitate the extrapolation of the freshwater ecotoxicity database to marine species. This surrogate approach assumes that the response of freshwater species is similar to marine species. This section examines the rationale for such an extrapolation via a preliminary review of the available comparative data. Details of available standard ecotoxicological test protocols for marine and estuarine organisms have also been collated and are presented. The future application of such protocols will permit appropriate effects testing for the purposes of risk assessment in the marine environment and greatly extend the ecotoxicity database for relevant species.

7.2 Applicability of the freshwater biota ecotoxicity database

Hutchinson *et al* (1998) report a comparison of the sensitivities of freshwater and marine biota (fish and invertebrates) to various substances based on the ECETOC Aquatic Toxicity (EAT) database (ECETOC, 1993b). Whilst the authors emphasised the preliminary nature of the observations based on a limited number of data, some trends were apparent. In the case of fish (see Table 2), the sensitivity of freshwater (FW) and saltwater (SW) species were within a factor of 10 for 91% of EC_{50} values (acute endpoints) and 93% of NOEC values (mainly chronic endpoints). Within the smaller database for invertebrates, freshwater and saltwater species responses were within a factor of 10 for 33% of EC_{50} values and 83% of NOEC values (see Table 3). Insufficient data in the EAT precluded a similar comparison of freshwater and marine plants and algae.

Table 2: Summary of EC_{50} sensitivity ratios (Hutchinson et al, 1998)

Endpoint	Fish (EC_{50})	Inverts (EC_{50})
FW > sensitive SW	23%	34%
FW = sensitive SW (ratio 0.5 - 2.0)	27%	8%
FW < sensitive SW	50%	58%
FW/SW ratio <10	91% (n = 22)	33% (n = 12)

Table 3: Summary of NOEC sensitivity ratios (Hutchinson et al, 1998)

Endpoint	Fish (NOEC)	Inverts (NOEC)
FW > sensitive SW	43%	33%
FW = sensitive SW (ratio 0.5 - 2.0)	29%	50%
FW < sensitive SW	28%	17%
FW/SW ratio <10	93% (n = 14)	83% (n = 6)

The ECETOC Aquatic Hazard Assessment II Task Force is updating the EAT database. This new database (EAT-3) contains over 5,400 quality reviewed endpoints, of which around 1,300 relate to SW species. A summary of comparisons for fish, invertebrates and algae for acute data (EC_{50} values) is presented in Table 4 [1]. In the case of fish, SW species were more sensitive (ratio >2.0) in 33% of cases. The sensitivity of FW and SW fish species were within a factor of 10 for 86% of EC_{50} values. Extreme ratios of <0.1 or >10 were equally likely. SW invertebrate species were more sensitive (ratio >2.0) than FW species for 31% of EC_{50} values. FW and SW invertebrate species responses were within a factor of 10 for 64% of EC_{50} values. Again, extreme ratios of <0.1 or >10 were equally likely. SW algal species were more sensitive (ratio >2.0) than FW species for 27% of EC_{50} values. FW and SW algal species responses were within a factor of 10 for 100% of EC_{50} values.

Table 4: Summary of EC_{50} sensitivity ratios (ECETOC EAT-3 database)

Endpoint	Fish (EC_{50})	Inverts (EC_{50})	Algae (EC_{50})
FW > sensitive SW	24%	51%	36%
FW = sensitive SW (ratio 0.5 - 2.0)	43%	18%	36%
FW < sensitive SW	33%	31%	27%
FW/SW ratio < factor 10	86% (n = 51)	64% (n = 39)	100% (n = 11)

A summary of comparisons for fish, invertebrates and algae for chronic/sub-chronic data (NOEC values) is presented in Table 5. In the case of fish, SW species of fish were more sensitive (ratio >2.0) in 18% of cases. The sensitivity of FW and SW species were within a factor of 10 for 71% of EC_{50} values. Ratios <0.1 were much more likely than ratios >10. Within the database for invertebrates, SW species were less sensitive (ratio >2.0) than FW species for 14% of NOEC values. FW and SW species responses were within a factor of 10 for all but one NOEC of <0.1 (86%). No comparison was made of algal NOECs due to a lack of comparable data.

[1] Comparisons are based on geometric means of observations for any given substance within the two media.

Table 5: Summary of NOEC sensitivity ratios (ECETOC EAT-3 database)

Endpoint	Fish (NOEC)	Inverts (NOEC)	Algae (NOEC)
FW > sensitive SW	53%	57%	nd
FW = sensitive SW (ratio 0.5 - 2.0)	29%	29%	nd
FW < sensitive SW	18%	14%	nd
FW/SW ratio < factor 10	71% (n = 17)	86% (n = 7)	nd

nd No data

A more comprehensive review including paired-species comparisons will be included in the Aquatic Hazard Assessment II Task Force report (ECETOC, 2001; Eadsforth *et al*, 2001).

It should be noted that the above comparison of the responses of both fish and invertebrates relate to disparate species and not paired species comparisons. The outcome of more direct comparisons is reported by LeBlanc (1984), who established the correlation between the responses of representative paired (i.e. a single FW and a single SW) species of algae, invertebrates and fish to a common set of non-pesticide organic compounds (see Table 6 and Figures 4-6).

Table 6: Comparison of sensitivity of freshwater and saltwater species to non-pesticide organics [n = 19] (from LeBlanc, 1984)

Trophic Level	Freshwater Species	Saltwater Species	r^2	Equation
Fish	Lepomis macrochirus	Cyprinodon variegatus	0.80 (p<0.01)	log L. macrochirus LC_{50} = (log C. variegatus LC_{50} * 0.87) + 0.32
Inverts	Daphnia magna	Mysidopsis bahia	0.80 (p<0.01)	log D. magna LC_{50} = (log M. bahia LC_{50} * 0.88) - 0.11
Algae	Selenastrum capricornutum	Skeletonema costatum	0.87 (p<0.01)	log S. capricornutum LC_{50} = (log S. costatum LC_{50} * 1.0) - 0.28

In the case of the algae, the SW species (*Skeletonema costatum*) was generally more sensitive (as reflected by the regression intercept of -0.28) but the differences between the two species were <1 log unit for all but one of the compounds. Sensitivity to 5 compounds differed by >0.5 log unit. The average difference was 0.31 log unit (factor ~2). This constitutes a relatively minor difference compared to other examples of interspecific differences of up to 4 orders of magnitude in responses of algae (both FW and SW) to individual test substances as collated by Lewis (1995). Variations in sensitivity as high as 5 orders of magnitude have been reported between different phylogenetic (taxonomic) groups of algae (Rojícková-Padrtová and Maršálek, 1999).

A similar pattern was observed with the invertebrates. *Mysidopsis bahia* was generally more sensitive than *Daphnia magna*, but the differences between the two species were <1 log unit for all but one of the compounds. A total of 7 compounds differed by >0.5 log unit. The average difference was 0.38 log unit (factor ~2). It should be noted however, that the duration of the mysid shrimp bioassay was 96 hours compared to 48 hours for *Daphnia* species and this will at least partially help to explain the reported differences.

In the case of the fish, only one compound differed by >1 log unit. A total of 4 compounds differed by >0.5 log unit with the FW species (*Lepomis macrochirus*) being more sensitive in 3 of these cases (this is reflected in the regression intercept of +0.32). The average difference was 0.31 log unit (factor ~2).

In addition, the relative sensitivities of aquatic organisms occupying the three different trophic levels in both freshwater and saltwater environments were also established. In most cases, the differences in sensitivity observed between the trophic levels in fresh water and salt water were as great or greater than that observed between the paired species from a common trophic level across the two media (see Table 7). This would appear to suggest a physiological similarity between species belonging to similar taxonomic groups, regardless of their freshwater or marine origins.

Table 7: Comparison of sensitivity of species from different trophic levels to non-pesticide organics [n = 19] (from LeBlanc, 1984)

Trophic Comparison	Freshwater Species r^2	SaltwaterSpecies r^2
Fish v Invertebrate	0.94	0.76
Invertebrate v Algae	0.67	0.71
Algae v Fish	0.62	0.84

Figure 4: Acute ecotoxicity of non-pesticide organics to Selenastrum capricornutum and Skeletonema costatum (from LeBlanc, 1984)

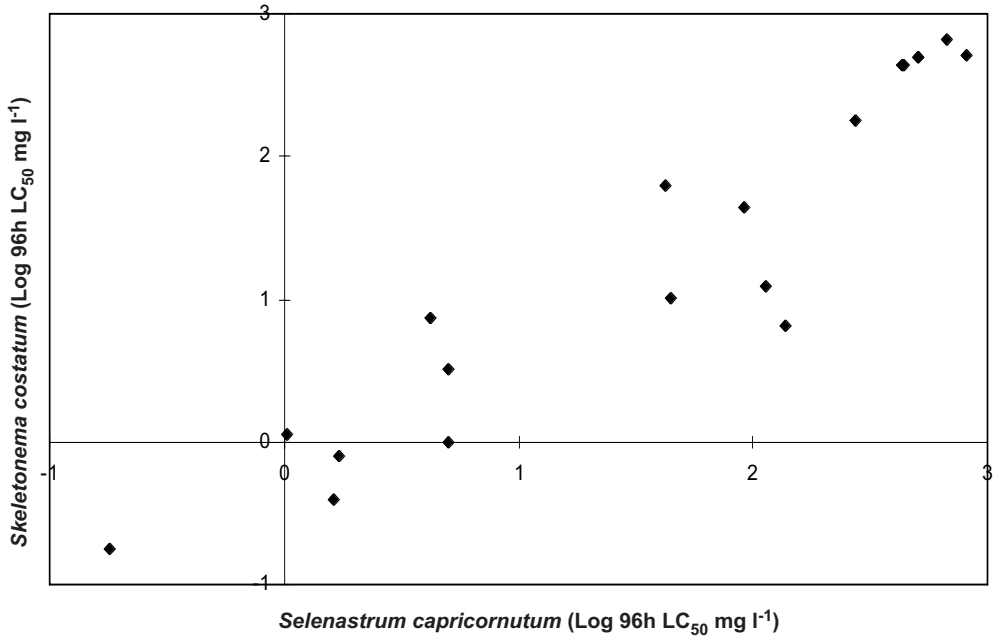


Figure 5: Acute ecotoxicity of non-pesticide organics to Daphnia magna and Mysidopsis bahia (from LeBlanc, 1984)

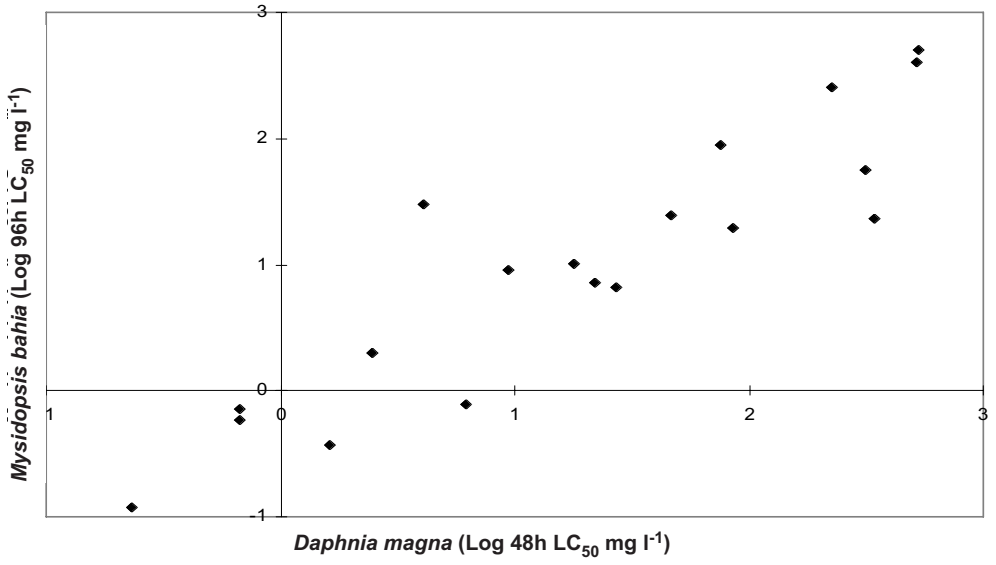
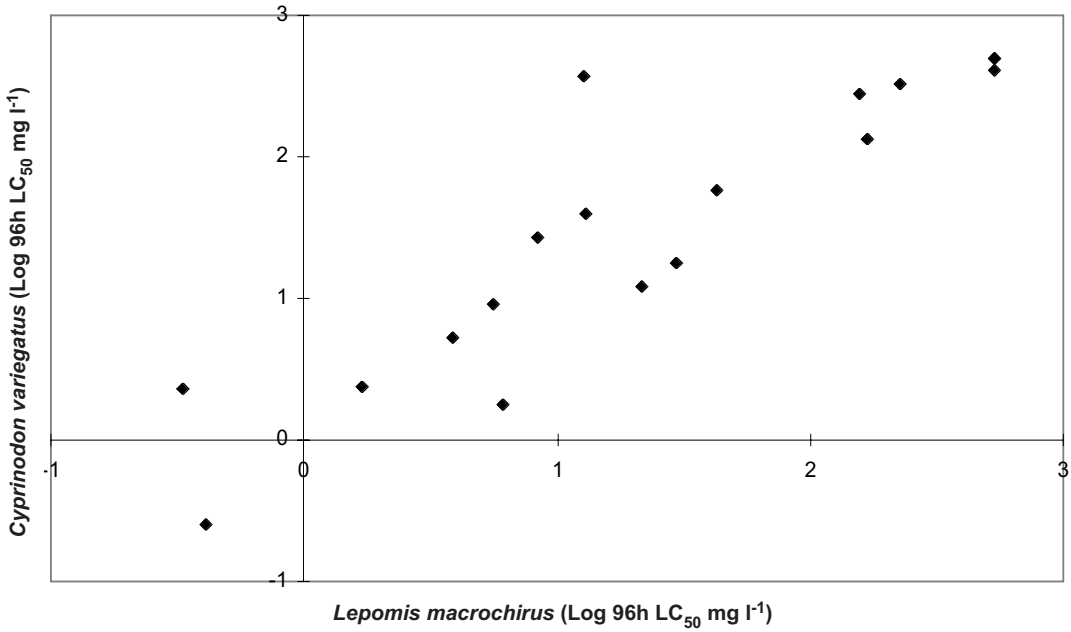


Figure 6: Acute ecotoxicity of non-pesticide organics to Lepomis macrochirus and Cyprinodon variegatus (from LeBlanc, 1984)

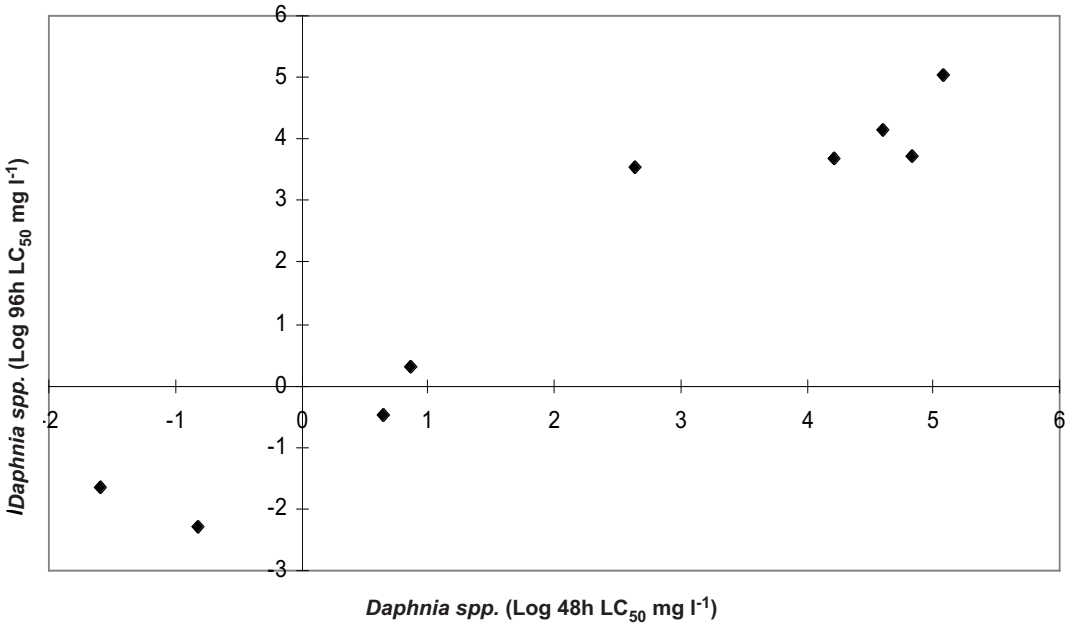


A subsequent comparison of the sensitivities of *Daphnia* spp. and *Mysidopsis bahia* to organic pesticides is also available from Robinson (1999) (see Figure 7). In 8 out of the 9 cases, *Mysidopsis bahia* was more sensitive than *Daphnia* spp. For three compounds the difference in sensitivity was >1 log unit. The average difference was reported as a factor of 2 (i.e. *Mysidopsis bahia* was on average twice as sensitive as *Daphnia* spp.). It should be noted however, that the author attributed the apparent greater sensitivity of *Mysidopsis bahia* to the differing duration of the bioassays (i.e. 48 hours for *Daphnia* spp. compared with 96 hours for *Mysidopsis bahia*). The derived relationship was consistent with that observed by LeBlanc (1984) for a direct comparison of other organics. A pooled data set (n = 28) yielded a similar relationship (see Table 8).

Table 8: Comparison of sensitivity of Daphnia spp. and Mysidopsis bahia to (i) pesticides [n = 9] and to (ii) pesticides and other organic compounds [n = 28] (from Robinson, 1999)

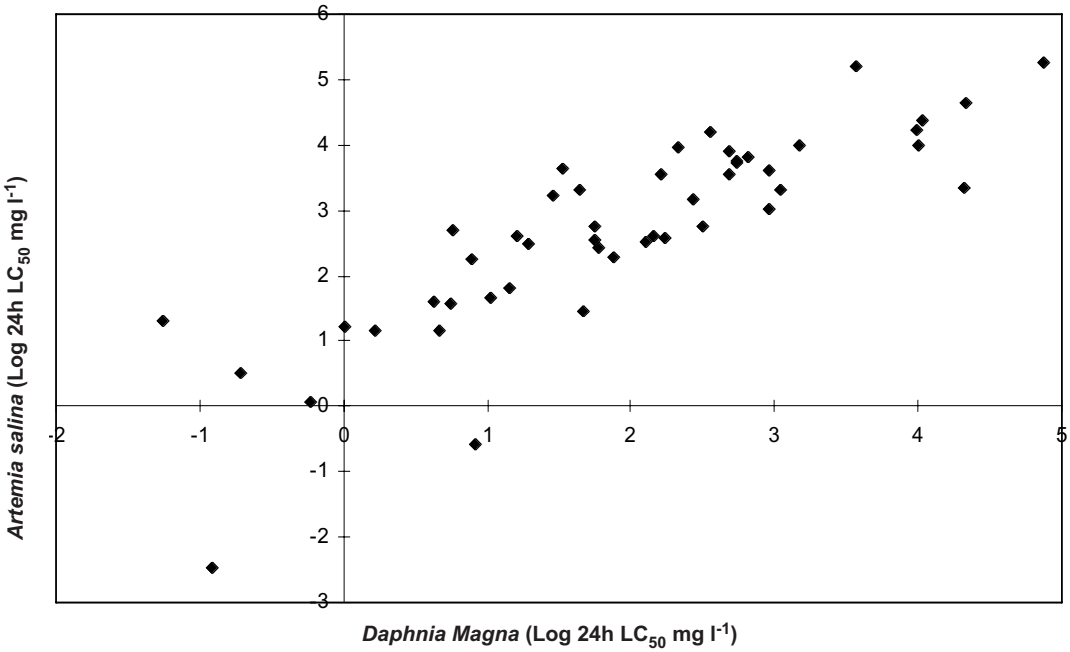
Data set	Equation	r^2
Pesticides	Mysidopsis bahia log LC_{50} (µmol l^{-1}) = (Daphnia spp. log EC_{50} (µmol l^{-1}) * 1.05)-0.47	0.94
Pesticides and other organics	Mysidopsis bahia log LC_{50} (µmol l^{-1}) = (Daphnia spp. log EC_{50} (µmol l^{-1}) * 1.03)-0.38	0.94

Figure 7: Acute ecotoxicity of pesticides to Daphnia spp. and Mysidopsis bahia



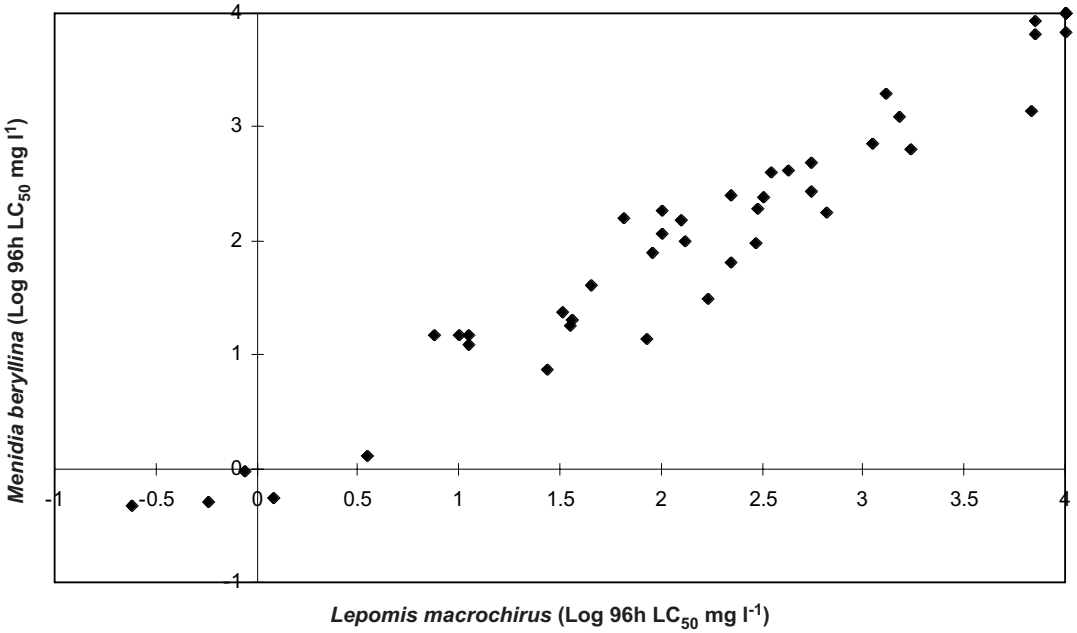
Comparisons can also be made between other pairs of FW and SW invertebrates. For example, Calleja *et al* (1994) tested 50 compounds with respect to their acute ecotoxicity to *Daphnia magna* and *Artemia salina* (amongst other species). Whilst brine shrimps are not marine species *per se*, their natural habitat are saline waters and some physiological parallels (i.e. osmoregulation) with marine invertebrate species can be assumed. For 21 of the compounds, there was >1 log unit difference in the sensitivity of the two species. Half of these compounds (i.e. 10) were metal salts. However, in 19 out of the 21 cases *Daphnia magna* was the more sensitive species. The r^2 value derived from the correlation of log-transformed 24h $L(E)C_{50}$ values for the two species is 0.71 (see Figure 8). This excludes malathion which is an extreme outlier that is markedly more ecotoxic to *Daphnia magna* than to *Artemia salina*.

Figure 8: Acute ecotoxicity of the 50 chemicals to Daphnia magna and Artemia salina (from Calleja et al, 1994)



Dawson *et al* (1977) similarly report a comparison of the acute ecotoxicity of 47 industrial chemicals to freshwater Bluegill Sunfish (*Lepomis macrochirus*) and saltwater Tidewater Silversides (*Menidia beryllina*) (see Figure 9). In the study, 96h LC_{50} values for the two species were remarkably consistent ($r^2 = 0.96$) for most compounds tested with no marked anomalies (log *Menidia beryllina* LC_{50} = (log *Lepomis macrochirus* LC_{50} * 0.98) - 0.07). The difference in the sensitivity of the two species to all compounds was <1 log unit. The sensitivity to six compounds differed by >0.5 log unit with the saltwater species being the most sensitive in all 6 cases. The greatest variation was observed for tetramethyl lead and thallium acetate with freshwater 96h LC_{50} values a factor of 6.2 and 5.5 greater than the respective saltwater value. The average difference in sensitivity was 0.21 log unit (factor <2).

Figure 9: Acute ecotoxicity of industrial chemicals to Lepomis macrochirus and Menidia beryllina (from Dawson et al, 1977)



Hemmer *et al* (1992) determined the relative sensitivities of two estuarine/coastal species, namely Topsmelt (*Atherinops affinis*) and Inland Silversides (*Menidia beryllina*), when exposed in 96h static acute toxicity tests to 11 chemicals. Additional comparisons with data for three freshwater species (*Lepomis macrochirus, Oncorhynchus mykiss* and *Pimephales promelas*) and one estuarine fish species (*Cyprinodon variegatus*) derived from the literature are also reported. A correlation matrix derived from the data in this study is presented below (Table 9).

Table 9: Matrix of correlation coefficients of log-transformed acute static 96h LC_{50} values from six species of fish (from Hemmer et al, 1992)

	A. affinis	M. beryllina	C. variegatus	P. promelas	L. macrochirus	O. mykiss
	Marine/Estuarine Species (SW)			Freshwater Species (FW)		
A. affinis	-	-	-	-	-	-
M. beryllina	0.98 (n = 11)	-	-	-	-	-
C. variegatus	0.91 (n = 9)	0.93 (n = 11)	-	-	-	-
P. promelas	0.82 (n = 10)	0.84 (n = 11)	0.77 (n = 8)	-	-	-
L. macrochirus	0.95 (n = 10)	0.97 (n = 11)	0.86 (n = 8)	0.80 (n = 8)	-	-
O. mykiss	0.95 (n = 8)	0.93 (n = 11)	0.73^{ns} (n = 6)	0.78 (n = 8)	0.69 (n = 7)	-

In almost all cases, there was a significant correlation ($p<0.05$) between the responses of any pair of species. Within each of the nine permutations comparing the responses of a freshwater species to a marine/estuarine species, freshwater species were more sensitive than marine/estuarine species in seven cases (highlighted in bold in Table 10).

Table 10: Differences between log-transformed 96h LC_{50} values between species pairs (from Hemmer et al, 1992). Comparisons are based on log units i.e. <0.5 log units difference

	A. affinis	**M. beryllina**	**C. variegatus**	**P. promelas**	**L. macrochirus**	**O. mykiss**
	Marine/Estuarine Species (SW)			**Freshwater Species (FW)**		
A. affinis	-	-	-	-	-	-
M. beryllina	AA>MB (9) 9 <0.5 2 >0.5 0 >1.0 n = 11	-	-	-	-	-
C. variegatus	AA>CV (7) 5 <0.5 2 >0.5 2 >1.0 n = 9	MB>CV (8) 6 <0.5 1 >0.5 2 >1.0 n = 9	-	-	-	-
P. promelas	AA>PP (7) 5 <0.5 2 >0.5 3 >1.0 n = 10	PP>MB (6) 5 <0.5 2 >0.5 3 >1.0 n = 10	PP>CV (6) 4 <0.5 1 >0.5 3 >1.0 n = 8	-	-	-
L. macrochirus	LM>AA (8) 8 <0.5 2 >0.5 0 >1.0 n = 10	LM>MB (10) 5 <0.5 4 >0.5 1 >1.0 n = 10	LM>CV (8) 3 <0.5 1 >0.5 4 >1.0 n = 8	LM>PP(8) 5 <0.5 4 >0.5 0 >1.0 n = 9	-	-
O. mykiss	AA>OM (6) 7 <0.5 1 >0.5 0 >1.0 n = 8	OM>MB (5) 6 <0.5 2 >0.5 0 >1.0 n = 8	OM>CV (4) 3 <0.5 0 >0.5 3 >1.0 n = 6	OM>PP (6) 5 <0.5 0 >0.5 3 >1.0 n = 8	LM>OM (5) 5 <0.5 2 >0.5 0 >1.0 n = 7	-

Numbers in brackets refer to the number of occasions that one species was the more sensitive of the pair, i.e. *A. affinis* was more sensitive than *M. beryllina* for 9 of 11 substances considered, although in most cases the difference between the two species was <0.5 log units. For no substance was the difference >1 log unit.

A hierarchy consistent with the established relative sensitivity of the pairs would be: *L. macrochirus* (FW) > *A. affinis* (SW) > *O. mykiss* (FW) > *P. promelas* (FW) > *M. beryllina* (SW) > *C. variegatus* (SW)

Overall, most comparisons (approximately 60% or greater) for individual compounds reveal that the difference in the responses of paired species are less than 0.5 log units. No consistent differences in variation could be discerned from comparisons made within or between media, i.e. variation within freshwater or marine/estuarine species appeared to be similar to that between freshwater and marine estuarine species.

Nendza and Klein (1990) studied structure-toxicity relationships using acute toxicity data to various freshwater and estuarine organisms from different taxonomic groups for 26 phenol and benzidine derivatives. No statistical discrimination could be made between freshwater and estuarine organisms and the ranking of sensitivities for taxonomic groups was similar. Zaroogian *et al* (1985b) similarly investigated QSAR predictions of marine effects based on freshwater data.

Nacci and Jackim (1985) report a comparison of a sea urchin (*Arbacia punctulata* - Echinoderm) acute embryo test (growth inhibition) with standard acute toxicity tests based on *Pimephales promelas* (fathead minnow) and *Daphnia magna* (water flea). Correlations and regression results are presented below (Table 11). In both cases, the sea urchin bioassay proved to be the least sensitive to a suite of eight organic compounds.

Table 11: Comparison of sensitivity of Daphnia magna and Pimephales promelas with Arbacia punctulata in acute toxicity test (from Nacci and Jackim, 1985)

Regression Equation	Correlation (n = 8)
D. magna 48h LC_{50} (mg l^{-1}) = 1.018 * Arbacia 4h EC_{50} (mg l^{-1}) + (-0.4184)	r^2 = 0.9909
P. promelas 96h LC_{50} (mg l^{-1}) = 1.0019 * Arbacia 4h EC_{50} (mg l^{-1}) + (-0.28682)	r^2 = 0.9292

Sorokin (1999) reports a comparison of the responses of the two most commonly used species for algal toxicity tests, i.e. the freshwater green alga *Selenastrum capricornutum* and the marine diatom *Skeletonema costatum*. The source data for this study was the Aquire database (US EPA, 1997b). A search yielded 2,070 records for the two species. This was reduced to 114 compounds that had been tested on both species and where a common endpoint (i.e. EC_{50}) was available. A common method of analysis (i.e. population growth readings) reduced the number of compounds to 16, 8 of which were metals and 8 organic compounds. A regression analysis of log transformed EC_{50} yielded an r^2 value of 0.23. In most cases (68%) reported responses of the two algal species were within one order of magnitude. Where differences existed (>factor 2), the marine species provided to be the less sensitive (Table 12).

Table 12: Summary of sensitivity ratios for Algae EC_{50}s (µg l^{-1}) (from Sorokin, 1999)

Endpoint	EC_{50} All Chemicals	EC_{50} Organics	EC_{50} Metals
FW Algae > tolerant SW algae	25%	50%	0%
FW Algae = sensitive SW algae	25%	25%	25%
FW Algae < tolerant SW algae	50%	25%	62.5%
FW/SW ratio <10*	68% (n = 16)	75% (n = 8)	62.5% (n = 8)

* Factor of 10 assumed to indicate no significant difference between values (after Hutchinson, 1998)

A comparison of marine and freshwater acute ecotoxicity data from the Aquire database (US EPA, 1997b) and other US EPA sources (US EPA, 1986) for selected species is also presented here. The species compared were selected on the basis that they are representative of their respective taxa/phyla and appear to be those most commonly employed in testing. They were *Skeletonema costatum* (marine algae), *Mysidopsis bahia* (marine crustacean), *Crassostrea virginica* (marine mollusc), *Cyrinodon variegatus* (marine fish), *Nereis arenaceodentata* (marine annelid), *Paracentrotus lividus* (marine echinoderm), *Selenastrum capricornutum* (freshwater algae), *Daphnia magna* (freshwater crustacean) *Pimephales promelas* (freshwater fish). This dataset allowed the influence of the availability of marine data upon the outcome of freshwater versus marine species comparisons to be assessed. Comparisons between the most sensitive endpoints from the freshwater and marine datasets were possible for 259 substances.

The outcome of the comparisons are detailed in Table 13 and Figure 10. Overall, increased availability of data from the six marine taxa did not appear to influence greatly the outcome of the analyses when comparing the median values from the respective distributions. In all cases, the median values of the ratios between the marine and freshwater endpoints were very similar and within the range 0.44 to 1.61 (where 1 denotes equal sensitivity and <1 greater sensitivity on the part of marine organisms). Classical freshwater ecotoxicity data (i.e. algae, *Daphnia* and fish) yielded more sensitive endpoints than the corresponding marine dataset (potentially containing up to 6 taxa including molluscs, echinoderms and annelids) in 54% of cases. In addition, freshwater endpoints tended to be more sensitive by larger margins than the contrary. Marine endpoints were more sensitive than freshwater endpoints for many individual substances, but less often and by smaller margins. These margins could nevertheless still be significant, with an extreme of 4 orders of magnitude for one particular compound reducing to 2 orders of magnitude at the 5-percentile. Of those comparative ratios below the 5-percentile (i.e. 0.016), no data were available for *D. magna* for approximately two-thirds of the substances considered. This is an important observation as it is a relatively sensitive species (see below).

As the number of marine taxa increased, the magnitude of the ratios at the 95-percentiles decreased. This meant that the degree to which freshwater taxa were more sensitive than marine taxa decreased as more marine data became available - which is perhaps to have been expected. The trend was less clear at the 5-percentile and perhaps even

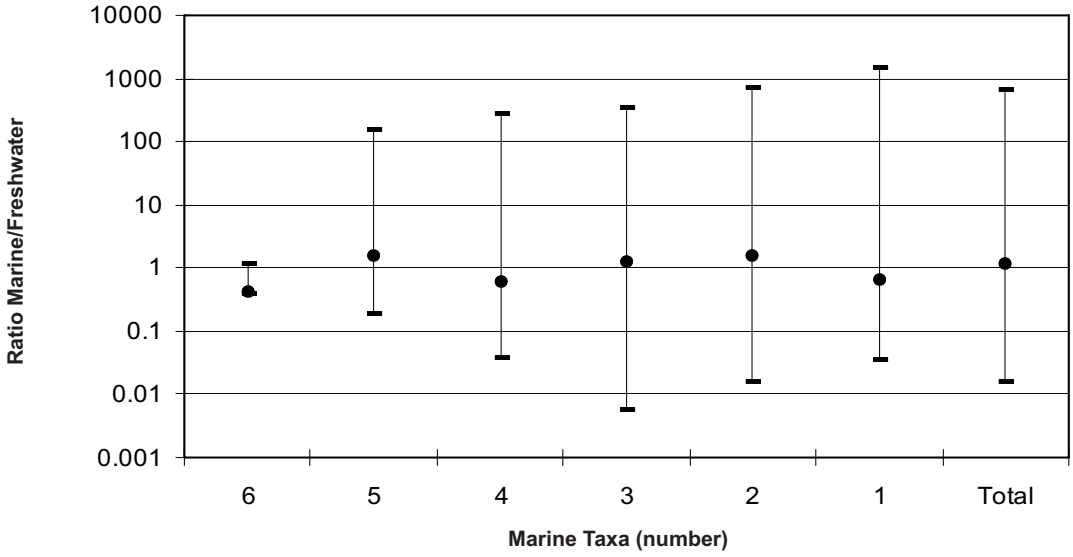
[2] Leptofos - Paucity of data meant that the response pf *M. bahia* which is relatively sensitive was compared with that of *P.promelas* which is relatively insensitive.

contradictory. As the availability of data from marine taxa increased from 3 through to 6, the extent to which marine species were more sensitive than freshwater species actually decreased. It should be noted however that the number of observations where available marine taxa number ≥5 is limited (n = 8).

Table 13: Comparison of sensitivity of key marine and freshwater taxa (from Aquire (US EPA, 1997b) and US EPA, 1986). Values of ratios <1 denote greater sensitivity of marine species

Marine Taxa	Median Ratio	Number	Ratio ≤1 (%)	Minimum Ratio	5%-ile Ratio	95%-ile Ratio	Maximum Ratio
6	0.44	3	83.2	0.39	0.40	1.20	1.28
5	1.53	5	36.6	0.104	0.19	156	192
4	0.63	29	59.0	0.0096	0.0395	283	2,100
3	1.3	95	38.2	0.0003	0.0059	356	1,120
2	1.61	99	46.0	0.0019	0.0162	748	16,667
1	0.65	28	55.9	0.0001	0.0368	1,506	50,000
Total	1.22	259	46.1	0.0001	0.0160	660	50,000

Figure 10: Comparison of sensitivity (median, 5-percentile and 95-percentile) of key marine and freshwater taxa (from Aquire (US EPA, 1997b) and US EPA, 1986). Values of ratios <1 denote greater sensitivity of marine species



Comparisons of the relative sensitivity of the same 9 taxa from the Aquire (US EPA, 1997b) database revealed that *Daphnia magna* was consistently the most sensitive species - particularly if the relevant endpoint was immobilisation (Appendix B, Table 23). The ratios of *D. magna* to the other species (both freshwater and marine) at the median ranged from 0.08 for *Nereis arenaceodentata* to 0.83 for *Mysidopsis bahia* (where values <1 imply greater sensitivity on the part of *D. magna*). The proportion of ratios ≤1 ranged from 55% for *M. bahia* (n = 149) to 93% for *N. arenaceodentata* (n = 25). Comparison of the minimum/maximum and 5-percentile/95-percentile ratios also revealed than when *D. magna* was the more sensitive taxa, it was by a greater margin than when the converse was true. The relative sensitivity of *D. magna* is further confirmed by regressions of *D. magna* against each of the remaining 8 taxa (Appendix B, Table 23).

Comparative Diversity: One potential criticism of the existing marine effects database is the general lack of data pertaining to certain key marine taxa such as molluscs or echinoderms. Implicit in such criticism is that such phyla are potentially more sensitive and that any effects assessment conducted in the absence of such data may not capture this sensitivity. However, such a criticism can also be leveled at the freshwater effects database which similarly lacks information relating to key taxa such as molluscs and insects. So this is a short-coming of the effects assessment process as a whole rather than solely for marine environments. It should be noted that an assumption of variation in the responses of species e.g. from different trophic levels and taxa is already implicit in the use of tiered assessment factors to derive PNECs (e.g. TGD).

One means with which to try to measure the relevance of the established screening approach to aquatic ecotoxicity testing is to gather information on relative importance of species within the ecosystem. Importance is, however, difficult to define, as it could be based on total species biomass, or on food web keystone species or on diversity of the phylum present in aquatic ecosystems. It is extremely difficult to estimate total species biomass or relative importance within the food web and so here an attempt has been made to use phylogenic data on fauna to determine species abundance within an ecosystem. However, there are certain disadvantages to this method which make quantification difficult. Firstly, there are certainly many more species which have yet to be discovered For example, in the last twenty years two new phyla, namely Cycliophora and Loricifera, have been discovered in the North-East Atlantic and many hundreds of new marine species are described worldwide each year. Secondly, phylologists classify species differently, dividing or grouping organisms into greater or smaller categories. Such divisions between specialists may cause differences of an order of magnitude in estimations of species abundance (May, 1988). The third disadvantage of using this methodology, is that whilst it provides an unbiased viewpoint, it fails to take into account that the food chain is essentially pyramidal in form and that species at higher trophic levels will probably be less numerous.

Overall biodiversity is higher in marine environments with 18 exclusively marine phyla among the 27 metazoan phyla. Examples of phyla typically not represented in freshwater environments include Ctenophora, Mesozoa, Echinodermata whilst others that are rarely present in freshwater ecosystems include Nemertina and Porifera. Figures 11 and 12 provide detail of the relative abundance of species in major phyla from the animal kingdom within freshwater and marine environments. The data have been compiled

from various sources (May, 1988; WRI, 1992; Barnes, 1963). The phylum Arthropoda has been separated into the two classes Crustacea and Insecta to highlight the differences between the marine and freshwater environment. Likewise, elements within the phylum Chordata have been highlighted separately.

Freshwater effects testing based on fish (9.7%) and *Daphnia* (Arthropoda - Crustacea 5.3%) would imply coverage of a total of 15% of species within the freshwater fauna. In the marine environment, the absolute number of phyla *per se* is greater with an overall predominance of Mollusca species (48%). Vertebrates (mainly fish) account for 6.6% and Arthropoda - Crustacea represent 11.4%. One implication of this observation is that if typical freshwater ecotoxicology data (i.e. fish and *Daphnia*) are employed in marine risk assessment, 82% of marine fauna species will not be represented by the freshwater data (with the absence of molluscs being most notable). Even if there is no significant difference in sensitivity between comparable freshwater and saltwater species, it would be difficult to ensure that the most sensitive marine species has been represented. One option would be to supplement the typical freshwater ecotoxicity data set with additional testing based on the use of hitherto unrepresented species such as molluscs and echinoderms or annelids. Analysis presented here (Table 13, Figure 10) is particularly useful in this respect. Classical freshwater ecotoxicity data (*S. capricornutum*, *D. magna* and *P. promelas*) yielded more sensitive endpoints than the corresponding marine dataset (potentially containing up to 6 taxa including molluscs, echinoderms and annelids) in just over half the cases (54%). Freshwater endpoints were also more sensitive by larger margins than the contrary. Marine endpoints were more sensitive than freshwater endpoints for many individual substances, but less often and by smaller margins. Likewise, the response of *D. magna* (Appendix B, Table 23) appears to be quite marked relative to responses observed in other acute bioassays employing commonly tested marine species (including a mollusc, an annelid and an echinoderm). In practice, issues around diversity may prove not to be of prime concern.

Figure 11: Species composition of freshwater fauna (after May, 1988, WRI, 1992 and Barnes, 1963)

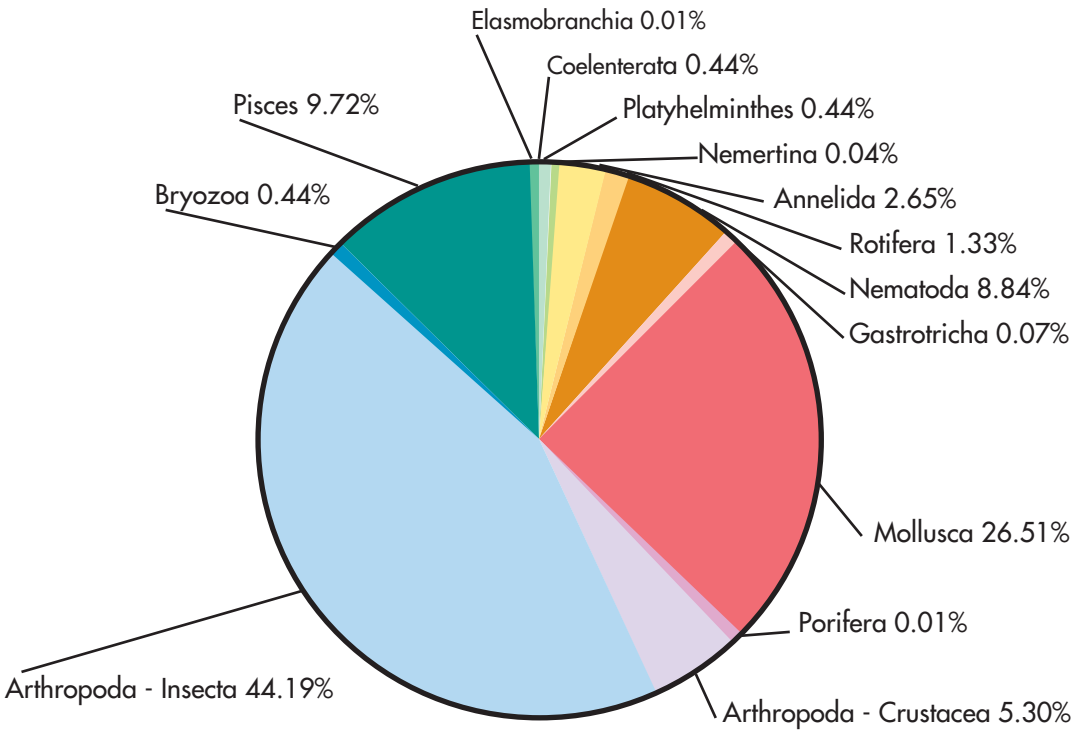
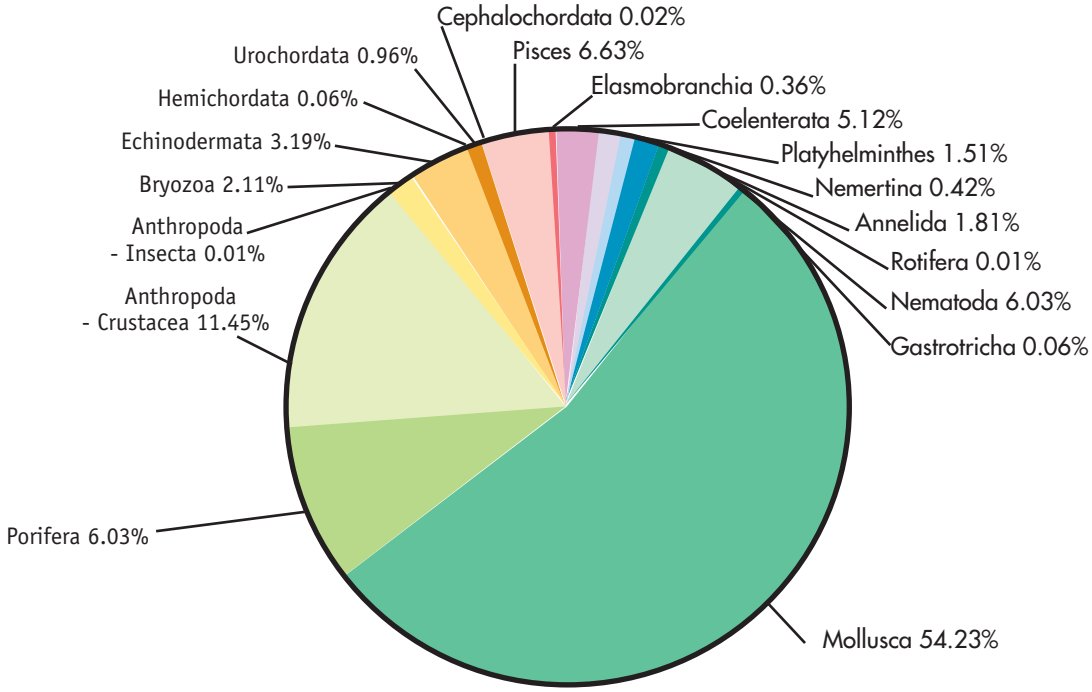


Figure 12: Species composition of marine fauna (after May, 1988, WRI, 1992 and Barnes, 1963)



Explaining Differences: The actual degree of correlation between freshwater and saltwater organisms will potentially be influenced by three factors:

- *Biological Differences*: Saltwater and freshwater organism differ in their physiology, phylogeny and life histories. This will influence sensitivity to toxicants. A potential criticism of the existing seawater effects testing database is the general lack of data pertaining to certain key marine taxa (e.g. Echinodermata, Mollusca, Cepaholopoda, Ctenophora). However, such a criticism could also be levelled at the freshwater effects database, which similarly lacks information relating to taxa such as molluscs and insects. However, it should be noted that an assumption of variation in the responses of species from different trophic levels and taxonomic groups is already implicit in the use of stepped-assessment factors to derive PNECs e.g. TGD (EC, 1996). Differences in physiology may also be responsible for differences in uptake and toxicity of certain chemicals to freshwater and marine crustaceans and fish (Rainbow, 1997a; Ferguson and Hogstrand, 1998; Tachikawa *et al*, 1991; Tsuda *et al*, 1990). Saltwater species may also have pelagic planktonic stages which can exhibit different sensitivities to chemicals (e.g. Wong *et al*, 1995; Lee *et al*, 1996). Finally, reproductive strategies of marine invertebrates are less responsive to changing environmental conditions, which might be expected to lead to differences in sensitivity to toxicants (Hutchinson *et al*, 1998). Any such differences would introduce a bias into any comparative study.

- *Chemical Speciation*: Differences in bioavailability in fresh and salt waters can be expected for a number of inorganic substances and can have a major impact on toxicity (Rainbow, 1997b). When reviewing toxicity data, it is important to recognise the possible differences between concentrations of total and active chemical species (e.g. Dixon and Gardner, 1998). Differences in the bioavailability of organic substances may occur as a consequence of phenomena such as adsorption to sediments and salting-out effects. Such differences are often acknowledged in water quality standards (which may be regarded as PNECs), with different standards for salt and fresh waters.

- *Test Methods:* Most internationally recognised test guidelines permit latitude in the way studies are performed, analysed and reported (e.g. identical growth data from algal tests can produce EC_{50} values an order of magnitude apart if expressed as final biomass rather than growth rate) (Nyholm, 1990). Differences between test methods for related freshwater and saltwater species are also a known source of major variability (Whitehouse *et al*, 1996). Coupled with a lack of diversity in the test species covered by standard test guidelines, such differences could introduce bias when extrapolating from freshwater toxicity data. Clearly, consistency between test methods for freshwater and saltwater species will help in making correlations between them. Furthermore, new test guidelines to redress the absence of key taxa may also be required.

Current Practice: In practice, there are several examples of existing situations where freshwater ecotoxicity data have been employed in lieu of or together with data for marine organisms for risk assessment purposes. These include substances undergoing transport in sea-going vessels (GESAMP, 1989), substances employed in the off-shore oil and gas industry (Karman *et al*, 1996) and contaminants monitored in the marine environment under the aegis of OSPAR for which Ecotoxicological Acceptable Concentrations (EACs) have been determined (OSPAR, 1998c). Other recent examples include a series of marine risk assessments for chlorinated organics in the OSPARCOM North Sea region conducted on behalf of Euro Chlor (i.e. chloroform, Zok *et al*, 1998; 1,2- dichloroethane, De Rooij *et al*, 1998a; 1,1,2-trichloroethane, De Rooij *et al*, 1998b; trichloroethylene, Boutonnet *et al*, 1998; tetrachloroethylene, De Rooij *et al*, 1998c). In each case pooled data for both marine and freshwater organisms available from IUCLID (1996) were employed in the effects assessment to derive the PNEC. This approach was justified as no significant differences in the sensitivity of marine and freshwater organisms from comparable tests were observed (Garny, 1998). Differences amongst freshwater species were as important as differences between freshwater and marine taxa.

7.3 Standard ecotoxicological test protocols (since species testing)

A substantial number of standardised ecotoxicological test protocols for marine and estuarine organisms have been developed by various national and international bodies (e.g. PARCOM, ISO, OECD, US EPA and ASTM). Details of these are summarised in Appendix B (see also Ward, 1995). Protocols exist for micro- and macro-algae, pelagic and benthic invertebrates (including crustacea, polychaetes, bivalves and echinoderms) and fish. It is notable that many of these protocols relate to regulatory submissions and as such publication of test results is apparently limited. This may contribute to the comparatively restricted (relative to the freshwater environment) effects database available for marine and estuarine fauna and flora.

Plant Testing: Plant communities are essential to the functioning of all ecosystems. Algal plankton form the base of most aquatic food chains, produce oxygen and are important in nutrient cycling. Macroalgae provide habitat and shelter for many members of near-coastal fauna. Marine algae also influence the physico-chemical quality of waters including light intensity, pH and dissolved oxygen. Given the importance of algae, phytotoxicity data are required to evaluate the impact of compounds that may enter the marine or estuarine environment. Most algal tests can be considered as chronic tests as they assess effects over several generations during a typical 3 - 4 day exposure. Many of the test species will have been chosen for their availability and ease of culture (Lewis, 1995) rather than ecological relevance or sensitivity. Toxicity tests with macroalgae are conducted less frequently. Whilst marine vascular plants are rare, a marine cordgrass (*Spartina alternifora*) has been used in seed germination test (Wang, 1992).

Invertebrate Testing: Given the great diversity of marine life, many hundreds of saltwater species representing many trophic levels will have been used in ecotoxicity tests. However, relatively few tests have been standardised for regulatory purposes. By definition, most of these species used are either readily cultured or easily collected. Many of the standard ecotoxicity testing with invertebrates are acute short-term tests of limited duration (i.e. 4d or less) with mortality as the endpoint. Nevertheless, established chronic tests (i.e. with growth and reproductive endpoints) are available for both pelagic (e.g. mysids) and benthic species (e.g. polychaetes).

Fish: The importance of fish species as representatives of higher trophic levels is self-evident in effects assessment practice. To this end, several saltwater fish species are available for acute toxicity testing. Amongst the most commonly tested are the sheepshead minnow, *Cyprinodon variegatus* (OECD, ASTM, US EPA), the Atlantic or inland silverside, *Menidia menidia* or *Menidia beryllina* (ASTM, US EPA) and the turbot, *Scopthalmus maximus* (PARCOM). Various protocols also exist for chronic early life stage (ELS) tests utilising the sheepshead minnow and the two species of silverside (ASTM, US EPA).

The standard organisms typically employed in standard tests during freshwater risk assessment are micro-algae, *Daphnia* and fish. They represent three trophic levels (i.e. primary producers, primary consumers and secondary consumers), three taxonomic groups (i.e. green algae, crustaceans and fish), two life forms (i.e. plankton and nekton)

and three feeding strategies (i.e. photosynthetic, herbivorous filter feeder and carnivore). Organisms used in marine ecotoxicity testing for the purpose of effects assessment can similarly be assigned to different trophic levels, in order to ensure the representativeness of the effects database. Standard tests are currently available to allow testing with various taxa representative of several trophic levels, life forms and feeding strategies. Whilst the majority of the bioassays are short-term or acute, chronic bioassays exist for fish, algae and both pelagic and benthic invertebrates. A matrix detailing the availability of test protocols for various taxonomic groups and compartments is presented in Table 13.

Table 13: Test protocols for various taxonomic groups and compartments

Test Level	Taxa	Water Column	Sediment
	Microalgae	✓	
	Macroalgae		
Acute	Fish	✓	
(i.e. mortality,	Crustaceans	✓	✓
immobilisation)	Molluscs	✓	✓
	Annelids		✓
	Echinoderms	✓	✓
	Microalgae	✓	
	Macroalgae	✓	
Sub-chronic/Chronic	Fish	✓	
(i.e. growth,	Crustaceans	✓	✓
reproduction)	Molluscs		
	Annelids		✓
	Echinoderms		

7.4 Field and mesocosm studies

Most evaluations of the effects of test substances upon aquatic organisms are based on the outcome of single species laboratory testing extrapolated to protective levels in the environment. Although the general applicability of such an approach is debatable, it has been suggested that the effects of non-persistent, acutely toxic compounds in field tests can be predicted from laboratory scale studies (SETAC-RESOLVE, 1992). One corollary is that the environmental effects of persistent, chronically toxic compounds are best assessed via multi-species based testing. However, well designed model ecosystem studies are costly to conduct and have typically only been used by a few laboratories. Their use is therefore limited to situations that are difficult to evaluate in conventional laboratory studies such as plankton dynamics or other key structural or functional endpoints. Overall, their use in routine effects assessment is therefore likely to be limited. The problems associated with marine mesocosm studies in general are reviewed by Graney *et al* (1995). The value of marine model ecosystem studies in ecotoxicology has also been reviewed by ECETOC (ECETOC, 1997). In general, biological

complexity (and stability) in such model ecosystems is a function of size with assemblages of benthic, planktonic and pelagial species being associated with large volume systems. Smaller systems typically focus upon benthic communities only (ECETOC, 1997).

7.5 Conclusions

Overall, the data that have been reviewed suggest a reasonable correlation, at least for acute data, between the ecotoxicological responses of freshwater and saltwater biota. There does not appear to be any marked difference in sensitivity between freshwater and saltwater biota that systematically applies across all trophic levels or taxa. When differences between comparable species from the freshwater and marine biota were evaluated (e.g. marine crustacea v freshwater crustacea, marine fish v freshwater fish) differences were consistently within a factor of 10 (<1 log unit) and usually somewhat less. Average differences in sensitivity for such paired species comparisons were more typically within a factor of 2. Where evaluated, differences between trophic levels or taxa within each medium were generally as significant or even more marked. This would appear to suggest a physiological similarity between species belonging to similar taxonomic groups regardless of their freshwater or marine origins. Variation between taxa is implicitly assumed in the use of assessment factors in current risk assessment practice. Reported differences in sensitivity between certain species pairs in some studies (i.e. *Daphnia* and *Mysidopsis*) is likely to be at least partially attributable to differing duration of experimental exposure rather than the sole result of underlying innate differences in sensitivity.

A substantial number of standardised ecotoxicological protocols exist for marine and estuarine organisms and are summarised here. These include protocols for taxa that are typically not tested in fresh water (i.e. molluscs or annelids) or indeed not represented in the freshwater environment in general (i.e. echinoderms). One potential criticism of the existing freshwater toxicological database vis-à-vis its applicability to the marine environment is the lack of data relating to certain key marine taxa such as molluscs, annelids or echinoderms. Analysis presented here appears to suggest that in practice, this may not be of prime concern. Classical freshwater ecotoxicity data (*S. capricornutum, D. magna* and *P. promelas*) yielded more sensitive endpoints more often than the corresponding marine dataset (potentially containing up to six taxa (including molluscs, echinoderms and annelids). In the same analysis, freshwater endpoints were also potentially more sensitive by larger margins than the contrary. The relative sensitivity of *D. magna* to a wide range of commonly tested marine and freshwater species was also noted. Overall the use of freshwater acute effects data in lieu of or in addition to saltwater effects data for risk assessment purposes is not contraindicated by the empirical data reviewed here. Use of pooled data is therefore recommended. Under such circumstances, PNEC values should be derived from the most sensitive endpoint regardless of the medium.

8. EFFECTS ASSESSMENT AND PNEC DERIVATION

8.1 Protection goals

The underlying aim of environmental risk assessment is the protection of the functionality of a specific ecosystem. Implicit in this approach is the assumption that certain levels of a contaminant in the environment, specifically those less than the PNEC, can be tolerated. The PNEC is derived mainly from laboratory tests designed to measure changes primarily in survival, growth or reproduction of test organisms, which are the key parameters in population dynamics. In current risk assessment it is assumed that the protection of all species will preserve functionality. Within the marine environment (although not necessarily exclusively) there is potentially a further emphasis relating to the impact upon larger marine species of mammal or birds (i.e. high trophic level species) and additional concerns regarding the unknown long-term effects of bioaccumulation and biomagnification in complex food chains.

8.2 Compartments

Separate PNECs values need to be derived for the relevant compartments of interest:

- water compartment,
- benthic compartment, and
- biota.

A similar differentiation is employed in the CHARM model (Karman *et al*, 1996). Within the water compartment, the presence of a contaminant can potentially impact the pelagic biota (e.g. phytoplankton, zooplankton, and most fish species). In the benthic compartment, a contaminant can potentially affect the sediment biota such as epi- and in-faunal organisms. The third compartment, the biota (representing organisms which are eaten by avian and mammalian predators), occurs within the other two compartments. For clarity, in this report, the relevant PNEC is termed $PNEC_{predator}$. It is important due to the potential for biomagnification along the food chain, particularly in higher animals (i.e. fish-eating birds and marine mammals).

8.3 Data evaluation

The evaluation of the quality of the available ecotoxicological data should be conducted according to standards described in the TGD (EC, 1996) (see Table 14). Only valid studies ought to be used to derive PNECs. Invalid or non-assignable studies should not be used. Additional guidance relating to quality criteria for the evaluation of ecotoxicological studies in the context of marine environmental risk assessment is available from OSPAR (OSPAR, 1998c).

Table 14: Quality criteria for acceptance of ecotoxicity data (EC, 1996)

Case	Detailed Description of Test	Accordance with Scientific Guidelines	Measured Concentration	Conclusion: Reliability Level
I	+	+	+	[1] valid without restriction
II	±	±	±	[2] valid with restrictions; to be considered with care
III	Insufficient	-	-	[3] invalid
IV	The information to give an adequate opinion is not available			[4] non-assignable

If studies employing freshwater species are to be employed in the effects assessment, some evaluation of their validity or significance to the marine environment (vis-à-vis e.g. physico-chemical conditions) needs to be made via expert judgment. A further important consideration is the integration of bioaccumulation in an environmental risk assessment scheme for the marine environment. This requires an evaluation of whether potentially bioaccumulative substances reach a steady-state body burden within an organism during ecotoxicity testing. If a steady-state body burden is reached, then the direct effects of bioconcentration are included and a PNEC derived from this testing is appropriate for risk assessment. For lipophilic substances, where a steady-state body burden may not be achieved during the duration of the test, the time to reach T_{95} should be established. This can be determined directly or estimated for fish using a QSAR (ECETOC, 1995). If the time to reach T_{95} is greater than the duration of a bioassay, the result of the bioassay can be adjusted to correct for the theoretical % body burden or the duration of testing can be extended. At present no equivalent QSAR or empirical database relating to time to reach steady-state exists for invertebrates, although intuitively it is often assumed that time to steady-state will typically be less in such organisms.

8.4 Assessment factors

In deriving a PNEC from a single species endpoint, an assessment factor is typically employed in order to extrapolate from the bioassay to the ecosystem. This approach is well established and forms the basis of the PNEC derivation prescribed by the TGD (EC, 1996). In general terms, lower assessment factors are applied when more data are available relating to more trophic levels. For example, a factor of 1,000 is typically applied to short-term ecotoxicity data. Greater confidence in the acceptability of the no-effect threshold is possible when data on additional species and extended duration of exposure are available. Chronic studies are typically recognised as being more relevant to risk assessment than acute data. Details of the assessment factors promulgated in the TGD are presented in Table 15. Whilst the basis of the rationale remains to be established categorically, it has proved to be a pragmatic approach and is undoubtedly conservative.

Such an approach was applied to the series of marine risk assessments for chlorinated organics in the OSPARCOM North Sea region conducted on behalf of Euro Chlor (as cited in Section 7.2).

Table 15: Assessment factors to derive PNEC values (EC, 1996)

Data	Assessment Factor
At least one short-term L(E)C_{50} from each of three trophic levels	1,000
One long-term NOEC	100
Two long-term NOECs from species from two trophic levels	50
Long-term NOECs from at least three species representing three trophic levels.	10
Field data or model ecosystem	Reviewed on a case by case basis

A similar approach has been applied in the derivation of marine EACs as developed under the aegis of OSPAR (OSPAR, 1998c). Such EACs are intended as effects benchmarks against which the results of environmental monitoring can be assessed in an attempt to identify possible areas of concern. The principle of the procedure for deriving EACs is also based upon the derivation of an extrapolated concentration based on ecotoxicological information. Dependent upon the extent of the data set, varying extrapolation factors are applied to the lowest NOEC or L(E)C_{50} value from the ecotoxicological data available (Table 16). EACs are only derived when data which meet predefined quality criteria are available from at least three species (which can include either marine or freshwater).

Table 16: Extrapolation factors used to derive an EAC (OSPAR, 1998c)

Data	Extrapolation Factor
Applied to the lowest acute L(E)C_{50} when the data available are few or the range of organisms is narrow.	1,000
Applied to the lowest acute L(E)C_{50} when there is an extensive database covering a phylogenically wide range of species, or to the lowest chronic EC_{50} or NOEC when few chronic data are available.	100
Applied to the lowest chronic NOEC for a sufficient and representative number of species.	10

EACs for a selection of PAHs, pesticides, PCBs and metals have already been derived for the water and sediment compartments and biota (OSPAR, 1998c). Values for the water compartment were denoted as firm or provisional on the basis of the predominance of chronic data from different taxonomic groups (i.e. 3 marine species including fish, invertebrates and algae). Within the sediment compartment, an EAC derived from

spiked sediment bioassays was denoted as firm if data were available for two marine sediment dwelling organisms from different taxonomic groups. If the sediment compartment EAC was derived via equilibrium partitioning, the value was considered firm when calculated using a measured K_p value and a firm EAC for the water compartment. Biota EACs were regarded as firm when derived from observed critical body burdens. Biota EACs derived from direct effects data via an appropriate BCF for fish or mussels were considered "*firm*" when a measured BCF and firm water compartment EAC were employed. Biota EACs relating to adverse effects due to secondary poisoning were considered firm if toxicity data were available from one bird and mammal species exposed via the food.

8.5 PNEC derivation

8.5.1 $PNEC_{water}$

Essentially a similar approach to the TGD (i.e. as detailed in Table 15) is envisaged for the marine environment effects assessment for the water compartment. PNEC values should be derived using all available freshwater and marine effects data as recommended previously (e.g. Garny, 1998). If additional testing is necessary to verify the effects assessment, then it is recommended that marine test protocols be employed if available.

8.5.2 $PNEC_{benthic}$

As for the water compartment, a similar approach to that outlined in the TGD is envisaged. Given that the potential concerns with regard to sediments as compared to the water column relate to the possible additional exposure via ingestion, it is recommended that only chronic studies entailing exposure via sediment are employed in deriving $PNEC_{benthic}$. In the absence of any ecotoxicological data for sediment-dwelling organisms, $PNEC_{benthic}$ may be calculated using the equilibrium partitioning method as presented in the TGD (EC, 1996) and based on OECD (1992) and Di Toro *et al* (1991). The underlying assumptions are that (i) sediment and water column organisms are equally sensitive, (ii) concentrations in sediment, interstitial water and benthic organisms are in equilibrium and can be predicted in any of the phases via partition coefficients and (iii) sediment/water partition coefficients can be measured or derived on the basis of a generic partition method from separately measurable characteristics of the sediment and chemical in question. However, the current TGD states that this methodology only considers the water phase, and does not take into account uptake of a chemical from sediments via ingestion. This approach is not consistent with the theory described by the references cited. Di Toro *et al* (1991) specifically state that "... assuming that the partitioning of the chemical between sediment organic carbon and pore water is at equilibrium ... the organism receives an equivalent exposure from a water-only exposure or from any equilibrated phase; either from pore water via respiration; from sediment carbon via ingestion; or from a mixture of the routes. Thus, the pathway of exposure is not significant". As a consequence of this apparent misinterpretation of the theory, the TGD attempts to correct for the possibility of increased exposure by ingestion by increasing the PEC, by an additional factor of 10 for compounds which are likely to adsorb (i.e. $K_{ow} > 5$).

8.5.3 $PNEC_{predator}$

The derivation of a PNEC for the biota compartment is more problematic. The comparative analysis of accumulation and toxicity data for marine fish, birds and mammals with residue data from monitoring campaigns suggests that it may be more appropriate to determine critical body concentrations rather than critical environmental concentrations for the purpose of risk characterisation in the marine environment (Nendza and Klein, 1990). Extrapolation of threshold levels from terrestrial species typically employed in avian and mammalian bioassays to marine birds and mammals is one possibility, although the validity of such extrapolations have yet to be established. One possible approach to the incorporation of secondary poisoning in the derivation of the $PNEC_{predator}$ is based on the food chain; water → fish or mussel → fish or mussel-eating predator. The method is based on a No Effect Concentration (NEC) for predators derived from mammalian or avian laboratory studies using assessment factors and is based on a methodology described by OSPAR to derive EAC for fish or mussels (OSPAR, 1998c). This in turn was based on the work of Romijn *et al* (1993) and Everts *et al* (1993) and aspects of the TGD (EC, 1996). A calorific conversion factor is used to compensate for differences in calorific content of laboratory food versus mussels or fish. The NEC for predators is employed in order to derive a concentration in mussel or fish tissues which is presumed not to result in adverse effects on predators. Although such a scheme has yet to be validated, it can be regarded as a provisional approach for deriving an initial indication of the potential for secondary poisoning (at least within simple food-chains). A similar approach could be employed in the assessment of human health risks from exposure via ingestion of marine food species.

8.6 Statistical extrapolation

As with the freshwater risk assessment process, the marine effects assessment performed with assessment factors may also be supplemented with a statistical extrapolation method where the database is sufficient to allow for its application. When data are available from chronic bioassays performed with representative taxonomic groups, statistical extrapolation methods can be used to derive a PNEC. The main underlying assumptions of such methods are that the distribution of species sensitivities follows a theoretical distribution function and that the species tested in laboratory bioassays are a random sub-set of this distribution (OECD, 1992). Validation of these methods via comparison with multi-species tests has been carried out for freshwater ecosystems (e.g. Emans *et al*, 1993). Additional work is required to validate their use for marine ecosystems.

9. RISK CHARACTERISATION

9.1 Marine risk assessment methodology

Existing schemes for the environmental risk assessment of chemicals (e.g. EU TGD (EC, 1996)) focus on the terrestrial and freshwater ecosystems. The principles have already been reviewed in Section 2. Current risk assessment practice for the freshwater and terrestrial ecosystems compares the PEC with the PNEC for the relevant ecosystem using data from representative species. Implicit in this approach is the assumption that there is a tolerable threshold of any chemical substance in the environment (i.e. the PNEC). An element of precaution (or margin of safety) is built into the approach via the use of conservative/worse-case assumptions within exposure and effects assessments. Any risk assessment framework for marine ecosystems should be consistent with existing practice and therefore emulate EU requirements under relevant legislation (i.e. Council regulation 793/93/EC and Council directive 67/548/EEC). This implies the adoption of common concepts (such as the comparison of exposure and effects) and common protection goals. The extension of the use of the readily comprehensible PEC/PNEC ratio (with a nominal safety threshold at unity) would provide continuity between the limnic and marine environments. Incorporation of additional experience from other examples of marine risk assessment frameworks will also be useful in this respect. Of particular interest is the use of EAC by OSPAR when benchmarking effects data against results from marine monitoring programmes. Whilst not explicitly positioned as risk assessment, the principles employed in this approach are effectively parallel to that employed in the practice of risk assessment.

9.2 Marine risk assessment objectives

The objective of marine risk assessment is the protection of the structure, function and thus sustainability of the marine ecosystem(s). This implies protection at the population/species level (and the acceptance of the possibility of some effect at the level of individual organisms at concentrations less than the PNEC). Within the context of this document, the process of risk assessment relates to adverse effects from anthropogenic emissions. Specific additional concerns surrounding the potentially irreversible long-term accumulation of hazardous substances within marine biota have also been highlighted (i.e. OSPAR, 1999b). Any risk assessment framework for the marine environment will therefore have to specifically address this concern.

9.3 Marine risk assessment scale

Risk assessment within the terrestrial and freshwater environments are conducted over different spatial scales. For example, preliminary release from a point source necessitates a local assessment in immediate proximity to the point of discharge, whilst further distribution and fate is dealt with at the regional scale. A similar differentiation may also be applied to the marine environment and a multi-stage/scale approach is proposed for the risk assessment of chemicals in marine ecosystems. Details and derivations of the proposed spatial scales for exposure assessment are as provided in Section 5 (i.e.

local, regional and continental scale). Derivation of PNECs for aquatic and sediment-dwelling organisms and avian and mammalian predators is described in Section 5. The proposed framework for marine risk assessment in summarised in Table 17, and a schematic of the approach to risk characterisation is given in Figure 10.

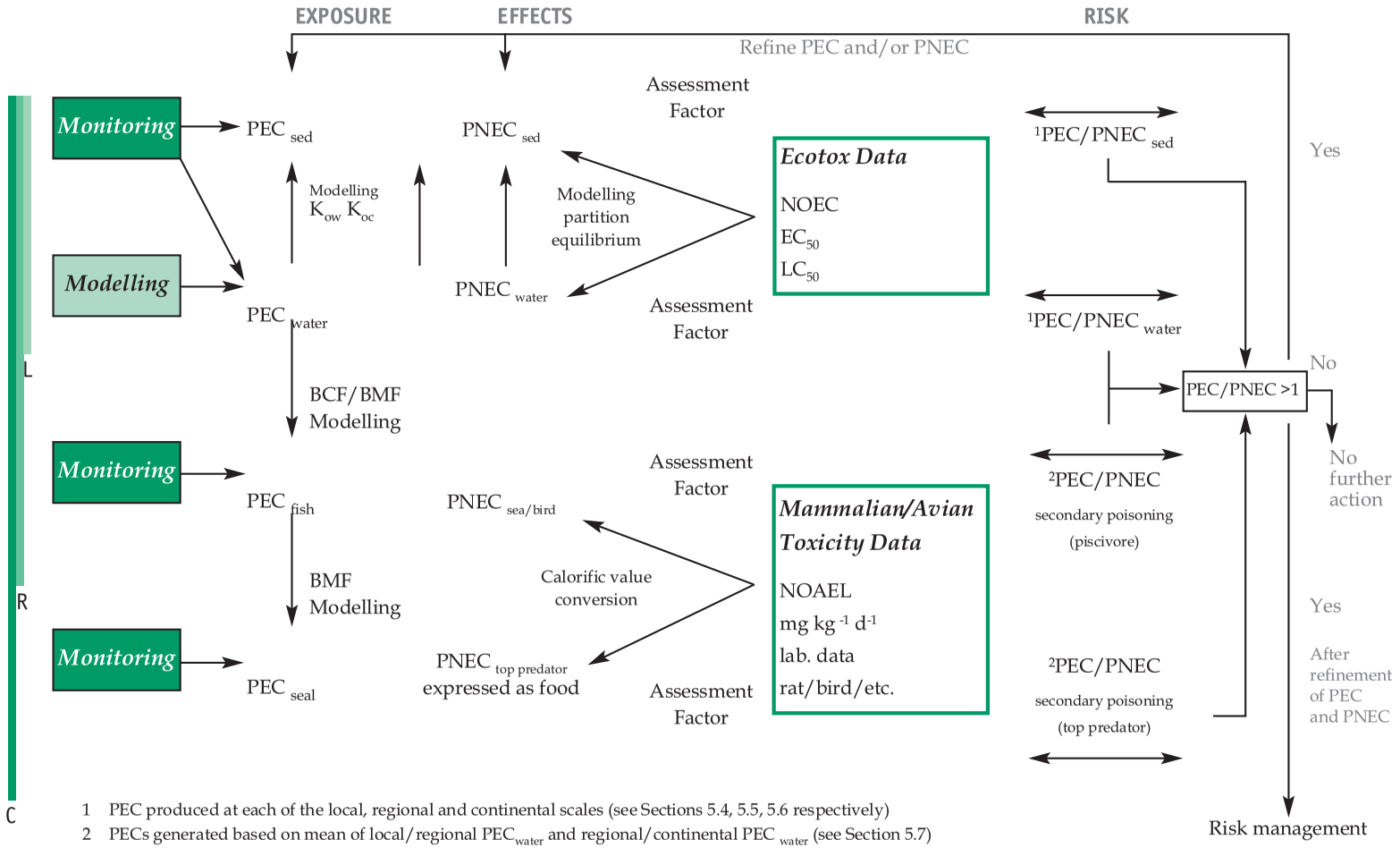
Table 17: Multi-scale marine risk assessment framework

Exposure/Scale	No Effect Concentration			
	$PNEC_{water}$	$PNEC_{sediment}$	$PNEC_{seal}$	$PNEC_{bear}$
$PEC_{local\ seawater}$	✓			
$PEC_{local\ sediment}$		✓		
$PEC_{regional\ seawater}$	✓			
$PEC_{regional\ sediment}$		✓		
$PEC_{fish\ (local\text{-}regional)}$			✓	
$PEC_{seal\ (local\text{-}regional)}$				✓
$PEC_{continental\ seawater}$	✓			
$PEC_{continental\ sediment}$		✓		
$PEC_{fish\ (regional\text{-}continental)}$			✓	
$PEC_{seal\ (regional\text{-}continental)}$				✓

9.4 CONCLUSIONS

Risk can be characterised (as the PEC/PNEC ratio) at the local, regional and continental scales for water and sediment. Risk to piscivorous mammals (e.g. seals) and birds, and to secondary mammalian predators (e.g. polar bears) can be characterised at the local-regional and regional-continental scales.

Figure 13: Schematic of approach to risk characterisation



1 PEC produced at each of the local, regional and continental scales (see Sections 5.4, 5.5, 5.6 respectively)

2 PECs generated based on mean of local/regional PEC_{water} and regional/continental PEC_{water} (see Section 5.7)

10. SUMMARY OF PROPOSED APPROACH TO EXTEND THE EU TGD TO THE MARINE ENVIRONMENT

10.1 Introduction

In many instances, some or all of a marine risk assessment will be site specific (relating to a known geographical area) and in such cases monitoring data representative of that area, or site specific models, may be available that can be used to define the distribution of a chemical resulting from a single or multiple emission sources. The following is a summary of the approaches that can be used when relevant monitoring data are not available and a site specific model is not appropriate. The assessment scheme is based on the generic model used in the EU TGD to represent the freshwater and terrestrial environment of Europe (EC, 1996), but identifying when and how this model can be modified to address the marine environment.

10.2 Spatial scales

As in the existing TGD, the spatial scales proposed are (marine) local, regional and continental; these are conceptually nested within a further "global" scale, representing the world's oceans, although the proposed risk assessment techniques are restricted to the 3 smaller scales.

10.3 Risk characterisation ratios

In all cases a PEC/PNEC ratio of 1 should be adopted as the critical value to determine whether a significant risk is indicated, as in the present TGD. The use of different "trigger" values for different environments or different categories of chemical will lead to confusion. Any precautionary allowances for uncertainty should be applied to the PNEC (or, if applicable, the PEC).

10.4 Local assessment

10.4.1 Spatial scale

As described in the TGD, in the immediate vicinity of a single source.

10.4.2 Water phase - derivation of $PEC_{local\ seawater}$

10.4.2.1 Land-based emissions (e.g. from production, formulation, processing and use)

As a reasonable worst case, based on discharges to the upper reaches of an estuary where local dilution may be no greater than in the freshwater river, but characterised by higher levels of suspended solids.

Therefore, to obtain C_{local}:

As TGD: Via sewage treatment plant (STP)
x10 dilution

But: Partition to 25 mg l^{-1} of suspended particulates ($SUSP_{water}$) containing 10% organic carbon (OC), as TGD.

Kp_{susp} derived from K_{ow} (or K_{oc}) as TGD.

Then: $PEC_{local\ seawater} = C_{local} + PEC_{regional\ seawater}$

10.4.2.2 Off-shore platforms (emissions from oil and gas exploration and production)

In the absence of specific data, the assumption is direct discharge without treatment and with a generic dilution factor, to sea water with the characteristics (in particular suspended solids level) of the regional environment. Therefore, to obtain C_{local}:

No STP
x1,000 dilution as generic default
Partition to 2 mg l^{-1} of suspended particulates ($SUSP_{water}$) containing 10% organic carbon (OC)

Then: $PEC_{local} = C_{local} + PEC_{regional\ seawater}$

10.4.2.3 River outflows

River outflows from catchment areas are subject to multiple point and diffuse sources but constitute a point source input to the marine environment. The principal need is to include this as an input to the regional model, but a PEC_{local} can also be estimated, if required, by assuming dilution of the river water with an equal volume of estuary water, with partitioning of the resulting concentration with the relevant level of suspended particulates. Therefore, $PEC_{local\ seawater}$ is calculated as follows:

Mean of $PEC_{regional\ freshwater}$ and $PEC_{regional\ seawater}$

Partition to 25 mg l^{-1} of suspended particulates ($SUSP_{water}$)

containing 10% organic carbon (OC), as TGD.

10.4.2.4 Other direct emissions (e.g. harbours, marinas, fish-farms)

Specific emission scenarios would be required, in particular to derive generic local dilution factors. A number of existing models could help to define these.

10.4.3 Water phase - derivation of $PNEC_{seawater}$

For chemical substances whose form or speciation (and therefore behaviour) is likely to be directly changed by the ionic composition of sea water (e.g. inorganics substances such as metals, and certain ionisable organic substances), their toxicity in the marine environment may also be different to that expressed in fresh water and marine toxicity data may be required to evaluate that difference. However, for substances whose speciation is not affected in this way, analysis of the available data provides no evidence of a systematic difference in sensitivity between marine and freshwater organisms. This conclusion is based not only on data for algae, crustaceans and fish (frequently tested in both environments) but also on data for phyla (e.g. echinoderms and molluscs) not found, or not frequently tested, in fresh water. A similar analysis also indicates that no greater bioaccumulation of chemicals by (water-breathing) marine organisms occurs, compared with fresh water, which supports the hypothesis that sensitivity will be unaffected unless the speciation of the chemical is affected by the ionic strength of the medium.

There are limited data on which to make these sensitivity comparisons, in particular for phyla (e.g. molluscs) which may be more significant in marine ecosystems (in terms of number of species and/or biomass) than in fresh water, and further research is in progress to examine the data more fully and to increase the database for comparison (e.g. www.cefic.org/lri). However, at the present time, it is reasonable to conclude that for most organic substances, freshwater toxicity data can be used equally with marine data to derive the PNEC, and that the assessment factors used to protect freshwater ecosystems will be equally protective for the marine environment.

Therefore, to derive a marine PNEC:

- develop guidance to detect and evaluate substances with physico-chemical properties which indicate speciation will be affected by the ionic composition of sea water.

For the remaining substances:

- pool data from marine and freshwater organisms and take lowest valid result;
- apply assessment factors, as in TGD.

10.4.4 Sediment phase - derivation of $PEC_{local\ sediment}$

Although K_{ow} and K_{oc} are, theoretically, increased slightly as a result of the ionic strength of sea water, the difference does not appear to be significant, especially considering the variability in measured K_{ow} values and the uncertainty in estimating K_{oc} from K_{ow}.

Therefore, in the absence of specific marine data, K_{ow} and hence K_{oc} values derived by standard (freshwater) techniques can be used directly to derive partitioning behaviour in the marine environment. The sediment depth relevant to the risk assessment is that which the organisms inhabit, which is, by definition, aerobic; if aerobic, there must be good exchange with the overlying water and near equilibrium conditions can be assumed.

10.4.4.1 Land-based and coastal emissions

Because the proposed "worst-case" scenario represents the upper reaches of an estuary, the sediment characteristics used for fresh water are applicable. Therefore, to obtain $PEC_{local\ sediment}$:

- Partitioning of $PEC_{local\ seawater}$ to sediment as in TGD (characteristics as suspended particulates, 10% organic carbon).

10.4.4.2 Off-shore platforms

Because of the relatively large depth of water around most platforms in Europe, the $PEC_{local\ seawater}$ cannot be assumed to represent the concentration above the local sediment. Furthermore, the discharge of cuttings and other solids may affect the transport of chemicals to the bottom sediments. Sediment biomonitoring, monitoring for specific substances, or site specific models are available for many platforms; if not available for the chemical of interest, data for chemicals with similar physico-chemical properties and emission characteristics can be used to derive $PEC_{local\ sediment}$ (or generic defaults).

$PEC_{regional}$ for off-shore discharges from oil and gas production platforms can be obtained through available models or monitoring date. A simple dilution factor cannot be applied to the regional case, because of the large water volumes included in a typical region.

10.4.5 Sediment phase - derivation of $PNEC_{sediment}$

As in the TGD; as for $PNEC_{aqua}$, direct use of freshwater toxicity data, or equilibrium partitioning to calculate $PNEC_{sediment}$ from $PNEC_{aqua}$ in absence of sediment measured data.

10.4.6 Secondary poisoning

For the marine environment, the ingestion of a chemical by fish-eating marine mammals (e.g. seals), or birds, is a particular concern. Although the TGD models this exact scenario, the concentration in the fish prey is predicted using only the bioconcentration factor (BCF) for fish. In order to allow for the possibility of an increased body burden in fish resulting from biomagnification, an additional factor (BMFactor) is proposed (for more hydrophobic substances) in calculating the PEC_{fish} (concentration in fish eaten by birds and mammals).

As in the TGD, the PEC_{fish} should be derived assuming that fish experience a concentration in water which is the mean of the PEC_{local} and $PEC_{regional}$. Therefore:

For log K_{ow} <5: $PEC_{fish} = BCF_{fish} \times 0.5(PEC_{local} + PEC_{regional})$ [as TGD]

For log K_{ow} ≥5 <8: $PEC_{fish} = BCF_{fish} \times 0.5(PEC_{local} + PEC_{regional}) \times BMFactor$

The BMFactor should be progressive, with a maximum value of 4 for log K_{ow} of 8 (BMFactor = log K_{ow} - 4). The maximum log K_{ow} of 8 is used to reflect the fact that the bioaccumulation potential of such highly hydrophobic substances declines rapidly above this value. If the BCF_{fish} is measured, rather than estimated from K_{ow}, then the BMFactor could be derived from the BCF and applied when BCF was greater than 5,000 (log BCF ≥ 3.7) using a similar formula (BMFactor = log BCF - 2.7, with maximum value of 4).

The PNEC for the mammalian (or avian) predator can be derived as currently described in the TGD.

10.5 Regional assessment

10.5.1 Spatial scale

If a local risk is predicted, it may be necessary to determine how far the impact extends into the surrounding region. Although concentrations will tend to decrease with distance from a source, due to dilution, partitioning and degradation, the residual concentrations from one source may combine with those from other point and diffuse sources and cumulate over time. Thus, a regional assessment should consider a particular or generic "reasonable worst case" scenario, subject to multiple sources but with sufficiently large spatial and temporal scales to allow any degradation and partitioning to occur. In the European context, it is logical to specify marine regions that are modelled in a way that parallels, and in the future can be integrated with, the existing EUSES model.

10.5.2 Water and sediment phases - derivation of $PEC_{regional}$

It is reasonable to envisage the marine region as the estuarine and coastal sea area that receives the river water flow and atmospheric load emanating from the terrestrial/freshwater conceptual "default" region (receiving 10% of European emissions). EUSES can be modified to provide a regional marine "box" with the appropriate size and characteristics. However, because of the difficulty in setting the size of a generic marine region, it may be preferable to assess chemicals on the basis of two (or more) regional scenarios. One could comprise a relatively small area and volume to represent, conceptually, an industrialised estuary and the immediately adjacent coastal waters, with the other scenario(s) to include a larger sea area that might be impacted. In modelling terms, these should be separate regional scales, not one "nested" within the other. The final definition of these areas requires more detailed sensitivity analysis of the parameters (e.g. size, depth and residence time). Other characteristics of the regional

"box" will also depend on the dimensions. For example, the organic carbon content of the sediments and suspended particulates for a small region (conceptually representing an estuary) should be higher than a larger region (conceptually including a larger proportion of the surrounding sea area).

As a starting point, Table 18 describes (in summary form) the modifications proposed to the current EUSES in order to represent the smaller of these regional scenarios.

Table 18: Proposed modifications to EUSES for marine$_{regional}$ scenario

Parameter	Value	Comment
Regional Area (km^2) North Sea area	4×10^3	10% of EUSES default area, approx 1% of
Area of water (%) to operate	99.7	Nominal land areas necessary to allow EUSES
Volume of water (m^3) Sea volume	4×10^{10}	From depth of 10 m, approx 0.1% of North
Suspended solids (mg l^{-1})	5	Reduced from EUSES default of 15 mg l^{-1}
Sediment organic carbon (%)	5	As EUSES default
Residence time of water (d)	96	Compares with EUSES default of 40 days for rivers. Set by adjusting the "fraction of flow from continental scale to region" (below)
Fraction of water flow from continental scale to region	0.01	
Total water flow through region (m^3 d^{-1})	4.17×10^8	Represents river inflow (EUSES regional river outflow, 6.9 x 107 m^3 d^{-1}) plus the exchange with the surrounding sea area, plus the rainfall in the region
Emissions (input) via water		Residual inputs resulting from 10% of total EU emissions being subjected to potential cumulation, degradation and partitioning in the terrestrial/freshwater environment, i.e. $PEC_{regional\ freshwater}$ x flow rate of 6.9×10^7 m^3 d^{-1}
Emissions (input) via atmosphere		Assume 50 % of (terrestrial) regional air enters marine box i.e. 0.5 x PEC_{air} x air flow rate of 1.85×10^{13} m^3 d^{-1}

Although laboratory experiments tend to suggest that biodegradation in sea water is slower than in standard degradation tests, observations of chemical persistence in the marine environment, notably in estuarine and coastal regions, show that degradation can be as rapid as in freshwater systems. Biodegradation kinetics in ecosystems tend to be correlated with the number of degraders, the availability of the chemical as a nutrient source and the presence of suspended solids. It is also essential that the degraders are given the opportunity to adapt to the contaminant. All these conditions are met in

estuaries and coastal regions prior to greater dilution of the contaminant concentration in the open sea zone. Therefore, considering that the biodegradation rate constants employed currently in EUSES (based on test data) are relatively conservative, there is no need to adjust the relationship for the marine environment, especially considering that a regional risk assessment is unlikely to be necessary for a chemical that satisfies a ready test (even without the 10-day window).

10.5.3 PNEC for aquatic and sediment organisms

The PNEC for aquatic organisms at the regional level would be the same as that at the local assessment. However, if additional or alternative regional scenarios were employed, the $PNEC_{sediment}$ would require normalisation to the level of organic carbon selected for the regional marine sediment.

10.5.4 Secondary poisoning

It is proposed that a second tier of secondary poisoning is relevant to the larger-scale marine environment. In addition to assessing the risk to birds and mammals eating fish, it is appropriate to assess the risk to mammals preying on other predatory mammals and birds (exemplified as polar bears eating seals). This can be achieved by applying a further adjustment factor to allow for possible biomagnification between the fish and the seal, to calculate the PEC_{seal} as the exposure estimate for its predators. In this case, the "seal" is assumed to be preying on fish which are exposed to the mean of the regional and continental $PEC_{seawater}$. Thus, for log $K_{ow} \geq 5 < 8$:

$$PEC_{seal} = PEC_{fish\ (regional\text{-}continental)} \times BMFactor$$

where: $$PEC_{fish\ (regional\text{-}continental)} = BCF_{fish} \times 0.5(PEC_{regional} + PEC_{continental}) \times BMFactor$$

It is proposed that the BMFactor is the same for both the first and second tier calculations, and is progressive, with a maximum of 4 (see Section 10.4.6, local secondary poisoning) . Therefore:

$$PEC_{seal} = BCF_{fish} \times 0.5(PEC_{regional} + PEC_{continental}) \times (BMFactor)^2$$

The PNEC for the predator ("polar bear") can be derived as described in the TGD for human risk assessment, making appropriate adjustments for such indices as feeding rate and calorific value of the food.

10.6 Continental assessment

A model that is a modified version of (or is designed to parallel) EUSES will require a larger (continental) area to be defined within which the region is "nested". Furthermore, because the marine region(s) may be set at a relatively small size, it may be considered appropriate on occasions to assess risk at this continental scale. It is proposed that, conceptually, the continental box is approximately the scale of the North Sea, or a large part thereof. The final dimensions and characteristics that are appropriate will depend on the final definition of the marine region(s). Table 19 summarises the inputs for the region proposed above.

Table 19: Proposed modifications to EUSES for marine$_{continental}$ scenario

Parameter	Value	Comment
Continental area (excluding region)	3.96×10^5	Approximate area of North Sea, ca. 100 times regional area, as TGD.
Area of water (km^2)	3.95×10^5	
Volume of water (m^3)	2.37×10^{13}	Approximately 60% of volume of North Sea[a]
Susp. solids concentration (mg l^{-1})	2	Reduced from EUSES default of 15 mg l^{-1}
Organic carbon in sediment (%)	1	Reduced from EUSES default of 5%[b]
Fraction of water flow from global scale to continental	0	Cannot be changed in current EUSES
Residence time of air (d)	2.15	
Residence time of water (d)	581	Approximate residence time of North Sea. Defined by total water flow (below) and volume.
Water flow through continental box (m^3 d^{-1})	4.08×10^{10}	Varied by setting population, to obtain desired residence time.

[a] The volume is derived from the area and a depth of 60m; this could be increased to 100m if considered necessary to set the volume approximately equal to that of the North Sea.

[b] The current version of EUSES does not allow different organic carbon (OC) contents to be set for the regional and continental scales; therefore, in practice, it is necessary to use a mean value of 3% OC for both scales when running the model.

11. RESEARCH RECOMMENDATIONS

11.1 Persistence and biodegradation

The concern with persistence of chemicals is that such chemicals may be accumulated within the cells of organisms and consequently bioconcentrate and biomagnify within ecosystems eventually causing an adverse effect. Furthermore little is known about the potential effects of non-bioaccumulating substances over multiple generations at low concentrations. The issues therefore are:

- what is persistence?
- what factors contribute to persistence?
- are persistent substances necessarily available for bioconcentration?
- what factors affect bioaccumulation in different species?
- what are the consequences of long-term exposure at low concentrations?

Persistence is not an inherent property of a chemical. Unlike physical and chemical properties it is dependent upon the diversity of a wide variety of external environmental parameters. For the correct assessment of the potential environmental impact, the partitioning of the chemical between the various environmental compartments must be understood. It is therefore essential to evaluate the rate at which the chemical may change, degrade or interact with the various environmental parameters, including biota, other chemicals and physical factors. Current approaches, based on simple biodegradation and hydrolysis studies, are too simple and greater understanding is needed. Although persistence may be defined by assigning half-lives within different environmental compartments under specified conditions, the relevance of these criteria, the extent to which they may be measured, or the level of variability that exists within the environment is unknown.

A major factor affecting the persistence of an organic chemical is its susceptibility to microbial breakdown. Research on biodegradability in the marine environment is recommended in the following areas:

- potential for extrapolation of existing test to the marine environment;
- test methodologies to determine half-lives;
- substrate specificity of marine microorganisms;
- microbial adaptation
- substrate affinity;
- substrate affinity and biodegradation rates with varying concentrations in waters and sediments;
- co-metabolism.

11.2 Ecotoxicity

To improve our understanding of the effects of chemicals in the environment work is needed to:

- develop more information on whether taxa that are common in marine ecosystems (e.g. molluscs, echinoderms) but not (or are poorly represented) in fresh water, are of similar sensitivity to the freshwater species commonly used in risk assessment. The goal would be to minimise additional testing requirements and establish how this can best be done and for which types of chemicals it is possible;
- assess whether predictions of a chemical's toxicity in salt water can be based on its structural and other properties using QSARs;
- establish whether there is a need for more high-quality marine data on the effects of a wide range of substance (particularly on algae and plant species) and if this need simply requires the generation of data or the development of tests to fill data gaps. Similarly, the reasons for any observed differences in the sensitivity of comparable freshwater and seawater species to individual compounds noted here should be investigated. For example, it is likely to be significant that the more marked differences between responses of species were often associated with metallic compounds. The potential influence of salinity on bioavailability is one issue that therefore needs to be addressed when extrapolating from the freshwater database to the marine environment in any effects assessment of metallic compounds (or indeed any other ionisable compounds);
- examine the feasibility of extrapolating data for risk assessment from fresh and marine waters and sediments;
- examine the value of bio-indicators in the form of community structure indices (not biomarkers) for their ability to integrate responses to multiple exposures to persistent and bioaccumulating substances particularly in marine sediments;
- develop the understanding of the relationship between critical body burdens and chronic toxicity end points. Validation by field studies should also be addressed.

11.3 Adsorption and bioavailability

Chemicals adsorbed onto suspended matter or sediment are subjected to substantial hydrodynamic distribution processes, i.e. transport, deposition and resuspension. Appropriate models for transport, adsorption and desorption as a basis for determining concentrations in sediments need to be developed.

11.4 Models and monitoring

To improve exposure assessments it is proposed that research should be considered to:

- understand how data on degradation, bioconcentration/biomagnification and bioaccumulation can be extrapolated within local, regional and global models. Examine the extent to which current models are capable of assessing the degradation and partitioning of chemicals in the marine environment;
- review existing models to identify how well they represent the flow of a chemical through the global environment including food chains. Develop monitoring activities to address the validation of such models. In particular investigate how data from body residues and population monitoring might be used to develop these models.

Estuarine environments are of particular importance because of the large number of substances which pass through from rivers and they are responsible for the final distribution of substances in the marine environments. Within the risk assessment framework there is a need to improve the understanding and forecasting techniques of the fate, distribution and impact of substances, in order to develop and implement a generic model framework to be applicable for any "virtual" European estuary which can be used to calculate PEC_{local} and $PEC_{regional}$. Any model developed should provide concentrations of substances in both sediment and water and also information on fluxes to open waters.

The models currently available for addressing biomagnification used for freshwater will need to be developed for marine systems to take account of the longer food chains and the migration and feeding habits of particular target species.

The extent to which environmental hazard assessment data for chemicals in fresh water and marine waters can be extrapolated for risk assessment of chemicals in sediments needs to be clarified. There is also a need to identify key factors influencing the behaviour of chemicals in the various compartments and assess whether guidelines for extrapolation of data between the compartments can be established. It is recognised from the outset that this task will be technically challenging as there may be different mechanisms operating within the different compartments which could significantly influence the environmental fate and behaviour of chemicals. For example, it is recognised that behaviour of chemicals (i.e. in terms of partitioning and bioaccumulation potential) in the environment will be influenced by their physio-chemical properties. These will also effect the behaviour and ultimately the endpoint of tests conducted to produce toxicity data for environmental hazard assessment. To complicate matters further it is recognised that there are a number of other factors which may effect significantly the endpoints of such tests for the different compartments. For example, in toxicity and bioaccumulation tests of poorly soluble compounds in water (using currently accepted practice of water accommodated fractions) there is only one potential route via the water phase whereas there may potentially be two uptake routes for such chemicals dosed into soils and sediment (exposure by the water phase and potentially by direct ingestion). Currently the effects of such factors on the endpoints of toxicity, biodegradation and

bioaccumulation tests are poorly understood. However, there is growing evidence that, for a number of chemicals, pore water concentrations in soils and sediments (which can be predicted from knowledge of the physico-chemical properties of the chemicals and composition of the soils/sediments) appear to ultimately control their toxicity irrespective of the compartment.

Can the key factors that control the behaviour of a chemical irrespective of the compartment be identified for various classes of chemicals? If so, guidelines should be developed for classes of chemicals to assist in deciding whether 1) their fate and effects can be adequately assessed by extrapolation of data for the aquatic environment or 2) aquatic tests provide suitable data or are tests with soils and sediments required to provide data to enable a risk assessment for these compartments to be conducted?

GLOSSARY

Acute Toxicity
The harmful properties of a substance which are demonstrated within a short period (hours for e.g. algae to days for e.g. crustaceans and fish) of exposure.

Advection
Physical transport or movement of a substance with its medium (air, water, sediment).

Allochthonous
Imported material or organisms from outside a defined ecosystem.

Assessment Factor
A factor applied to a data point or set when assessing a substance in order to derive a safe level of that substance in the environment.

Autochthonous
Material produced or organisms grown within a defined ecosystem.

Background Concentration
The concentration of a chemical in a medium prior to the action under consideration or the concentration that would have occurred in the absence of the prior action*. For some chemicals geogenic sources will contribute to the background concentration.

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

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.

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 due to water-borne exposure.

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.

* From Van Leeuwen, C.J. and Hermens, J. (1996).

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.

Catchment
The area from which rainfall flows into a river.

Chronic Toxicity
The harmful properties of a substance which are demonstrated only after long-term exposure in relation to the life of the test organism.

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.

Emission
Release of a substance from a source, including discharges, into the wider environment. *

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).

Existing Chemicals
Chemicals listed in the EINECS (EU legislation).

Exposure
1) Concentration, amount or intensity of a particular physical or chemical agent or environmental agent that reaches the target population, organism, organ, tissue or cell, usually expressed in (numerical) terms of substance concentration, duration, and frequency (for chemical agents and microorganisms) or intensity (for physical agents such as radiation), and
2) process by which a substance becomes available for absorptionby the target population, organism, organ, tissue or cell by any given route. *

Fate
Disposition of a material in various environmental compartments (e.g. soil or sediment, water, air, biota) as a result of transport, partitioning, transformation, and degradation. *

* From Van Leeuwen, C.J. and Hermens, J. (1996).

Half-life
The time in which the concentration of a substance will be reduced by 50%.

Halophilic
Organisms with an obligate requirement for saline conditions.

Hazard
The set of inherent properties of a substance or mixture which makes it capable of causing adverse effects in man or to the environment when a particular level of exposure occurs. Cf. risk. *

Heterotrophic
The process of the formation of organic matter from organic carbon and inorganic nutrients.

LC_{50} Value (Median Lethal Concentration)
A statistically derived concentration which, over a defined period of exposure, is expected to cause 50% mortality in the test population.

Local Scale
A specific concept in EU Environmental Risk Assessment, which defines a specific or local release site. Further details may be found in the TGDs.

LOEC (Lowest Observed Effect Concentration)
The lowest test concentration at which the substance is observed to have a "statistically significant" and unequivocal effect on the test species.

Mixing Zones
Sections of water downstream of a discharge, within which the discharge and the receiving water have not yet been fully mixed.

Model
A formal representation of some component of the world or a mathematical function with parameters which can be adjusted so that the function closely describes a set of empirical data. A *mathematical* or *mechanistic* model is usually based on biological, chemical or physical mechanisms, and its parameters have real world interpretations. By contrast, *statistical* or *empirical* models are curve-fitted to data where the mathematical function used is selected for its numerical properties. Extrapolation from mechanistic models (e.g. Pharmacokinetic equations) usually carries higher confidence than extrapolation using empirical models (e.g. the logistic extrapolation models). A model that can describe the temporal change of a system variable under the influence of an arbitrary "external force" is called a dynamic model. To turn a *mass balance* model into a *dynamic* model, theories are needed to relate the internal processes to the state of the system, expressed eg. in terms of concentrations. The elements required to build dynamic models are called *process* models. *

* From Van Leeuwen, C.J. and Hermens, J. (1996).

Monitoring
Long-term, standardised measurement, evaluation, and reporting of specified properties of the environment, in order to define the current state of the environment, and to establish environmental trends. Surveys and surveillance are both used to achieve this objective.

New Chemicals
In the EU, those produced since 18th September 1981. They are not listed on the EINECS.

NOEC (No Observed Effect Concentration)
The highest tested concentration below the LOEC where the stated effect was not observed. The NOEC is usually connected with chronic effects.

Oligotrophic
Nutrient deficient.

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 TGDs, the PEC predicted for the vicinity of a point source eg, a production or formulation site, or for a wastewater treatment plant.

$PEC_{regional}$
In the EU TGDs, 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 which is regarded as a level below which the balance of probability is that an unacceptable effect will not occur.

Reasonable Worst Case
Reasonably unfavourable but not unrealistic situation. Combining the most adverse environmental circumstances and worst-case release parameters necessarily results in an unrealistic over all worst-case estimation, which is extremely unlikely to occur. *

Receiving Water
Surface water (e.g. in a stream, river or lake) that has received a discharged waste, or is about to receive such a waste (e.g. just upstream or up-current from the discharge point). *

* From Van Leeuwen, C.J. and Hermens, J. (1996).

Residence (or Retention) Time
The mean time a substance remains in a compartment. It is obtained by dividing the amount of a substance in the compartment by the flux of that substance into the compartment.

Risk
The probability of an adverse effect on man or the environment resulting from a given exposure to a chemical or mixture. It is the likelihood of a harmful effect or effects occurring due to exposure to a risk factor (usually some chemical, physical or biological agent). Risk is usually expressed as the probability of an adverse effect occurring, ie. the expected ratio between the number of individuals that would experience an adverse effect in a given time and the total number of individuals exposed to the risk factor. *

Risk Management
A decision making process that entails the consideration of political, social, economic and engineering information together with risk-related information in order to develop, analyse and compare the regulatory options and select the appropriate regulatory response to a potential health or environmental hazard.*

Secondary Poisoning
The product of trophic transfer and toxicity.

Speciation
Determination of the exact chemical form or compound in which an element occurs in a sample, for example whether arsenic occurs in the form of trivalent or pentavalent ions or as part of an organic molecule, and the quantitative distribution of the different chemical forms that may coexist.*

Steady-state
The non-equilibrium state of a system in which matter flows in and out at equal rates so that all of the components remain at constant concentrations (dynamic equilibrium). In a chemical reaction, a component is in a steady-state if the rate at which the component is being synthesised (produced) is equal to the rate at which it is being degraded (used). In multimedia exposure models and bioaccumulation models it is the state at which the competing rates of input/uptake and output/ elimination are equal. An apparent steady-state is reached when the concentration of a chemical remains essentially constant over time. Bioconcentration factors are usually measured at steady-state.*

Sulphate-reducing Bacteria (SRB)
Anaerobic bacteria that obtain energy by the dissimilatory reduction of sulphate to sulphide.

T_{95}
The time taken to reach 95% of the steady-state concentration.

* From Van Leeuwen, C.J. and Hermens, J. (1996).

TGD
EU Technical Guidance Document in support of risk assessment for new and existing chemicals.

TOC
Total organic carbon, often expressed as mg/l in water or kg OC/kg solid. The organic matter content of soil and sediment is often determined by measurement of organic carbon. Typically, about half of all natural organic matter consists of carbon (OC ≈ 0.6 x OM).*

Turnover Time
The time required to replace a quantity of substance equal to the amount in the compartment

Validation (of a Physically-based Model)
The process of establishing that the predictions of a model agree with the results of an experiment, and that the agreement is not fortuitous but the result of the correctness and the applicability of the theories which are intended to capture the natural processes which the model intends to predict.

Validity (of a Physically-based Model)
A property of a model which requires that it be 1) useful, 2) able to capture natural processes, 3) able to reproduce natural patterns.

* From Van Leeuwen, C.J. and Hermens, J. (1996).

LIST OF ABBREVIATIONS

BCF	Bioconcentration Factor
CHARM	Chemical Hazard Assessment and Risk Management of off-shore exploration and production chemicals
CFU	Colony Forming Unit
DREAM	Dose-related Risk and Effect Assessment Model
EACs	Ecotoxicological Assessment Criteria
EC	Effect Concentration
EIF	Environmental Impact Factor
EUSES	European Union System for the Evaluation of Substances
HELCOM	Helsinki Commission
K_d	Partition coefficient for adsorption of the chemical onto a specific substance, i.e. sewage sludge or sediment (l kg^{-1})
K_{oc}	Partition coefficient between organic carbon and water (l kg^{-1})
K_{ow}	Octanol-water partition coefficient
MEC	Monitored Environmental Concentration
NOEC	No Observed Effect Concentration
OC	Organic Carbon
OSPAR	Oslo-Paris convention for the protection of the marine environment of the North-East Atlantic
PARCOM	Paris Commission
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Exposure Concentration
POPs	Persistent Organic Pollutants
PNEC	Predicted No Effect Concentration
ppm	Parts per million
STP	Sewage Treatment Plant
TGD	Technical Guidance Document
WWTP	Wastewater Treatment Plant

APPENDIX A

Modelling the marine environment using EUSES

Regional

EUSES (version 1.00) allows environmental concentrations to be predicted at the regional scale using a Mackay-type level III multi-media model (see Section 2.2). The existing default parameters for this model represent an environment that can be visualised as a highly industrialised region with an area of 40,000 km^2, receiving 10% of the total European emissions of the substance. However these parameters can be reset to represent different scenarios, including a marine region that can be visualised as the estuarine and coastal sea region that receives the river water flow from the terrestrial/freshwater default region. Table 18 shows the default values and the proposed alternatives for a marine region.

The principal changes are to decrease the overall area (to be more representative of the impact zone of a major European river) and to increase the proportion of that area which is water to 99.7%, with an increased depth of 10 metres. The resulting water area is approximately 1% of the North Sea area and less than 0.1% of the North Sea volume.

The suspended solids concentration, which affects chemical partitioning, can be varied and is currently set at 5 mg l^{-1}. This is intermediate between the proposed value of 25 mg l^{-1} for the generic local marine scenario (upper reaches of an estuary) and the value (2 mg l^{-1}) proposed (below) for the continental scale.

For this (conceptual) estuarine/coastal region, it is considered appropriate for the organic carbon content of the sediment to remain at the existing TGD default of 5%, although a lower value (1% organic carbon) is more appropriate at the continental scale. However, the current version of EUSES does not allow different organic carbon contents to be set for the regional and continental scales. Therefore, in practice, it is necessary to use a mean value of 0.03 (3% organic carbon) for both scales when running the model.

The "fraction of water flow from continental scale to region" sets the water flow (residence time) through the box (equivalent to river flow entering in the terrestrial scenario); this is set to give a residence time of 96 days. It is necessary to set the number of inhabitants to a low level, otherwise additional water flow is assumed from wastewater.

Continental

In EUSES, the regional model is nested within a continental scale box, which is necessary to provide background concentrations in the inflowing water and air to the regional model. In addition, the flow of water through the continental model is used to define the flow from the continental to the regional (as "Fraction of water flow from continental scale to region"). Although it is possible to adjust the area of the continental model (for example, to match that of the North Sea), there is no direct way of changing the continental water flow (to ensure a representative water residence time) except by artificially inflating the continental population. Therefore the continental parameters

are set using a water volume (2.37×10^{13}) approximately 40% smaller than the North Sea, and the population set to 2×10^{11}, to obtain an average residence time of 581 days, a reasonable approximation of the average for the North Sea. The proposed Continental parameters are given in Table 21.

Emissions

The emissions to the marine regional model can be entered directly in units of kg per year to air and to surface water. The emission to surface water is derived from the predicted water concentration (PEC_{water}), obtained by running the default (terrestrial) model, and the corresponding regional river outflow (9×10^7 m^3 d^{-1}). The output provides both PEC_{water} (dissolved) and PEC_{water} (total); it is proposed to use the total value as a precautionary conservative estimate. The emissions to wastewater and soil must be set to zero.

The emissions to the marine air are calculated from the PEC_{air} (terrestrial) and the flow of air across the proposed regional marine area. The atmospheric volume is 4×10^{12} m^3 and the residence time of the air is 0.216 days; therefore the air flow is 1.85×10^{13} m^3 d^{-1}. It would be conservative to assume that 50% of the air flow to the marine region was derived from the terrestrial region.

The continental emissions must also be entered. Since the region receives 10% of the continental emissions, but is nested within the continental box, the continental emissions are 9 times the regional values.

Example

A non-biodegradable organic substance with log K_{ow} of 7.0, with regional (Standard TGD) emissions of 10,000 kg per year to air, 100,000 kg per year to wastewater (93% removal in STP) and 43,000 kg per year direct to surface water, gave the following results:

Regional (freshwater) PEC_{water} (total)	*0.98 µg l^{-1}*
Regional (freshwater) PEC_{water} (dissolved)	*0.51 µg l^{-1}*
Regional PEC_{air}	*6.35×10^{-7} mg m^{-3}*
Regional $PEC_{sediment}$	*11.5 mg kg^{-1}*

Using the PEC_{water} (total), these calculate to give inputs to the regional marine model of 32,200 kg yr^{-1} to water and 2,142 kg yr^{-1} to air, which resulted in:

Regional (marine) PEC_{water} (total)	*0.224 µg l^{-1}*
Regional (marine) PEC_{water} (dissolved)	*0.167 µg l^{-1}*
Regional PEC_{air}	*1.13×10^{-6} mg m^{-3}*
Regional $PEC_{sediment}$	*3.76 mg kg^{-1}*
Continental (marine) PEC_{water} (total)	*0.0098 µg l^{-1}*
Continental (marine) PEC_{water} (dissolved)	*0.0085 µg l^{-1}*

Table 20: Changes in EUSES defaults for marine regional modelling

Parameter	EUSES default value	Regional marine value
Regional Area (km^2)	4×10^4	**4×10^3**
Area fraction water	0.03	**0.997**
[Area of water (km^2)]	[1,200]	**[3,988]**
Area fraction natural soil	0.6	**0.001**
Area fraction agricultural soil	0.27	**0.001**
Area fraction industrial soil	0.1	**0.001**
Mixing depth natural soil (m)	0.05	0.05
Mixing depth agricultural soil (m)	0.2	0.2
Mixing depth industrial soil (m)	0.05	0.05
Atmospheric mixing height (m)	1,000	1,000
Depth of water (m)	3	**10**
[Volume of water (m^3)]	[3.6×10^9]	**[4×10^{10}]**
Susp. solids concentration (mg l^{-1})	15	**5**
Depth of sediment (m)	0.03	0.03
Fraction of organic carbon in sediment	0.05	0.05
Fraction of aerobic sediment	0.1	0.1
Average annual precipitation (mm yr^{-1})	700	700
Wind speed (m s^{-1})	3	3
Fraction of water flow from continental scale to region	0.034	0.01
[Residence time of air (d)]	[0.7]	**[0.216]**
[Residence time of water (d)]	[40]	**[96]**
Fraction of rain infiltrating soil	0.25	0.25
Fraction of rain running off soil	0.25	0.25
Connection to STP (%)	70	(70)
[River (sea) water inflow (m^3 d^{-1})]	[6.56×10^7]	**4.09×10^8**
[Wastewater flow (m^3 d^{-1})]	[4×10^6]	**0**
Rain (0.7 m per year) (m^3 d^{-1} net flow)	[2.09×10^7]	**[7.7×10^6]**
[Total water flow through region (m^3 d^{-1})]	[9.0×10^7]	**[4.17×10^8]**

Changes are highlighted in bold. [ ] indicates value calculated from other parameters.

Table 21: Changes in EUSES defaults for marine continental modelling

Parameter	EUSES default value	Continental marine value
Total Area (including region) (km^2)	3.56×10^6	**4×10^5**
Continental area (excluding region)	3.52×10^6	**3.96×10^5**
Area fraction water	0.03	**0.997**
[Area of water (km^2)]	[105,600]	**[3.95×10^5]**
Area fraction natural soil	0.6	**0.001**
Area fraction agricultural soil	0.27	**0.001**
Area fraction industrial soil	0.1	**0.001**
Depth of water (m)	3	**60**
[Volume of water (m^3)]	[3.17×10^{11}]	**[2.37×10^{13}]**
Susp. solids concentration (mg l^{-1})	15	**2**
Fraction of organic carbon in sediment	5	**1a**
Fraction of water flow from global scale to continental	0	0
[Residence time of air (d)]	[6.41]	**[2.15]**
[Residence time of water (d)]	[166]	**[581]**
[Water flow through continental box (m^3 d^{-1})]	[1.91×10^9]	**[4.08×10^{10}]**

[a] The current version of EUSES does not allow different OC contents to be set for the regional and continental scales; therefore, in practice, it is necessary to use a mean value of 0.03 (3% OC) for both scales when running the model.

Changes are highlighted in bold. [ ] indicates value calculated from other parameters

APPENDIX B

Selected standard ecotoxicological test protocols for marine and estuarine organisms

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms

Taxa/Species	Exposure	Parameters Period	Method
International Standardisation Organisation (ISO)			
Skeletonema costatum or Phaeodactylum tricornutum (marine algae)	72 hours 96 hours	Growth (EC_{50}/NOEC)	ISO 10253
Acartia and Tisbe spp, (copepod)	48 hours	Mortality (LC_{50})	ISO 14669
International Maritime Organisation (IMO) Convention for the Prevention of Pollution from Ships (MARPOL, 1978)			
Chaetogammarus marinus (gammarid)	96 hours	Mortality (LC_{50})	MARPOL
Mysidopsis bahia (mysid shrimp)	96 hours	Mortality (LC_{50}) and behaviour (EC_{50})	MARPOL
Crangon crangon (brown shrimp)	96 hours	Mortality (LC_{50}) and behaviour (EC_{50})	MARPOL
Acartia tonsa (copepod)	48 hours	Mortality (LC_{50})	MARPOL
Paris Commission (PARCOM)			
Skeletonema costatum (marine algae)	72 hours	Growth (IC_{50})	PARCOM
Chaetogammarus marinus (gammarid)	96 hours	Mortality (LC_{50})	PARCOM
Mysidopsis bahia (mysid shrimp)	96 hours	Mortality (LC_{50}) and behaviour (EC_{50})	PARCOM
Acartia tonsa (copepod)	48 hours	Mortality (LC_{50})	PARCOM
Tisbe battagali (copepod)	48 hours	Mortality (LC_{50})	PARCOM
Corophium volutator or Corophium arenarium (amphipod)	10 days	Mortality and sediment re-working (LC_{50} and EC_{50})	PARCOM
Abra alba (bivalve)	96 - 120 hours	Mortality and sediment re-working (LC_{50} and EC_{50})	PARCOM

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms (continued)

Taxa/Species	Exposure	Parameters Period	Method
Crassostrea gigas (pacific oyster)	24 hours	Embryolarval development (EC_{50})	PARCOM
Arenicola marina (oligochaete)	10 days	Mortality and sediment re-working (LC_{50} and EC_{50})	PARCOM
Echinocardium cordatum (sea urchin)	14 days	Mortality and sediment re-working (LC_{50} and EC_{50})	PARCOM
Scophthalmus maximus (turbot) or Cyprinodon variegatus (sheepshead minnow)	96 hours	Mortality (LC_{50})	PARCOM
Organisation for Economic Co-operation and Development (OECD)			
Cyprinodon variegatus (sheepshead minnow)	96 hours	Mortality/behaviour test, semi-static	OECD 203
US EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)			
"Estuarine and Marine Organisms" "Fish Early Life Stage/Aquatic Invertebrate Life Cycle" "Fish Life Cycle"		US EPA FIFRA OPP 72.3 US EPA FIFRA OPP 72.4 US EPA FIFRA OPP 72.5	
American Society for Testing and Materials (ASTM)			
Skeletonema costatum (diatom), Thalassiosira pseudonana (diatom), Dunaliella tertiolecta (flagellate) and Phaeodactylum tricornutum (golden-brown alga)	96 hours	Growth (IC_{50})	ASTME1218-97a
Marine and Estuarine Amphipods			ASTM E1667-92
Gonyaulax polyedra and Pyrocystis lunula (bioluminescent dinoflagellates)	G. polyedra 96 hours (acute) and 7 days (chronic) P. lunula 4 hours	Bioluminescence (IC_{50})	ASTM E1924-97

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms (continued)

Taxa/Species	Exposure	Parameters Period	Method
Champia parvula (redalgae)	48 hours exposure + 5 - 7 days development	Sexual reproduction (IC_{50}, NOEC and LOEC)	ASTM E1498-92
Crassostrea virginica (eastern oysters), Crassostrea gigas (pacific oysters) Mercenaria mercenaria (quahogs) and Mytilus edulis (bay mussels)	48 hours	Embryo-larval ;ortality and development (EC_{50} and LC_{50})	ASTM E724-94
14 species saltwater fishes and 23 species saltwater shrimps, crabs, bivalves and polychaetes)	2 - 8 days	Mortality and immobilisation invertebrates (copepods, (LC_{50}, EC_{50} and IC_{50})	ASTM E729-96
Cyprinodon variegatus (sheepshead minnow), Menidia menidia (atlantic silverside) and Menidia peninsulae (tidewater silverside)	28 days	Early life stage development and growth (EC_{10}, EC_{50} and EC_{90})	ASTM E1241-92
Rhepoxynius abronius, Eohaustorius estuarius, Ampelisca abdita, Grandidierella japonica and Leptocheirus plumulosus (marine and estuarine infaunal amphipods)	10 days	Mortality and sublethal effects (reburial) LC_{50} and EC_{50})	ASTM E1367-92
Neanthes arenaceodentata and Neanthes virens (marine and estuarine infaunal polychaetous annelids)	10 days (static) 20 - 28 days (renewal)	Mortality and growth (LC_{50}, EC_{50}, LOEC and NOEC)	ASTM E1611-94
Holmesimysis costata and Neomysis mercedis (mysid shrimp)	96 hours	Mortality (LC_{50},LOEC and NOEC)	ASTM E1463-92
Mysidopsis bahia, M. begelowi and M. almyra (mysid shrimp)	"Life Cycle" (median time to first brood release in control plus ≥7 days)	Mortality, growth and reproduction (EC_{10}, EC_{25} and EC_{50})	ASTM E1191-97

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms (continued)

Taxa/Species	Exposure	Parameters Period	Method
Neanthes arenaceodentata, Capitella capitata, Ophryotrocha diadema and Dinophilus gyrociliatus (marine and estuarine polychaetous annelids)	96 hours (acute),10 - 28 days (chronic) and 10 days - 3 months (life cycle)	Mortality, growth and reproduction (LC5, LC_{50}, EC_5,EC_{50}, LOEC and NOEC)	ASTM E1562-94
Arbacia punctulata (atlantic sea urchin), Strongylocentrus droebachiensis (green sea urchin), Strongylocentrus pupuratus (purple sea urchins) and Dendraster excentricus (sand dollar)	48 - 96 hours	Embryo-larval and development (LC_{50}, EC_{50}, LOEC and NOEC)	ASTM E1563-95
US EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS)			
Skeletonema costatum (marine algae)	96 hours	Growth (IC_{50})	US EPA OPPTS 850.5400
Cyprinodon variegatus (sheepshead minnow), Menidia menidia (atlantic silverside) and Menidia penisulae (tidewater silverside)	96 hours	Mortality (LC_{50})	US EPA OPPTS 850.1075
Crassostrea virginica (eastern oysters)	96 hours	Shell deposition (EC_{50})	US EPA OPPTS 850.1025
Mysidopsis bahia (mysid shrimp)	96 hours	Mortality (LC_{50} and NOEC)	US EPA OPPTS 850.1035
Penaeus aztecus (brown shrimp), Penaeus duorarum (pink shrimp) or Penaeus setiferus (white shrimp)	96 hours	Mortality (LC_{50} and NOEC)	US EPA OPPTS 850.1035
Cyprinodon variegatus (sheepshead minnow)		Life cycle toxicity	US EPA OPPTS 850.1050
Crassostrea virginica (eastern oysters), Crassostrea gigas (pacific oysters) Mercenaria mercenaria (quahogs) and Mytilus edulis (bay mussels)	48 hours	Embryo-larval ;ortality and development (EC_{50})	US EPA OPPTS 850.1055
Cyprinodon variegatus (sheepshead minnow), Menidia menidia (atlantic silverside) and Menidia peninsulae (tidewater silverside)	96 hours	Mortality (LC_{50} and NOEC)	US EPA OPPTS 850.1075

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms (continued)

Taxa/Species	Exposure	Parameters Period	Method
Mysidopsis bahia (mysid shrimp)	28 days	Mortality (LC_{50}) and "chronic criteria" i.e. growth and reproduction (MATC)	US EPA OPPTS 850.1350
Cyprinodon variegatus (sheepshead minnow), Menidia menidia (atlantic silverside) and Menidia penisulae (tidewater silverside)	28 days	Early life stage development (LOEC and NOEC)	US EPA OPPTS 850.1400
US EPA Toxic Substances Control Act (TSCA)			
Skeletonema costatum (marine algae)	96 hours	Growth inhibition (EC_{10}, EC_{50} and EC_{90})	US EPA TSCA 797.1050
Skeletonema costatum, Thalassiosira pseudonana (diatom)	96 hours	Growth inhibition (EC_{10}, EC_{50} and EC_{90})	US EPA TSCA 797.1075
Mysidopsis bahia (mysid shrimp)	96 hours	Mortality (LC_{50})	US EPA TSCA 797.1930
Mysidopsis bahia (mysid shrimp)	28 days	Mortality (LC_{50}) and "chronic criteria" i.e. growth and reproduction (MATC)	US EPA TSCA 797.1950
Penaeus aztecus, Penaeus duorarum or Penaeus setiferus (penaeid shrimps)	Acute	-	US EPA TSCA 797.1970
Crassostrea virginica (oyster)	Acute	-	US EPA TSCA 797.1800
Cyprinondon variegatus (sheepshead minnow), Menidia menidia (atlantic silverside) Menidia penisulae (tidewater silverside)	28 days	Early life stage development and OEC and NOEC)	US EPA TSCA 797.1600

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms (continued)

Taxa/Species	Exposure	Parameters Period	Method
US EPA Ocean Disposal Bioassay Working Group			
Palaemonetes pugio, Palaemonetes vulgaris and Palaemonetes intermedius (grass shrimp)	96 hours	Larval mortality	Bioassay Procedures for the Ocean Disposal Permit Program EPA-600/9-78-010
Cyprinodon variegatus (sheepshead minnow)	Life Cycle (4 - 6 months)	Embryo-larval mortality, growth and reproduction	Bioassay Procedures for the Ocean Disposal Program EPA-600/9-78-010
Cyprinodon variegatus (sheepshead minnow)	Invitro	Brain acetylcholin-esterase (AChE) inhibition	Bioassay Procedures for the Ocean Disposal Permit Program EPA-600/9-76-010
Crassostrea virginica (eastern oysters)	Short-term	-	Bioassay Procedures for the Ocean Disposal Permit Program EPA-600/9-76-010
Mysidopsis bahia (mysid shrimp)	Acute	-	Bioassay Procedures for the Ocean Disposal Permit Program EPA-600/9-78-010
Mysidopsis bahia (mysid shrimp)	Entire Life Cycle (3 - 4 months)	Mortality, growth and reproduction	Bioassay Procedures for the Ocean Disposal Permit Program EPA-600/9-78-010

Table 22: Selected standard ecotoxicological test protocols for marine and estuarine organisms (continued)

Taxa/Species	Exposure	Parameters Period	Method
	Miscellaneous		
Corophium volutator (estuarine amphipod)	28 days	Mortality and growth	Allen et al (2000); Sims et al (2000)
Leptocheirus plumulosus(estuarine amphipod)	28 days	Mortality, growth and reproduction	Allen et al (2000); Sims et al (2000)
Arenicola Marina (polychaete)	100 days	Mortality and sublethal effects (casting rate)	Allen et al (2000)
Ophryotrocha spp. (polychaete)	35 days	Mortality and reproduction	Crane and Shepherd (1996)

Table 23: Comparison of the sensitivity of Daphnia magna relative to selected freshwater and marine test species

Taxa	Algae	Crustacea	Mollusc	Fish	Annelid	Echinoderm	Algae	Fish
Medium	Marine				Fresh water			
Species	Skeletonema costatum	Mysidopsis bahia	Crassostrea virginica	Cyprinodon variegatus	Nereisarena-ceodentata	Paracentrotus lividus	Selenastrum capricornutum	Pimephales promelas
Comparative Pairs	106	149	106	162	25	18	105	154
Ratio at 5%	0.00009	0.00297	0.00030	0.00016	0.00011	0.00046	0.00047	0.00005
Ratio at 25%	0.0188	0.1520	0.0063	0.0338	0.0100	0.0097	0.0688	0.0298
Ratio at Median	0.43	0.83	0.20	0.22	0.08	0.12	0.54	0.39
Ratio at 75%	1.82	4.21	2.55	0.91	0.354	0.74	4.43	1.36
Ratio at 95%	158	23.7	30.9	9.00	2.08	6.875	743	16.5
Minimum Ratio	0.0000016	0.00000067	0.00001167	0.00000004	0.00068	0.0003535	0.00000029	0.0000006
Maximum Ratio	3,600	769	10,000	1,606	3.46	11.83	36,000	5,955
% at Ratio <1	61.9	55.2	66.6	76.7	92.6	77.4	58.6	67.3
% at Ratio 0.5	51.8	42.9	57.1	62.2	88.4	70.5	46.1	55.0
% at Ratio 2	75.5	68.9	72.3	86.9	94.8	82.5	68.0	79.5
% <Factor 2	23.7	26.0	15.2	24.7	6.4	12.0	21.9	24.5
% at Ratio 0.1	35.0	18.9	44.7	40.0	57.3	47.8	28.8	35.2
% at Ratio 10	87.4	87.5	92.5	95.2	n/a	98.1	80.9	93.3
% <Factor 10	52.4	68.6	47.8	55.2	42.7	50.3	52.1	58.1
Correlation	0.48	0.74	0.42	0.61	0.68	0.39	0.40	0.50
Intercept	0.465	0.071	-0.115	0.620	0.430	0.0175	0.079	0.360
Slope	0.399	0.693	0.260	0.509	0.424	0.303	0.380	0.411
Significance (p)	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001	<0.001

Table 23: Comparison of the sensitivity of Daphnia magna relative to selected freshwater and marine test species.

Taxa	Algae	Crustacea	Mollusc	Fish	Annelid	Echinoderm	Algae	Fish
Medium	**Marine**				**Fresh water**			
Comments	Over 100 paired comparisons made with *D. magna. Daphnia magna* was the more sensitive in nearly 62% of cases. Approx. 52% of observations are within a factor of 10. Only 24% within a factor of 2. A highly significant correlation between the responses of the two species was observed.	Nearly 150 paired comparisons possible between *D. magna* and *M. bahia. D. magna* was the more sensitive in over 55% of cases. Approx. 69% of observations are within a factor of 10. Exactly 26% within a factor of 2. A highly significant correlation between the responses of the two species was observed.	Over 100 paired comparisons made between *C. virginica* and *D. magna. D. magna* was the more sensitive in 67% of cases. Approx. 39% of observations are within a factor of 10. Some 15% of pairs were within a factor of 2. A highly significant correlation between the responses of the two species was observed.	A large number (162) of paired comparisons was possible between *C. variegatus* and *D. magna. D. magna* was the more sensitive in close to 77% of cases. Approx. 55% of observations are within a factor of 10. Nearly 25% are within factor of 2. A highly significant correlation between the responses of the two species was observed.	A more limited number of comparisons made with *D. magna (25). D. magna* was the more sensitive in 93% of cases. Approx. 47% of observations are within a factor of 10. The proportion within a factor of 2 was only 6%. A highly significant correlation between the responses of the two species was observed.	Data availability permitted a comparisons with *D. magna* for 18 substances. *D. magna* was the more sensitive in approx. 77% of cases. The proportion of observations within a factor of 10 was 50%. The proportion within a factor of 2 was nearly 12%. No statistically significant correlation between the responses of the two species was observed.	Comparisons were with *D. magna* for over 100 substances. *D. magna* was the more sensitive in approx. 59% of cases. Over 52% of observations were within a factor of 10. The proportion within a factor of 2 was 22%. A highly statistically significant correlation between the responses of the two species was observed.	Comparisons were with *D. magna* for over 150 substances. *D. magna* was the more sensitive in approx. 67% of cases. Over 58% of observations were within a factor of 10. The proportion within a factor of 2 was 25%. A highly statistically significant correlation between the responses of the two species was observed.

BIBLIOGRAPHY

Alexander M. 1985. Biodegradation of organic chemicals. *Environ Sci Technol* 18:106-111.

Allen YT, Kirby SJ, Reed J, Thain JE. 2000. Development and application of chronic and sublethal sediment bioassays. Third SETAC World Congress 21-25 May 2000, Brighton, UK, Abstract/Poster 5B/p10.

AMAP. 1998. AMAP Assessment Report: Arctic pollution issues. In Wilson SJ, Murray JL, Huntington HP, eds, *Arctic Monitoring Assessment Programme (AMAP)*, Oslo, Norway.

Arnosti C, Jorgensen BB, Sagemann J, Thamdrup B. 1998. Temperature dependence of microbial degradation of organic matter in marine sediments: polysaccharide hydrolysis, oxygen consumption and sulfate reduction. *Mar Ecol Prog Ser* 165:59-70.

Austin B. 1988. Marine Microbiology. Cambridge University Press.

Baart AC, Boon JG. 1998. Scremotox: A screening model for comparative ecotoxicological risk assessment- North Sea. Delft Hydraulics Project Z2182 for Ministry of Housing, Spatial Planning and Environment, Den Haag, Netherlands.

Barber MC, Suarez LA, Lassiter RR. 1991. Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Lake Ontario salmonids. *Can J Fish Aquat Sci* 48:318-337.

Barnes RD. 1963. Invertebrate zoology, Second edition. WB Saunders Company.

Bartholomew GW, Pfinder FK. 1983. Influence of spatial and temporal variations on organic pollutant biodegradation rates in an estuarine environment. *Appl Environ Micro* 45:103.

Bauer JE, Capone DG. 1988. Effects of co-occurring aromatic hydrocarbons on degradation of individual polycyclic aromatic hydrocarbons in marine slurries. *Appl Environ Micro* 54: 1649-1655.

Berg EL. 1982. *Handbook for Sampling and Sample Preservation of Water and Wastewater*. US EPA No. 600/4-82-029, Cincinnati, Ohio, USA.

BMT Marine Information Systems. 1999. PROTEUS. Pollution Risk Offshore Technical EvalUation Systems. Southampton; UK.
http://www.bmtmis.com/Products/Proteus/prodproteus.htm

Boethling RS, Howard PH, Beauman JA, Larosche ME. 1995. Factors for intermedia extrapolation in biodegradability assessment. *Chemosphere* 30:741-752.

Boutonnet J-C, de Rooij C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998. Trichloroethylene. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - Trichloroethylene. *Environ Monit Assess* 53:467-487.

Brandes LJ, den Hollander HA, van de Meent D. 1996. Simplebox 2: A nested multi-media fate model for evaluating the environmental fate of chemicals. Report No 719101 029. National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

BUA. 1999. Marine Risk Assessment: Concept and Criteria. Report No. 220 of the project group "Marine Risk Assessment" of the GDCh-Advisory Committee on Existing Chemicals (BUA). Hirzel S, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany.

Calleja MC, Personne G, Geladi P. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of *in vitro* cytotoxicity chemicals to aquatic non-vertebrates. *Arch Environ Contam Toxicol* 26:69-78.

Canton JH, Wegman RCC, Vulto TJA, Verhoef CH, van Esch GJ. 1978. Toxicity, accumulation and elimination studies of *-hexachlorocyclohexane (*-HCH) with freshwater organisms of different trophic levels. *Wat Res* 12:687-690.

CEFAS. 1999. Guidelines for the UK revised Off-shore Chemicals Notification Scheme, July 1999, Burnham-on-Crouch, Essex, UK.

Clark KE, Gobas FAPC, Mackay D. 1990. Model of organic chemical uptake and clearance by fish from food and water. *Environ Sci Technol* 24:1203-1213.

Connell DW. 1990. Biomagnification of lipophilic compounds in terrestrial and aquatic systems. In Connell DW, ed, *Bioaccumulation of xenobiotic compounds*, CRC Press, Boca Raton, Florida, USA.

Crane M, Shepherd B. 1996. Development of sediment toxicity tests and bioassays with marine meiofaunal polychaetes. Report from Royal Holloway University of London (School of Biological Sciences) to WRc, Medmenham, UK, for the England and Wales Environment Agency.

Crawford CC, Hobbie JE, Webb KL. 1974. Utilisation of dissolved free amino acids by estuarine micro-organisms. *Ecology* 55:551.

Cripe CR, Walker WW, Pritchard PH, Bourquin AW. 1987. A shake flask for estimation of biodegradability of the organic fraction of chemical wastes. *Ecotoxicol Environ Saf* 14:239-251.

Davies RP, Dobbs AJ. 1984. The prediction of bioconcentration in fish. *Wat Res* 18:1253-1262.

Dawson G, Jennings AJ, Drozdowski D, Rider E. 1977. The acute toxicity of 47 industrial chemicals to fresh and saltwater fishes. *J Haz Mat* 1 :303 - 318.

De Rooij C, Boutonnet J-C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998a. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - 1,2-Dichloroethane. *Environ Monit Assess* 53:425-445.

De Rooij C, Boutonnet J-C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998b. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - 1,1,2-Trichloroethane. *Environ Monit Assess* 53:447-466.

De Rooij C, Boutonnet J-C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998c. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - Tetrachloroethylene. *Environ Monit Assess* 53:489-508.

Delft Hydraulics - IVM. 1999. MAM PEC. Marine Antifoulant Model to Predic Environmental Concentrations. Delft Hydraulics, Delft, The Netherlands.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541-1583.

Dixon E, Gardner MJ. 1998. Reactive aluminium in UK surface waters. *Chemical Speciation and Bioavailability* 101:1-7.

Durell G, Neff J, Melbye A, Johnsen S, Garpestad E, Gruner H. 2000. Monitoring and assessment of produced water originating contaminants in the Ekofisk region of the North Sea. SPE International Conference on Health, Safety, and the Environment in Oil and Gas Exploration and Production. June 2000, Stavanger, Norway.

Eadsforth CV, Lemaire P, Tencalla F, Roman G, Wierich P, Webb S, Holt MS. 2001. Analysis of the ECETOC aquatic toxicity (EAT-3) database. I. Comparative toxicity of chemical substances to freshwater and saltwater organisms. *Submitted to Chemosphere*.

EC. 1996. Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. Office for official Publications of the European Communities, Luxembourg.

ECB. 1998. draft document ECB4/33/98-rev.1, November 26 1998, European Commission, Joint Research Centre, European Chemicals Bureau, Ispra, Italy.

ECB. 1999a. Revision of the Technical Guidance Document regarding the risk assessment of individual chemicals. ECB 4/10/99 (Rev 3), European Commission, Joint Research Centre, European Chemicals Bureau, Ispra, Italy.

ECB. 1999b. OSPAR-EC approach to marine environment risk assessment. ECB 4/22/99, European Commission, Joint Research Centre, European Chemicals Bureau, Ispra, Italy.

ECETOC. 1988. Now ISO 11734, Water quality - Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of biogas production.

ECETOC. 1993a. Estimation of the biodegradation of chemicals in the marine environment. Technical Report No. 54. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1993b. Aquatic toxicity data evaluation. Technical Report No. 56. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1995. The role of bioaccumulation in environmental risk assessment: the aquatic environment and related food webs. Technical Report No 67. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1997. The value of aquatic model ecosystems studies in ecotoxicology - Technical Report No 73. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1999a. Monitoring and modelling of industrial organic chemicals, with particular reference to aquatic risk assessment. Technical Report No 76. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1999b. GREAT-ER user manual. Special Report No 16. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2001. Aquatic toxicity database EAT3. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium. (In press)

Emans JJB, Van De Plassche EJ, Canton JH, Okkerman PC, Sparenburg PM. 1993. Validation of some extrapolation methods used for effects assessment. *Environ Toxicol Chem* 12 :2139-2154.

Ernst W. 1988. Appraisal of poorly degradable pollutants in the estuarine zone. *GWF-Wasser/Abwasser* 129:212.

Esser HO, Mosser P. 1982. An appraisal of problems related to the measurement and evaluation of bioaccumulation. *Ecotoxicol Environ Saf* 6:131-148.

EUSES. 1996. The European Union System for the Evaluation of Substances. National Institute of Public Health and the Environment (RIVM), The Netherlands. Available from the European Chemicals Bureau (EC/JRC), Ispra, Italy.

Evans MR, Gilbert JL, Riddle AM, Beling EM, Roberts GC, Snape JR, Gannon D, Gray A, Nyholm N, Madsen EL, Amann R. 2000. Review of persistence of chemicals in the terrestrial and marine environments. Report No BL6834/A. Brixham Environmental Laboratory, AstraZeneca, Brixham, UK.

Everts JW, Eys Y, Ruys M, Pijnenburg J, Visser H, Luttik R. 1993. Biomagnification and environmental quality criteria: a physiological approach. ICES *J Mar Sci* 50:333-335.

Falk-Petersen R, Gatten RR, Sargent JR. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: seasonal changes in the lipid class composition of *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa raschii* (M. Sars) and *T. inermis* (Krøyer). *J Exp Mar Biol Ecol* 54:209-224.

Ferguson EA, Hogstrand C. 1998. Acute silver toxicity to seawater-acclimated rainbow trout - influence of salinity on toxicity and silver speciation. *Environ Toxicol Chem* 17:589-593.

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir CGM. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol-water partition coefficient. *Environ Toxicol Chem* 17:951-961.
Galushko A, Minz D, Schink B, Widdel F. 1999. Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. *Environ Microbiol* 1:415-420

Garny V. 1998. Principles and purposes. In Calow P, ed, *Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea*, p 5-9. [Circulated as DYNAMEC 98/04/NGO.3-E*(L)]

GESAMP. 1989. The evaluation of the hazards of harmful substances carried by ships: revision of GESAMP reports and studies number 17. International Maritime Organisation: Report and Studies number 35. International Maritime Organisation, London, UK.

Goulder R. 1986. Seasonal variation in the abundance of heterotrophic activity of suspended bacteria in two lowland rivers. *Freshwater Biology* 16:21.

Graney RL, Giesy JP, Clark JR. 1995. Field Studies. In Rand GM, Fundamentals of aquatic toxicology. Taylor and Francis, London, UK.

Greene, D.H.S. and Selivonchick, D.P. (1987). Lipid metabolism in fish. *Prog Lipid Res* 26:53-85.

Groot S, Villars MT. 1995. Monitoring water quality in the future. Vol 5: Organisational aspects. Ministry of Housing, Spacial Planning and Environment, The Netherlands.

Gschwend PM, Zafiriou OC, Mantoura RFC, Schwarzenbach RP, Gagosian RB. 1982. Volatile organic compound at a coastal site I - seasonal variations. *Environ Sci Technol* 16:31-38.

Hashimoto S, Azuma T, Otsuki A. 1998. Distribution, sources and stability of haloacetic acids in Tokyo Bay, Japan. *Environ Toxicol Chem* 17:798-805.

Hawker DW. 1989. Bioaccumulation of metallic substances and organometallic compounds. In Connell DW, ed, *Bioaccumulation of xenobiotic compounds*. CRC Press, Boca Raton, FL, USA.

Hemmer MJ, Middaugh DP, Comparetta V. 1992. Comparative acute sensitivity of larval topsmelt, *Atherinops affinis* and inland silverside, *Mendia beryllina* to 11 chemicals. *Environ Toxicol Chem* 11:401-408.

Henderson RJ, Tocher DR. 1987. The lipid composition and biochemistry of freshwater fish. *Prog Lipid Res* 26:281-347.

HMSO. 1986. The sampling and initial preparation of sewage and waterworks sludges, soils, sediments, plant materials and contaminated wild life prior to analysis. In *Methods for the Examination of Waters and Associated Materials, 2nd edition*. Her Majesty's Stationary Office, London, UK.

Hodson RE, Azam F, Carlucci AF, Fuhrman JA, Karl DM, Holm-Hansen O. 1981. Microbial uptake of dissolved organic matter in McMurdo Sound Antartica. *Mar Biol* 61:89-94.

Holt MS, Fox K, Griessbach E, Johnsen S, Kinnunen J, Lecloux A, Murray-Smith R, Peterson DR, Schroeder R, Sivani M, ten Berge WFJ, Toy RJ, Feijtel TCM. 2000. Monitoring, modelling and environmental exposure assessment of industrial chemicals in the aquatic environment. *Chemosphere* 41:1799-1808.

Horowitz A, Shelton DR, Cornell CP, Tiedje JM. 1982. Anaerobic degradation of aromatic compounds in sediments and digested sludge. *Dev Ind Microbiol* 23:435-444.

Hutchinson TH, Scholz N, Guhl W. 1998. Analysis of the ECETOC Aquatic Toxicity (EAT) database IV - Comparative toxicity of chemical substances to freshwater versus saltwater organisms. *Chemosphere* 36:143-153.

IFREMER. 2000. Personal communication.

IUCLID. 1996. International Uniform Chemical Information Database. European Chemical Bureau, Ispra, Italy [CD-Rom].

Jackim E, Morrison G, Steele R. 1977. Effects of environmental factors on radiocadmium uptake by four species of marine bivalves. *Mar Biol* 40:303.

Johnsen S, Frost TK, Hjelsvold M, Utvik TI. 2000. The Environmental Impact Factor - a proposed tool for produced water impact reduction, management and regulation. SPE paper 61179, presented at the 2000 HSE meeting, Stavanger, Norway.

Johnsen S, Utvik TIR, Durell G, Reed M. 1998. Dilution and bioavailability of produced water compounds in the northern North Sea. A Combined Modelling and Field Study. SPE paper SPE 46269. Presented at the 1998 HSE meeting, Caracas, Venezuela.

Johnson and Luttik. 1999. EC Project 96/559/3040/DEB/E2. Utilisation of more environmentally friendly antifouling products.

Karman CC, Vik EA, Schobben HPM, Øfjord GD, van Dokkum HP. 1996. Charm III main report. TNO-MEP R 96/355. Institute of Environmental Sciences, Energy Research and Process Innovation (TNO MEP), Department of Ecological Risk Studies, Den Helder, The Netherlands.

Kerr RP, Capone DG. 1988. The effect of salinity on the microbial mineralisation of two polycyclic aromatic hydrocarbons in estuarine sediments. *Marine Environ Res* 26:181-198.

Kostka JE, Thamdrup B, Nohr Glud R, Canfield DE. 1999. Rates and pathways of carbon oxidation in permanently cold Arctic sediments. *Mar Ecol Prog Ser* 180:7-21.

Kuehnemann P, Battersby NS. 1992. Use of ^{14}C-most-probable-number method to enumerate active heterotrophic bacteria in aqueous samples. *Chemosphere* 25:591.

Larson RJ, Ventullo RM. 1986. Kinetics of biodegradation of nitrilotriacetic acid (NTA) in an estuarine environment. *Ecotoxicol Environ Saf* 12:166-179.

Laughlin RB, Guard HE, Coleman WM. 1986. Tributyltin in sea water: Speciation and octanol-water partition coefficient. *Environ Sci Technol* 20:201-204.

LeBlanc G. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. *Environ Toxicol Chem* 3:47-60.

Lee RF, Ryan CR. 1979. Microbial degradation of organochlorine compounds in estuarine waters and sediments. In Bourquin AW, Pritchard PH, eds, *Microbial degradation of pollutants in marine environments: Proceedings of the workshop. EPA-600/9-79-012*. US Environmental Protection Agency, Cincinnati, USA.

Lee RF, O'Malley K, Oshima Y. 1996. Effects of toxicants on developing oocytes and embryos of the blue crab, *Callinectes sapidus*. *Mar Environ Res* 42:125-128.

Lewis MA. 1995. Algae and vascular plant tests. In Rand GM, ed, *Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment*. Taylor Francis, Washington DC, USA, pp 135-170.

Madsen T, Rasmussen HB, Nilsson L. 1995. Anaerobic biodegradation potentials in digested sludge, a freshwater swamp and a marine sediment. *Chemosphere* 31:4243-4258.

MARPOL. 1978. The evaluation of the hazards of harmful substances carried by ships (Annex II). International Convention for the Prevention of Pollution from Ships, 1973, as modified by the Protocol of 1978 relating thereto (MARPOL 73/78). International Maritime Organisation (IMO). http://www.voeding.tno.nl/PS.cfm-PNR=chem117e

Massie LC, Ward AP, Davies JM. 1985. The effects of oil exploration and production in the Northern North Sea: Part 2 - Microbial biodegradation of hydrocarbons in water and sediments, 1979-1981. *Mar Environ Res* 15:235-262.

May RM. 1988. How many species are there on earth? *Science* 247:1441-1449.

Meyer-Reil LA, Köster M. 1992. Microbial life in pelagic sediments: the impact of environmental parameters on enzymatic degradation of organic material. *Mar Ecol Prog Ser* 81:65-72.

Mille G, Almallah M, Bianchi M, van Wambeke F, Bertrand JC. 1991. Effect of salinity on petroleum biodegradation. *Fres J Anal Chem* 339:788-791.

Miller MM, Wasik SP, Huang GL, Shiu WY, Mackay D. 1985. Relationships between octanol-water partition coefficient and aqueous solubility. *Environ Sci Technol* 19:522-529.

Murphy PG. 1971. The effect of size on the uptake of DDT from water by fish. *Bull Environ Contam Toxicol* 6:20-23.

Nacci DE, Jackim E. 1985. Rapid aquatic toxicity assay using incorporation of tritiated-thymidine into sea urchin, Arbacia punctulata embryo: Evaluation of toxicant exposure procedures. *Aquatic Toxicology and Hazard Assessment ASTM STP* 891:382-394.

Nendza M, Klein W. 1990. Comparative QSAR study on freshwater and estuarine toxicity. *Aquatic Toxicology* 17:63-74.

Nyholm N. 1990. Expression of results from growth inhibition tests with algae. *Arch Environ Contam Toxicol* 19:518-522.

Nyholm N. 1991. The European system of standardised legal tests for assessing the biodegradability of chemicals. *Environ Toxicol Chem* 10:1237-1246.

Nyholm N, Kristensen P. 1992. Screening methods for assessment of biodegradability of chemicals in seawater - Results from a ring test. *Ecotoxicol. Environ Saf* 23:161-172.

Nyholm N, Damborg A, Lingaard-Jorgensen P. 1992. A comparative study of test methods for assessment of the biodegradability of chemicals in seawater - screening tests and simulation tests. *Ecotoxicol Environ Saf* 23:173-190.

OECD. 1992. Report of the workshop on effects assessment of chemicals in sediment. OECD Environment Monograph No.60. Paris, France.

OECD. 1998. OECD 300 series. Tenth addendum to the OECD guidelines for the testing of chemicals. Section 3 Degradation and accumulation. OECD Paris.

OECD. 2000a. Simulation test - Aerobic transformation in surface water. Draft proposal for a new guideline. Paris, France.

OECD. 2000b. Aerobic and anaerobic transformation in aquatic sediment systems. Draft proposal for a new guideline 308. Paris, France.

Oren A, Gurevich P, Azachi M, Henis Y. 1992. Microbial degradation of pollutants at high salt concentrations. *Biodegradation* 3:387-398.

OSPAR. 1998a. OSPAR Strategy with regard to hazardous substances. OSPAR 98/14/1, Annex 34. OSPAR Commission, London, UK.

OSPAR. 1998b. Terms of reference for the ad hoc working group on the development of a dynamic selection and prioritisation mechanism for hazardous substances (DYNAMEC). ASMO 98/17/1, Annex 6.

OSPAR. 1998c. Report of the Third OSPAR Workshop on Ecotoxicological Assessment Criteria (The Hague: 25-29 November 1996). OSPAR Commission, London, UK. [Circulated as DYNAMEC(1) 99/2/3-E(L)].

OSPAR. 1999a. Point of departure forthe further development of risk assessment methodology for the marine environment - The basis of an OSPAR/EC approach. DYNAMEC 99/10/1-E, Annex 7. OSPAR Commission, London, UK.

OSPAR. 1999b. Draft framework for a common approach on risk assessment methodology for the marine environment. DYNAMEC (2) 99/10/1, Annex 6. OSPAR Commission, London, UK.

Osswald P, Courtes R, Bauda P, Bryers JD, Sunde E. 1995. Xenobiotic biodegradation test using attached bacteria in synthetic seawater. *Ecotoxicol Environ Saf* 31:211- 217.

Pärt P. 1989. Bioavailability and uptake of xenobiotics in fish. In Landner L, ed, *Chemicals in the aquatic environment*. Springer Verlag, Berlin, Germany.

Pritchard PH, Bourquin AW, Frederickson HL, Maziarz T. 1979. System design factors affecting environmental fate studies in microcosms. In *U.S. Environmental Protection Agency Proceedings of the workshop: Microbial degradation of pollutants in marine environments*, EPA-600/9-79-012. Gulf Breeze, FL, USA, pp 251-271.

Pritchard PH, O'Neil EJ, Spain CM, Ahearn DG. 1987. Physical and biological parameters that determine the fate of p-chlorophenol in laboratory test systems. *Appl Environ Micro* 53:1833-1838.

Quiroga JM, Sales D. 1989. Effect of temperature on kinetic of anionic surfactant degradation in sea-water. *J Disp Sci Technol* 10:773-784.

Rainbow PS. 1997a. Ecophysiology of trace metal uptake in crustaceans. *Estuarine, Coastal and Shelf Science* 44:169-175.

Rainbow PS. 1997b. Trace metal accumulation in marine invertebrates: marine biology or marine chemistry? *J Mar Biol Assoc*, 77:195-210.

Rankin JC, Davenport JA. 1981. Animal Osmoregulation. Blackie and Sons Ltd, Glasgow, UK.

Rheinheimer G, Gericke H, Wesnig J. 1990. Biodegradation of organic chemicals at environmentally relevant concentrations. Report from UBA (Umweltbundesamt), Berlin, Germany.

Rivkin RB, Anderson MR, Lajzerowicz C. 1996. Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. *Aquat Microb Ecol* 10:243-254.

RIVM, VROM, WVC. 1994. Uniform System for the Evaluation of Substances (USES), version 1.0. National Institute of Public Health and Environmental Protection (RIVM), Ministry of Housing, Spatial Planning and the Environment (VROM), Ministry of Welfare, Health and Cultural Affairs (WVC). Distribution No. 11144/150. Ministry of Housing, Spatial Planning and the Environment, The Hague, The Netherlands.

RIVM, VROM, WVC. 1998. Uniform System for the Evaluation of Substances (USES), version 2.0. National Institute of Public Health and Environmental Protection (RIVM), Ministry of Housing, Spatial Planning and the Environment (VROM), Ministry of Welfare, Health and Cultural Affairs (WVC). RIVM report 679102044. Ministry of Housing, Spatial Planning and the Environment, The Hague, The Netherlands.

Roberts JR, McGarrity JT. 1985. Modelling of biotic uptake. In Sheehan P, Korte K, Klein W, Bourdeau P, eds, *Appraisal of tests to predict the environmental behaviour of chemicals*, John Wiley and Sons, London, UK.

Robinson PW. 1999. The toxicity of pesticides and organics to Mysid shrimps can be predicted from *Daphnia* spp. toxicity data. *Wat Res* 33:1545-1549.

Rojícková-Padrtová R, Maršálek B. 1999. Selection and sensitivity comparisons of algal species for toxicity testing. *Chemosphere* 38:3329-3338.

Romijn CFAM, Luttik R, vd Meent D, Sloog W, Canton JH. 1993. Presentation of a general algorithm to include effect assessment on secondary poisoning in the derivation of environmental criteria. Part 1. Aquatic food chains. *Ecotoxicol Environ Saf* 26:61-85.

Saarikoski J, Lindström R, Tyynelä M, Viluksela M. 1986. Factors affecting the absorption of phenolics and carboxylic acids in the guppy (*Poecilia reticulata*). *Ecotox Environ Saf* 11:158-173.

Saltzmann HA. 1982. Biodegradation of aromatic hydrocarbon in marine sediments of three North Sea oil fields. *Mar Biol* 72:17-26.

Sargent JR, Falk-Petersen S. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: lipids and fatty acids of *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa raschii* (M. Sars) and *Thysanoessa inermis* during mid-winter. *Mar Biol* 62:131-137.

Sargent JR, Henderson RJ. 1986. The biological chemistry of marine copepods. In Corner EDS, O'Hara SCM, eds, *Lipids*, 59-108.

Schwarzenbach RP, Gschwend PM, Imboden DM. 1993. Environmental Organic Chemistry. Wiley-Interscience, New York, USA.

SETAC-RESOLVE. 1992. Proceedings of a workshop on aquatic microcosms for ecological assessment of pesticides (Wintergreen, Virginia, USA, October 1991). SETAC Foundation for Environmental Education and the RESOLVE Programme of the World Wildlife Fund, p 56.

SFT. 1999. Miljøovervåking av petroleumsvirksomheten på norsk sektor. SFT Guidelines 99-01. ISBN 82-7655-164-5.

Shimp RJ. 1989. LAS biodegradation in estuaries. *Tenside Surf Det* 26:390-395.

Shimp RJ, Young RL. 1987. Comparison of OECD and radiolabeled substrate methods for measuring biodegradation in marine environments. *Ecotox Environ Saf* 14:223-230.

Shimp RJ, Larson RJ, Boethling RS. 1990. Use of biodegradation data in chemical assessment. *Environ Toxicol Chem* 9:1369-1377.

Sims I, Holmes D, Fleming R, Dewitt T. 2000. The comparative sensitivity of two invertebrates used for assessing sediment toxicity. Third SETAC World Congress, Brighton, UK 21-25 May 2000, Abstract/Poster 5B/p015.

Smith RL, Klug MJ. 1987. Flowthrough reactor flasks for study of microbial metabolism in sediments. *Appl Environ Micro* 53:371.

Solbe JF, Buyle B, Guhl W, Hutchinson T, Laenge R, Mark U, Munk R, Scholz N. 1993. Developing hazard identification for the aquatic environment. *Sci Tot Environ* Supplement 47-61.

Sorokin N. 1999. MSc Thesis Dissertation. Royal Holloway and Bedford New College, University of London, London, UK.

Spain JC, van Veld PA, Monti CA, Pritchard PH, Cripe CR. 1984. Comparison of p-nitrophenol biodegradation in field and laboratory test systems. *Appl Environ Micro* 48:944.

Steber J, Herold C-P, Limia JM. 1995. Comparative evaluation of anaerobic biodegradability of hydrocarbons and fatty derivatives currently used as drilling fluids. *Chemosphere* 31:3105-3118.

Street JR. 1984. Assessment of biodegradability in the marine environment. In Persoone G, Jaspers E, Claus C, eds, *Ecotoxicological testing for the marine environment*. State University of Ghent and Institute for Marine Scientific Research, Ghent, Belgium, Vol 2, p 491.

Tachikawa M, Sawamura R, Okada S, Hamada A. 1991. Differences between freshwater and seawater killifish (*Oryzias latipes*) in the accumulation of pentachlorophenol. *Arch Environ Contam Toxicol* 21:146-151.

Thomann RV. 1989. Bioaccumulation model of organic chemical distribution in aquatic food-chains. *Environ Sci Technol* 23:699-707.

Thomann RV, Connolly JP. 1984. Model of PCB in Lake Michigan lake trout food chain. *Environ Sci Technol* 18:65-71.

Thouand G, Block JC. 1993. The use of precultured inocula for biodegradability tests. *Environ Technol* 14:601-614.

Thouand G, Capdeville B, Block JC. 1996. Pre-adapted inolcula for limiting the risk of errors in biodegradability tests. *Ecotoxicol Environ Saf* 33:261-267.

Tsuda T, Aoki S, Kojima M, Harada H. 1990. Differences between freshwater and seawater-acclimated guppies in the accumulation and excretion of tri-n-butyltin chloride and triphenyltin chloride. *Wat Res* 24:1373-1376.

UN. 1992. Agenda 21: Programme for action for sustainable development. Rio declaration on environment and development. The final text of agreements negotiated by governments at the UN conference on environment and development, 3-14 June, 1992, Rio de Janeiro, Brazil. E.93.1.11. UN Dept. of Public Information.

UN/ECE. 1996a. Report of the thirteenth session of the executive body for the convention on long-range transboundary air pollution. United Nations Economic Commission for Europe. ECE/EB.AIR/46. January 2.

UN/ECE. 1996b. Guidelines on Water Quality Monitoring and Assessment of Transboundary Rivers. ISBN 9036945402.

UNEP. 1995. United Nations Environment Programme Governing Council Decision 18/32: Persistent organic pollutants. May 1995. http://www.chem.unep.ch/pops

UNEP. 1997. United Nations Environment Programme Governing Council Decision 19/13C: International action to protect human health and the environment through measures which will reduce and/or eliminate emissions and discharges of persistent organic pollutants, including the development of an international legally binding instrument. February 1997. http://www.chem.unep.ch/pops

US EPA. 1982. Handbook for sampling and sample preservation of water and wastewater. Environmental Monitoring and Support Laboratory Office of Research and Development, Report EPA-600/4-82-029. Cincinnati, Ohio, USA.

US EPA. 1986. Acute toxicity handbook of chemicals to estuarine organisms (Mayer FL, Jr). EPA/600/X-86/231 (September 1986). US Environmental Protection Agency, Office of Research and Development, Gulf Breeze, Florida, USA.

US EPA. 1994. PLUMES model. http://www.epa.gov/epa_ceam/wwwhtml/plumes.htm.
US EPA. 1995. LERAM. Littoral Littoral Ecosystem Risk Assessment Model for Prediction of Risk of Chemical Stressors Entering the Aquatic Environment. US EPA, Duluth, MN, USA. (http://www.epa.gov/earth100/records/leram.html)

US EPA. 1996. CORMIX. Cornell Mixing Zone Expert System. Centre for Exposure Assessment Modelling, Athens, GA, USA. http://www.epa.gov/ceampubl/cormix.htm.

US EPA. 1997a. EXAMS. Exposure Analysis Modeling System. US-EPA, Centre for Exposure Assessment Modelling, Athens, GA, USA. http://www.epa.gov/ceampubl/exams.htm.
US EPA. 1997b. MED-Duluth's Ecotoxicology Data Systems. US EPA, http://www.daylight.com/products/databases/Aquire.html.

Utvik TIR, Durell GS, Johnsen S. 1999. Determining produced water originating PAH in North Sea water: Comparison of sampling techniques. *Mar Poll Bull* 38:977-989

Van Leeuwen CJ, Hermans J. 1996. Risk assessment of chemicals. Research Institute Toxicology University, Utrecht, Ed. Kluwer, Dordrecht, Netherlands.

Van Veld PA, Spain JC. 1982. Degradation of selected xenobiotic compounds in three types of aquatic test system. *Chemosphere* 12:641-649.

Varskog A. 2000. Presentation on MERMAID. SETAC Europe, Brighton, 22-25 May 2000. http://www.mm-s.no/

Wakeham SG, Canel EA, Doering PH. 1986. Behaviour of aliphatic hydrocarbons in the coastal seawater. Mesocosm experiments with ^{14C}decane. *Environ Sci Technol* 20:574.

Walker WW, Cripe CR, Pritchard PH, Bourquin AW. 1988. Biological and abiotic degradation of xenobiotic compounds in in vitro estuarine water and sediment/water systems. *Chemosphere* 17:2255-2270.

Wang W. 1992. Use of plants for assessment of environmental contaminants. *Rev Environ Contam Toxicol* 126:87-127.

Wania F. 1995. Bioaccumulation of persistant organic contaminants in the Arctic - speculations, illustrations, and preliminary model calculations. Poster presented at the fifth SETAC Europe Congress June 26-28, 1995, Copenhagen, Denmark.

Ward GS. 1995. Saltwater Tests. In Rand GM, ed, *Fundamentals of aquatic toxicology*, Taylor and Francis, London, UK.

Ward DM, Brock TD. 1978. Hydrocarbon biodegradation in hypersaline environments. *Appl Environ Micro* 35:353-359.

Ward EJ, Winfrey MR. 1985. Interactions between methanogenic and sulphate reducing bacteria in sediments. *Adv Aquatic Microbiology* 3:141.

Wheeler PA, Gosselin M, Sherr E, Thibault D, Kirchman DL, Benner R, Whitledge TE. 1996. Active cycling of organic carbon in the central Arctic ocean. *Nature* 380:697-699.

Whitehouse P, Crane M, Redshaw CJ, Turner C. 1996. Aquatic toxicity tests for the control of effluent discharges in the UK - the influence of test precision. *Ecotoxicology* 5:155-168.

Widdel F. 1985. Sulphate-reducing bacteria and their ecological niches. *Soc Appl Bacteriol Symposium Series* 13:157-184.

Wong CK, Cheung JKY, Chu KH. 1995. Effects of copper on survival, development and growth of *Metapenaeus ensis* larvae and postlarvae (Decapoda: Penaeidae). *Mar Pollut Bull* 31:416-419.

WRc. 1989. Handbook on the design and interpretation of monitoring programmes, ISBN 0 902156 72 l.

WRI - World Resources Institute. 1992. Relative number of described species in major taxa on http://www.wri.org/wri/biodiv.

Xie WH, Shiu WY, Mackay D. 1997. A review of the effects of salts on the solubility of organic compounds in seawater. *Marine Env Res* 44:429-444.

Zaroogian GE, Heltshe JF, Johnson M. 1985a. Estimation of bioconcentration in marine species using structure-activity models. *Environ Toxicol Chem* 4:3-12.

Zaroogian GE, Heltshe JF, Johnson M. 1985b. Estimation of toxicity to marine species with structure-activity models developed to estimate toxicity to freshwater fish. *Aquat Toxicol* 6:251-270.

Zok S, Boutonnet J-C, de Rooij C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998. Euro Chlor risk assessment for the marine environment OSPARCOM Region: North Sea - Chloroform. *Environ Monit Assess* 53:401-424.

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