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SUMMARY

The process of risk assessment of substances aims at safeguarding the integrity of complex environments and ecosystems. In this context, a No-Effect Concentration for environmental organisms needs to be predicted (= PNEC) on the basis of a limited amount of ecological and ecotoxicological data available. Most of the substance-specific data have been generated on single species under laboratory conditions, and empirically-derived assessment factors are currently used for the extrapolation to the real environment. It is the purpose of this report to explore in detail the value of aquatic model ecosystem studies in predicting the effect of substances in the "real world" ecosystem.

The relevant scientific literature was thoroughly screened and the various types of studies found were described separately for the three broad groups of studies, i.e., static freshwater, flowing freshwater and marine systems. Large differences exist among the reported studies concerning the test conditions chosen, particularly location, duration, size and complexity. The Task Force concluded that it seems inappropriate at this stage, to recommend a single standard test design or a set of designs. Each study should be tailored to address the specific issues or data requirements that have arisen from earlier stages of testing.

To enable safe concentrations to be forecast by means of assessment factors backed up by sound scientific data, a two-step procedure was followed:

- prediction from chronic single-species No-Observed-Effect-Concentrations (NOECs) to model ecosystem NOECs;

- prediction from model ecosystem NOECs to field NOECs (=PNECs).

To establish the potential usefulness and the role of model ecosystems in risk assessment, NOECs obtained from well-designed model ecosystem studies were compared with NOECs obtained from laboratory single-species tests on the one hand and with field studies on the other.

A database has been assembled containing high quality published information on the toxicity of substances in ecosystem studies and those from chronic single-species tests. Those ecosystem studies which provided values for both NOECs as well as the corresponding Lowest-Observed-Effect-Concentrations (LOECs) were selected following a critical review of the literature. From a total of 1108 data points only 248 studies fulfilled this criterion. They covered 34 substances. The data from single-species tests were extracted from the ECETOC Aquatic Toxicity (EAT) database, complemented with company and additional literature data of comparable quality.

The ratios between the most sensitive single-species NOECs and the most sensitive multi-species NOECs were compared, irrespective of ecological relevance. This was considered to be a reasonably conservative approach for the derivation of assessment factors. In the evaluation of model ecosystem studies for a particular substance, however, it is necessary to select, from the various endpoints recorded, the lowest one which is ecologically significant. Such evaluations, performed with three substances in this report, also demonstrate the high degree of conservatism of the above assessment factor.

For the prediction from chronic single-species NOECs to model ecosystem NOECs, the median value for the ratios (which ranged from 0.02-77.5 with log-normal distribution) was found to be 1.45 with a 90%ile value of 8.14. This suggests that an assessment factor of about 8 for the extrapolation from the lowest chronic single-species NOEC-value to a NOEC-value in a model ecosystem would be safe.

For the second step a comparison was made between results from model ecosystems and results from field studies. The conclusion was that results from the model ecosystem studies of sufficient complexity could be considered as realistic for the real world situation.

This means that an assessment factor of 8 is equally suitable for the prediction of a safe environmental concentration (PNEC) on the basis of chronic single-species NOECs.

1. INTRODUCTION

The environmental risk assessment of a substance is generally based on a comparison of its Predicted Environmental Concentration (PEC) with the Predicted No-Effect Concentration (PNEC). PNEC values are typically calculated from single-species acute or chronic laboratory toxicity tests using an appropriate assessment factor (US-EPA, 1984; OECD 1992; EEC 1996). It is assumed that where the PEC exceeds the PNEC (i.e., PEC/PNEC >1), there could be a potential for environmental effects. The process allows for, where necessary, a stepwise refinement of both the PEC and/or the PNEC independently from each other (ECETOC, 1993a; EEC, 1996). When the PEC/PNEC ratio exceeds unity and there appears to be a necessity of refining the PNEC, ecosystem studies are considered to be a suitable instrument for the derivation of a more realistic PNEC.

The principal purpose of model ecosystem studies in ecotoxicology is to provide data on the effects, and sometimes also fate, of substances under conditions which are more representative of the 'real world' than single-species laboratory tests. This is expressed in terms of greater realism concerning for example, exposure conditions and in the variety of data that can be collected, such as effects on several taxa examined in the same test. Furthermore, model ecosystem studies allow the examination of effects on endpoints based on functional or structural aspects at the ecosystem level. Thus these studies provide the opportunity to gain further insight into the ecological significance of the effects seen.

To explore in detail the value of the various model ecosystem studies in predicting the effect of substances in the environment, ECETOC established a Task Force with the following Terms of Reference:

- collate and critically evaluate the existing literature on biocoenosis studies;
- describe the techniques involved in biocoenosis studies;
- compare the test results obtained from experiments using biocoenosis and single-species approaches;
- evaluate the value and the consequences of using biocoenosis approaches to ecotoxicology testing.

This report is one of a series of ECETOC Technical Reports published in recent years that deals with the general and specific aspects of environmental risk assessment (ECETOC 1993a; 1993b; 1994a;

1994b; 1996). It considers the value of aquatic model ecosystem studies in the environmental risk assessment process, describing the various types of studies and their uses (Section 3), reviewing the possible extrapolation of results from chronic single-species studies to model ecosystems and from model ecosystems to the 'real world' (Section 4) and finally presents conclusions and recommendations (Section 5).

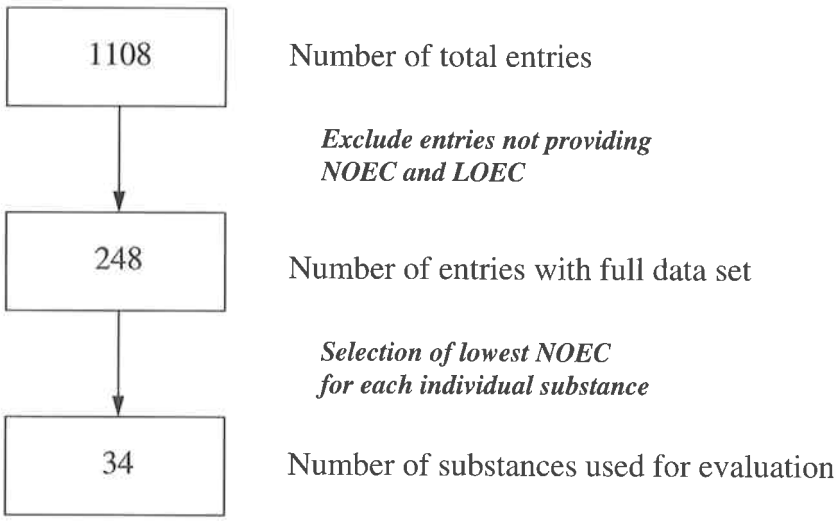
2. BACKGROUND

Various workshops were held and guidance documents were issued within recent years discussing test design and interpretation of results for freshwater model ecosystem studies (SETAC Europe, 1991; SETAC-RESOLVE, 1992; Graney *et al*, 1994; Hill *et al*, 1994). The results of these workshops were considered by the Task Force along with other relevant scientific literature.

The literature on model ecosystem studies was collected by electronic and manual searches. In view of the limited number of suitable terrestrial studies available in the open literature the report has been confined to a review of aquatic studies. The papers on aquatic studies were subdivided into three broad groups, i.e., flowing freshwater, static freshwater and marine systems.

Initially some 1108 literature references (Step 1 in Figure 1) were reviewed. These publications provided the basis for the analysis of the different test designs which have been developed for model ecosystem studies. The results of this analysis are summarised in Section 3 and reported in detail in Appendix A.

Figure 1: Process of Data Selection



For the quantitative analysis of the results of model ecosystem studies with those of single-species tests, however, the studies were in general only considered further if:

- they were well documented, published in peer-reviewed journals or in comprehensive, widely respected reviews;
- the data were supported by adequate chemical analysis;

- they reported both Lowest Observed Effect Concentrations (LOEC) *and* No Observed Effect Concentrations (NOEC);

- they were judged to be scientifically sound in design and execution (expert judgement).

Only 248 of the 1108 single model ecosystem studies fulfilled these criteria and were included in the database. For nine out of the 248 entries no reliable single-species values could be found. The remaining 239 entries comprised 34 different chemicals.

For the 34 chemicals identified above, chronic single-species toxicity NOEC values were extracted either from the ECETOC Aquatic Toxicity (EAT) data base (ECETOC 1993b) which has well-defined quality criteria for data acceptance or from other sources which were individually assessed for quality using criteria broadly in line with those of the EAT data base.

In some cases, where no chronic NOEC values were available, acute LC_{50}/EC_{50} values were taken and a factor of 10 was used to extrapolate from acute to chronic data (see Appendix D for detail). The approach was considered justified because of the generic aspect of this study and the additional conservatism in respect to the calculation of the NOEC ratios.

The relationship between the relative sensitivity of endpoints from single-species chronic toxicity tests, model ecosystem studies and field monitoring has been analysed in order to provide information on the extent to which the results of model ecosystem studies can be used to refine a PNEC derived by applying an assessment factor to the results of single-species tests. Ideally, such a comparison of the data of single-species tests to those of multi-species tests should be performed on the basis of threshold concentrations. Because test design normally does not allow the determination of a precise threshold concentration, comparisons are made on the basis of the NOECs.

Since the cost and effort involved in mounting model ecosystem studies usually limits the number of concentrations employed, dilution factors applied to most ecosystem studies range from 3 to 10 rather than from $\sqrt{2}$ to 2 as usually applied in single-species tests. Consequently the difference between the NOEC and the actual (unknown) threshold is generally greater in ecosystem studies than is the case for single-species tests. This potentially greater internal safety margin may provide for an additional factor of up to 8 in comparison to that of single-species tests. Hence the approach chosen resulted in additional conservatism.

All results refer to the substance itself; in the case of heavy metals the results are calculated on the basis of the cationic species.

A number of terms is used consistently throughout the report: The term *biocoenosis* is defined as an assemblage of organisms (plants, animals and bacteria) inhabiting a single biotope which interact with each other and their abiotic environment. It is synonymous with *community*. An *ecosystem* is defined as a natural unit consisting of a biocoenosis and its abiotic environment interacting to produce a stable system. *Model ecosystem* is part of the (natural) ecosystem comprising the main structural and functional parts of a real-world ecosystem but in a man-made structure. It is the last term which describes best the kind of studies which are reviewed in this report.

3. AQUATIC MODEL ECOSYSTEMS AND THEIR USE

3.1 GENERAL ASPECTS

The principal purpose of model ecosystem studies in ecotoxicology is to provide data on the fate and/or effects of substances under conditions which are more representative of the 'real world' than single-species laboratory tests. This is expressed in terms of greater realism concerning e.g., exposure conditions and in the variety of data that can be collected, e.g., effects on several taxa can be examined in the same test.

The choice of the test system (i.e., type of ecosystem to be used for testing) and the test design (i.e., location, size, duration and biological complexity) must be tailored for each study based on the existing knowledge of the fate and effects of the substance. It is therefore not possible, nor desirable, to define in advance details of the test systems or test design to be used. It is however, possible to indicate a number of more general aspects of test system and test design which should be considered when determining the type of study to be undertaken.

Three broad groups of model ecosystem studies can be identified: static freshwater, flowing freshwater and marine (usually static or with relatively-long replacement times). The variety of model ecosystem studies that have been used to assess the effects of substances within each of these three broad groups is large. Sections 3.2, 3.3 and 3.4 indicate the range and variety of these different model ecosystem studies and a more comprehensive description of them is given in Appendix A.

The principal aim of this report is to assess the value of model ecosystem studies in predicting the *effects* of substances in the environment. However, these studies can also provide useful information on aspects of exposure. Model ecosystem studies will, by their very nature, ensure that exposure is more realistic than in experiments carried out in less-complex systems.

There are various levels of biological organisation at which endpoints can be determined in an ecosystem. They range from effects on cells or organs of a test organism (i.e., sub-individual effects) via effects on individuals and on populations up to effects on community function and structure. The nature of the effects data required can be used to guide the choice of the test system and experimental design. The expectation is that as the size, duration and biological complexity of the test system increases so will the likelihood of detecting effects at the higher levels of biological organisation. Of course, there will be limits beyond which increasing size, duration and complexity do not bring concomitant rewards. In practice very large systems may reduce the chance to detect effects due to the difficulty of controlling variability between replicates as complexity of the test system

and duration of the study increase. As size and duration of the studies increase costs are also likely to rise. This should not be confused, however, with the enhanced predictive and explanatory power of large test systems. Due to their size these systems can accommodate fish and become excellent surrogates for natural systems.

Selection of the appropriate test system and experimental design must be based on a thorough knowledge of the capabilities of the various systems and the data requirements. Maximum value is likely to come from studies where relatively stable ecological communities are established in a replicated form and effects are examined based on population, community functional and structural endpoints. Figures 2, 3 and 4 illustrate the relationships between size, location, duration, type of community and biological complexity for static freshwater, flowing freshwater and marine model ecosystem studies reviewed.

One important element of all model ecosystem studies is the mode of application of the test substance. In general, it is desirable that this should reflect what occurs in the real world in terms of the rate, frequency and nature of application.

General chemicals and metals typically enter natural waters as components of effluent discharges (treated or untreated). Three exposure scenarios can be distinguished:

- short-duration spikes of contamination, for instance caused by accidental releases;
- intermittent contamination for example, by effluents from industrial plants with batch processes;
- continuous discharges, which is the case for most industrial and domestic sewage effluents.

Inland effluents are generally discharged into flowing fresh waters; model stream ecosystems have therefore often been used to examine their potential effects.

Discharges to the marine environment have been studied by both single additions to static systems and by continuous additions to model ecosystems with rather long residence times.

Pesticides under normal conditions of use may enter the aquatic environment by spray-drift or run-off following commercial applications to the land, though in some instances they will be deliberately applied to water (e.g., aquatic herbicides). Commercial applications of pesticides are typically of short duration, a maximum of hours, as single or intermittent events, and seasonal. Static freshwater model ecosystems have been most widely used to study the effects of pesticides.

Where spray-drift is the route of entry to be simulated it is desirable that the frequency and rate of application to the model ecosystem should be representative of commercial use. Typically model ecosystem studies of spray-drift have involved single or repeat "oversprays" of the model ecosystem at application rates extending from the commercial rate to rates that might represent the spray-drift onto a waterbody from an application to a crop some distance away.

Run-off following application of pesticides is a complex phenomenon. The duration and the nature of episodes vary depending upon rates of application, frequency of application, soil conditions, weather etc. In view of this complexity it is not possible to indicate in advance what might be the appropriate method of application of a pesticide in a particular study. Applications may range from single or repeat treatments with a slurry of soil-adsorbed pesticide to a static water model ecosystem (e.g., a pond), representing the result of a run-off event induced by heavy rain after a crop treatment, to a more or less continuous application of low concentrations of dissolved pesticide to a flowing water model ecosystem (e.g., streams) representing an input from tile drains.

When effects on non-target organisms of deliberate applications to aquatic systems are to be assessed it is important that the conditions of the study follow those recommended for commercial applications.

Dosing of the test substance into the model ecosystem should, as far as possible, simulate the 'real world' discharges/releases in terms of concentration, duration and other factors that may be relevant (e.g., presence of suspended solids and other dissolved organic matter).

An important element in correctly applying a substance in any model ecosystem study is to ensure, as far as is practicable, that once in the test system the substance has the same bioavailability as it would have in the real world.

Bioavailability can be influenced by many factors for example, water quality (e.g., pH, concentrations of suspended solids and dissolved organic matter) and the possible routes of uptake (e.g., via the water only, or via food and water). Many substances enter fresh and marine waters via waste water treatment plant effluents. In these situations tests should ideally be carried out under conditions that simulate the presence of the test substance in a treated effluent. However, in practice this can be difficult or impossible.

Model ecosystem studies will by their nature ensure that exposure is more realistic than will have been the case in experiments carried out in less-complex systems, but positive efforts should be made to maximise the realism of exposure. This enhanced realism, for example, in a plankton study should mean that exposure is carried out in the presence of realistic concentrations of dissolved organic