PROFILE

Ecotoxicological Effects Assessment in the Netherlands: Recent Developments

KEES VAN LEEUWEN
Directorate-General for Environmental Protection
Chemical Substances Division
P.O. Box 450, 2260 MB Leidschendam, The Netherlands

ABSTRACT / In a recently published annex to the National Environmental Policy Plan of the Netherlands (1989), attention was paid to ecotoxicological effects assessment. The proposed procedure was based on the advice of the Health Council of the Netherlands (1989) on risk assessment of toxic chemicals for ecosystems. The various extrapolation methods described by the Health Council are critically discussed in this paper. The extrapolation method of Van Straalen and Denneman (1989) is evaluated for eight chemicals and 11 aquatic species. Conclusions are drawn about the quality and quantity of the ecotoxicological data needed for aquatic effects assessment. For the soil—a compartment that is often at risk—ecotoxicological effects assessment is not possible because suitable ecotoxicological test methods still have to be developed.

Environmental risk assessment is becoming an increasingly important issue, particularly in view of the large numbers of pollutants that are potentially harmful to the functioning of ecosystems. Lessons from the recent past of several "do-nothing decades" have taught us that prevention of aquatic and terrestrial (including groundwater) pollution is much cheaper than cleanup. The high costs involved in cleanup operations of, e.g., polluted soils, aquatic sediments, or dump sites are painful reminders of the recent past in which dilution, adsorption, or leaching were used as an excuse for not taking preventive measures. This "out-of-sight–out-of-mind policy" has led to a grave deterioration of our environment—of our common future (Brundtland 1987)—for in many places restoration is out of the question, whatever our financial possibilities.

The risk assessment methodologies and management philosophies for the protection of human and environmental health in the Netherlands have been published recently (Premises for Risk Management 1989). In the Netherlands the objective for the so-called general environmental quality is to offer protection to 95% of the species in ecosystems. This percentage has been arbitrarily chosen and implies that 5% of the species may suffer detrimental effects. Furthermore, it is assumed that protecting the structure (the qualitative and quantitative distribution of species) will also safeguard the functional characteristics of ecosystems. In nature reserves the preservation of ecosystems and species play a key role. It therefore means that these *general* risk limits for ecosystems might not offer sufficient protection and that more stringent limits could be needed to protect certain species. In drawing up the policy for chemicals, no account has yet been taken of these *special* requirements and characteristics of ecosystems (Premises for Risk Management 1989). It is the intention of the Minister for the Environment in the Netherlands to make optimum use of the still limited knowledge of ecotoxicology (including the extrapolation procedures) for the protection of ecosystems against chemical pollution (Health Council of the Netherlands, 1989), among other things, to achieve a policy that is founded on sustainable development (National Environmental Policy Plan 1989).

Environmental hazard assessment is a two-component exercise involving separate exposure (PEC) and effect (NEC) determinations (Van De Meent 1989) (Figure 1). In order to make predictions about environmental concentrations, models are applied. The various models have recently been summarized by the OECD (1989a). Effects assessment is the process whereby "acceptable" toxicant levels in ecosystems are estimated on the basis of laboratory determined "no observed effect concentrations" (NOECs), $L(E)C_{50}$ values, or QSAR estimates (quantitative structure–activity relationships; cf. Hermens 1989) of acute toxicity (USEPA 1984). These "acceptable" toxicant levels are environmental concentrations below which certain adverse effects on species in ecosystems (e.g., survival, reproduction, and growth) are unlikely to occur (see Table 1 for definitions). These "acceptable" concentrations are obtained by applying safety factors (also called margins of safety, application factors, extrapolation factors, or assessment factors) on the laboratory toxicity data, usually in the range of 10 to 10,000 (OECD 1989b).

The various stages that can be distinguished in hazard assessment are initial (preliminary), interme-

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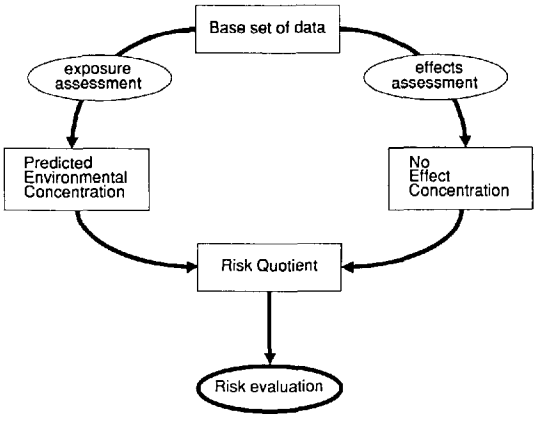

Figure 1. Systematic procedure for environmental hazard assessment through estimation of exposure and effect concentrations.

Table 1. Meaning of terms commonly used in the field of hazard and risk assessment (OECD, 1989b)

Effects assessment: The identification and quantification of the potential adverse effects of chemicals on individuals, populations, or ecosystems by means of laboratory testing or field observations (examples of endpoints: dose causing death, reproductive failure, or reduction of species diversity).

Exposure assessment: Estimation of the exposure of target organisms resulting from the release, transport, and fate of a chemical in the environment (examples of endpoints: environmental concentrations, intake per unit of body weight).

Hazard assessment: Integration of the effect and exposure assessment to determine the probable nature and magnitude of the hazard resulting from the release of a chemical into the environment (examples of endpoints: comparison of predicted environmental concentration with no-effect concentration).

Risk assessment: Quantitative or semiquantitative estimation of the probability of clearly defined environmental effects occurring as a result of the exposure to a chemical (examples of endpoints: estimates of the probability of reductions in population numbers).

diate (refined), and comprehensive hazard assessment (Table 2). Initial hazard assessment is generally based on acute or few chronic effects data. (Semi)chronic ecotoxicological tests are the basis for intermediate hazard assessment, whereas (semi)field studies provide the basis for comprehensive hazard assessment. The different tiers of tests resulting in rough to precise estimates of PECs and NOECs make hazard assessment an iterative process.

The rationale for extrapolation is that if no safety factors are applied, large parts of ecosystems will remain unprotected, as standard laboratory tests cover

Table 2. Stages in hazard assessment

Stage	Required effect data
Initial	acute toxicity tests
Intermediate	(semi)chronic toxicity tests
Comprehensive	(semi)field studies

only a minor part of the variety of responses that may actually occur in ecosystems. This has been substantiated by Slooff (1985). For 25–30% of the chemicals tested in acute as well as chronic aquatic toxicity experiments with a limited number of different species, the standard set of test species (one alga, one crustacean, and one fish) failed to cover the toxicity levels found for the few other species tested within one order of magnitude. Because only a few species were tested and many species cannot be kept or cultured under laboratory conditions, the actual percentage of unprotected species in ecosystems will be even higher. Another conclusion drawn in this study is that the relative susceptibility of species depends on the type of chemical studied. This means that the concept of "the most sensitive species" is really a myth.

Safety factors also are applied to the results of toxicity studies with white laboratory rats for the protection of human health. A safety factor of 100 is generally put on the no effect level (NEL) observed in a chronic feeding study with rats to derive a NEL for man for noncarcinogenic substances. However, protection of human health is relatively easy, as extrapolation only takes place from one mammalian species to another. The general tendency in both mammalian and ecotoxicological extrapolation is for lower extrapolation factors as the number and quality of data on different toxicological endpoints increase.

The various aspects of environmental hazard assessment, including a critical discussion of the various extrapolation methods, have recently been described in a report by the Health Council of the Netherlands (1989). Before going into a short description of the various ecotoxicological extrapolation methods described in the report of the Health Council, a few statements are necessary.

1. What species and functions of ecosystems are to be protected, and at what levels, is largely a political choice. Ecotoxicological effects assessment comprises both the definition of unacceptable effects and the determination of the concentration of a certain chemical that induces these effects. The definition of unacceptable effects is closely linked to that of important species (or processes) to be protected from these effects. It is recognized that the terms "unacceptable" and "important" imply value judgements (Stephan 1986) and

therefore often lead to much debate. Stephan (1986) has given seven major unacceptable direct or indirect effects that pollutants can have on important species: (1) unacceptable reduction in survival, (2) unacceptable reduction in growth, (3) unacceptable reduction in reproduction, (4) unacceptable level of avoidance, (5) unacceptable percentage of gross deformities or visible tumors in organisms, (6) unacceptable concentrations of toxic residues in consumed tissues, and (7) unacceptable flavor in consumed tissues.

Species can be selected on the basis of their ecological function (trophic level), their morphological structure, and their route of exposure (Health Council of the Netherlands 1989). Social, economic, and recreational factors also may play an important role, as illustrated by the ecological objective for the river Rhine, "the return of the salmon before the year 2000," one of the results of the ministers' conference on the pollution of the Rhine, which was held in Rotterdam in 1986 after the Sandoz calamity. It is important to note that the return of the salmon can only be approached as a broad ecological objective, for the salmon cannot be placed outside an integral ecological context; in other words, for the return of the salmon, great parts of the ecosystems of the Rhine have to be restored.

Because ecosystems can tolerate some stress and occasional adverse effects, protection of all species at all times and places is not deemed necessary. With data available for a large number of appropriate taxa from an appropriate variety of taxonomic and functional groups, a reasonable level of protection can probably be provided if all except a small fraction of the taxa are protected (Stephan and others 1985). In the Netherlands the maximum permissible level of a chemical for the so-called general environmental quality is reached if the concentration of a chemical equals that at which 95% of the species in an ecosystem is protected. The negligible level is defined as 1% of this upper limit (Premises for Risk Management 1989).

2. *The general trend in risk assessment is to generate more information from less testing* (Dobson 1988). The assessment of toxic effects on ecosystems implies exposure of complex biological systems, with their great varieties of species and exposure routes, and direct and indirect relations between species. The toxicity of compounds in these systems depends greatly on life-stage (Van Leeuwen and others 1985), feeding conditions (Kooyman and others 1983), other stress factors (e.g., competition), animal behavior, the presence of other compounds (Deneer 1988; Van Leeuwen and others 1987a), and many other physicochemical and biological factors.

A glance at any handbook on ecology or animal or plant taxonomy tells us that current standard ecotoxicological testing with fish, *Daphnia*, and algae presents nothing more than a caricature of what ecosystems really are. The actual situation is even worse: for at least 95% of all existing chemicals short-term toxicity data on these "representatives" of several important ecological functions are not available (for regulatory agencies).

Furthermore, we should bear in mind that for the aquatic environment the situation is far better than for the terrestrial environment, as politicians and ecotoxicologists have only recently "discovered" the terrestrial environment. At this moment ecotoxicological effects assessment for the soil—an environmental compartment that is often at risk—is hardly possible as an adequate number of soil ecotoxicity test guidelines is not available (cf. OECD 1989b). This is one reason why an integrated soil research program (ISRP 1989) is carried out in the Netherlands.

3. *In general, effects assessment for chemicals on the basis of field tests is not feasible.* It is often suggested that field studies may provide the ultimate answer in effects assessment. This view has been put forward in a number of articles by Cairns (e.g., 1986a,b) and others. Field studies are needed for various reasons (Crossland and others 1986, La Point and Perry 1989), but their role in the management of chemicals should not be overestimated; much depends on the questions that need answering. Field tests can only provide clear answers to clear questions, and very often these questions cannot be formuated clearly, simply because very limited standardized physicochemical and ecotoxicological data are available. This means that research efforts will only be cost effective and useful for decision making if the entire process of hazard assessment is conducted in the framework of a sound scientific approach (Table 2).

Standardization (harmonization) of field studies is virtually impossible because the type of field study to be done depends on the question to be answered and may differ from chemical to chemical, from application to application, and from site to site. The need for standardization even becomes doubtful when we consider the loss of ecological and environmental reality it would imply.

Many discrepancies between laboratory studies and field studies are due not only to various ecological reasons but also arise from a lack of knowledge on the actual exposure concentrations in the field situation (La Point and Perry 1989). Knowledge on the physicochemical behavior of chemicals in ecosystems (niche partitioning) is indispensable in field studies (Cross-

land 1986). As one field study cannot be representative for all ecosystems, the application of extrapolation factors remains necessary. In this respect, multispecies or field tests do *not* differ from standardized single-species tests.

Current research on the scope of refined or ultimate hazard assessment should therefore focus on developing methods of greater (geo)chemical and ecological realism if we want to close the gap between laboratory studies and the actual situation in the field. A first step for closing this gap would be to increase our knowledge considerably on the physicochemical behavior of chemicals by performing, e.g., realistic adsorption and degradation studies, and further to extend the number of ecotoxicological laboratory tests, i.e., to cover a broader range of taxa for effects assessment of chemicals beyond green algae, crustaceans, and fish, as has been suggested by the US EPA (Stephan and others 1985) and the Health Council of the Netherlands (1989). The US EPA requires tests on at least eight different taxa in order to derive quality criteria for the aquatic environment (Table 3).

Second, complex field studies should be preceded by toxicity studies at the population level under more or less realistic conditions, because it is impossible to explain toxicological effects on ecosystems in terms of causal relationships unless we understand the physicochemical behavior of chemicals and the dynamics of at least a number of key species and processes (cf., Kooyman and Metz 1984, Van Leeuwen and others 1987b).

Apart from the problems associated with the harmonization, costs, and extrapolation of field studies, effects in the field are difficult to interpret for the following reasons:

A. Many of the effects observed in the field do not necessarily originate from toxicological stress. Toxicity is only one of the many possible physical, (physico)chemical, or biological causes of effects that may be detected in the environment.

B. The primary test is the "so what!" test. As cause and effect are further removed or clouded by complex interactions, it is impossible to address the "so what!" question in a manner which is not undercut by social, political, or economic reality (Herricks and Schaeffer, 1984).

C. In view of the laboriousness of monitoring all species in a community, only lumped variables in multispecies tests are normally observed, and many effects will thereby escape notice (Cairns 1986b, Kooyman 1985, Nienhuis and Scholten 1989).

D. If any effects are, in fact, found in the variables observed, there is the problem of disentangling them from the scatter or of avoiding the error of the second kind in the statistical analysis of the results. Again, the labor involved in conducting field test prevents an adequate number of (independent) duplicates being run at the same time (Kooyman 1985, La Point and Perry 1989, Crossland and Wolff 1988). In addition, consecutive observations on a single experimental unit are highly interdependent. The complexity of the underlying processes makes it hard to take this dependence into account in the statistical analysis. Furthermore, experimental communities tend to diverge rapidly in their development, so that only coarser kinds of acute effects stand a reasonable chance of being detected (Kooyman 1985).

E. Stress in multispecies tests usually decreases rapidly after inoculation, because the toxic chemical is (bi-

Table 3. Required information to derive a criterion for freshwater aquatic organisms and their uses (Stephan and others, 1985)

1. Results of acceptable acute tests (see Section IV[a]) with at least one species of freshwater animal in at least eight different families such that all of the following are included:
   a. the family Salmonidae in the class Osteichthyes
   b. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
   c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
   d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)
   e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
   f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
   g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
   h. a family in any order of insect or any phylum not already represented.
2. Acute–chronic ratios (see Section VI[a]) with species of aquatic animals in at least three different families provided that of the three species:
   a. at least one is fish
   b. at least one is an invertebrate
   c. at least one is an acutely sensitive freshwater species (the other two may be saltwater species).
3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII[a]). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.
4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX[a]).

[a]Reference is made to the report of Stephan and others (1985).

ologically) degraded or becomes otherwise less available. In single-species tests, the level of stress is usually kept fixed by continuous or intermittent replacement of the test media. Supplying a continuous dose in a multispecies test resolves the problem only partially (Kooyman 1985, Crossland 1986).

F. There may be processes of adaptation or selection of resistant individuals. The quantitative importance of such processes is hard to assess and interpret. Individuals that survive because of their resistance to one chemical may be more vulnerable to another (Kooyman 1985).

4. Extrapolation or more testing? A difficult position for industry. The general trend in risk assessment systems for pollutants as observed by Dobson (1988), that more and more information is extracted from less testing, is a fact. The present position of industry is uncomfortable. Their general approach is to do no more testing as is strictly required, and I fully agree with this point of view. This businesslike approach holds especially for environmental matters and is motivated by economic reasons and other reasons, as well as by the fact that the environment cannot "claim" toxicological damage. In addition, environmental damage is difficult to quantify, although the current practice in the Netherlands of passing the costs of particular soil cleanup operations on to the industries involved is rapidly changing this point of view. In fact, the present situation would imply or at least suggest that the physicochemical and (eco)toxicological information provided derived from only few tests suffices for most management decisions.

On the other hand, great problems may arise when, on the basis of the limited information and the application of extrapolation factors, strong recommendations are made to reduce or even ban the use of certain products, as is the case with fabric softeners (cationic surfactants) in the Netherlands. The Dutch annual use of these chemicals is large (2000 tons expressed as active ingredient). The chemicals appeared to be relatively persistent and quite ecotoxic (similar chemicals are used as industrial pesticides, i.e., disinfectants) (Van Leeuwen 1989). The confrontation of industry with these conclusions, which were based on a limited number of data and on the application of a very reasonable extrapolation factor (a factor 13 on the lowest NOEC value of tests with one fish, one daphnid, and two algal species), rapidly changed this cost-effective view: ecosystem studies had to be performed before any measure could be taken!

From a management point of view it is difficult to live with this attitude of sitting and waiting for the ultimate truth. It will only encourage further delays in the management of chemicals; in other words when can research be ended and decision making begin? The answer is: not until an agreement is reached on extrapolation procedures or on a simple set of extrapolation factors, preferably in an international context. This is the only way to come to an objective, mutually acceptable way of effects assessment for ecosystems. But that is not all. The battery of ecotoxicity tests both for the aquatic and terrestrial environment must be extended by a number of species in order to improve the predictability of effects assessment on the basis of toxicity tests. Recommendations of this kind have been made by both the US EPA (Stephan and others 1985) and the OECD (1989b). The development of test methods is a task for the environmental protection agencies from both industry and government. The report of the Health Council of the Netherlands (1989) is not intended as a recipe but as a step for further discussions on ecotoxicological hazard assessment.

Assessing Risk of Toxic Chemicals for Ecosystems: Advice of the Health Council of the Netherlands

In the report of the Health Council of the Netherlands several extrapolation procedures are discussed. Here, attention will be paid only to several important aspects and methods.

Species-to-Species Variation in Sensitivity

In the methods of Kooyman (1987), Van Straalen and Denneman (1989) (here called the Van Straalen method) and the method of the US EPA (Stephan and others 1985, Erickson and Stephan 1984) (here called the EPA method) assumptions are made about the distribution of the sensitivity of different species to toxicants (*inter*species variation). In all these methods it is assumed that interspecies variation in sensitivity follows a symmetrical distribution (Figure 2). On the basis of experimental toxicity data, Kooyman (1987) has shown that this is a realistic assumption.

The most general assumption for this distribution resembles the normal distribution (Figure 2). This distribution is similar to the one taken for the variation of sensitivities within a species (*intra*species variation). It is the basis of many methods for the calculation of LC_{50} and EC_{50} values from toxicity tests (Finney 1971).

The extrapolation methods of both Kooyman and Van Straalen are based on the assumption that the LC_{50} and NOEC values for both the test species and for the species in the community can be conceived of as independent random trials from a log-logistic distribution. This assumption is equivalent to the generally

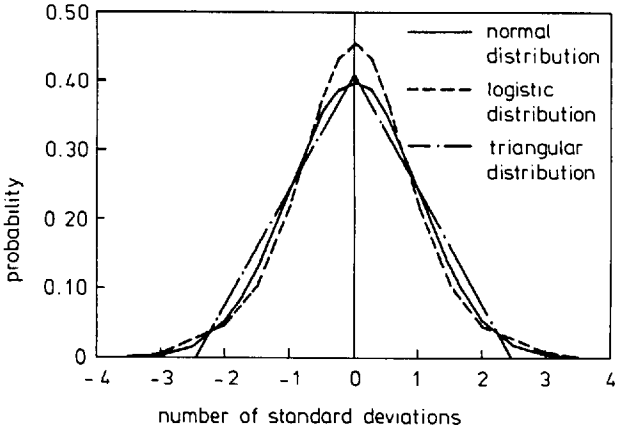

Figure 2. Probability density functions with a mean and standard deviation of 0 and 1, respectively (Health Council of the Netherlands 1989).

accepted function for intraspecies variation in sensitivity, i.e., the basic assumption in many LD_{50} and $L(E)C_{50}$ estimation programs. This distribution is very similar to that of the normal probability distribution (Figure 2), but the logistic distribution has some practical advantages as computations are simpler than those based on the normal distribution (Hewlett and Plackett 1979).

In the EPA method it is assumed that species sensitivities can be described by a triangular distribution (Figure 2). This distribution is as arbitrary as is the choice of a log-logistic distribution, but it implies the assumption that there is a threshold value below which effects would not occur. In fact, the triangular distribution offers the possibility of calculating a 100% protection level. It is the opinion of the Committee of the Health Council of the Netherlands that as long as it is not clear whether scientific threshold values for chemicals can be given, the assumption that such threshold values exist is too rigorous. The method of the EPA may only be applied if minimal requirements on quality and quantity of (partly specified) research data are met (Table 3). These minimum requirements will be discussed later.

In the methods of Slooff and others (1986) and Blanck (1984), no assumptions are made on the nature of the distribution function. The Blanck method contains the far-reaching assumption that the range of sensitivities in all species is the same as that of the few species analyzed by the author. Because of its serious objections to the EPA method and the Blanck method, the Committee of the Health Council of the Netherlands has advised against using these methods. In this paper no further attention will be given to these two methods.

Log-logistic Distribution

The distribution of LC_{50} and NOEC values depends on various factors. Ideally, it would only depend on differences in sensitivity between species, partly caused by differences in accumulation and metabolism. Additionally, differences in toxic effects of a chemical may have their influence. This holds especially for chemicals with a very specific mode of toxic action, e.g., neurotoxic chemicals and herbicides. Furthermore, the observations upon which the choice for a certain distribution is based are influenced by factors such as life-stage, experimental errors, speciation, and adsorption to solids or suspended matter (differences between the test media) that could change the biological availability of a chemical and therefore the $L(E)C_{50}$ and NOEC values. These differences contribute to the variation in the measured ecotoxicological parameters. It means that in the normal practice of ecotoxicology the assumption of a log-logistic distribution of sensitivity between species may often be violated.

Another factor is that many LC_{50} tests do not fulfill the requirement of chronic toxicity. Kooyman's method is based on chronic LC_{50} values, whereas in practice often only acute LC_{50} values are available. On the same grounds, the choice of a log-logistic distribution of NOEC values is debatable in the Van Straalen method too and perhaps even more, as in chronic tests not one but different endpoints of toxicity are expressed as NOEC values (e.g., effects on survival, growth, reproduction, population growth, hatching, behavior, etc.). Despite all these remarks, the log-logistic probability distribution seems to be a realistic assumption (Health Council of the Netherlands 1989), for which experimental evidence has been given (Kooyman 1987).

Extrapolation Method of Slooff

The method of Slooff and others (1986) is based on linear regression analyses between three sets of data: acute toxicity, chronic toxicity, and (semi)field toxicity. The equations have been calculated to predict chronic toxicity on the basis of acute toxicity data and to predict "safe" concentrations for ecosystems on the basis of acute or chonic data derived from single-species tests. The empirical relations are not supported by theoretical explanations, and the method is not based on an explicit principle with respect to the protection of ecosystems.

One criticism is that the method is only applicable to chemicals that have a pattern of toxicological action similar to the chemicals for which these equations were calculated. Another criticism is that the lowest acute or chronic value is used for the calculation of the NOEC

for ecosystems. If toxicity data of more species become available, the probability increases of finding lower acute or chronic L(E)C_{50} or NOEC values. These then will be used for extrapolation. The uncertainty value in this extrapolation method is a constant and does not depend on the number of data available. This is very unreasonable as uncertainty generally decreases as more information becomes available. Because of these and several other reasons, the Slooff method is not considered to be a suitable extrapolation method.

Methods of Kooyman and Van Straalen

These methods are founded on statistical principles and requirements. In both methods it is assumed that the LC_{50} and NOEC values derived from single-species tests, and for the species present in the observed community, may be conceived as mutually independent random trials from a log-logistic probability distribution. Each species tested is not representing any other species but is, in fact, one estimate of sensitivity. With several of such estimates, the overall range of sensitivity for all species then can be determined. This is, in reality, the objective in ecological effects assessment.

The principle of the method of Kooyman (1987) is that all species must be protected to the extent that with great probability the LC_{50} of the most sensitive species is not exceeded. In the Kooyman method this concentration is calculated from the geometrical mean LC_{50} value of a number of species divided by an application factor. This so-called hazardous concentration for sensitive species (HCS) is the concentration at which the LC_{50} of the most sensitive species present in the observed community exceeds that concentration by a specified probability. The HCS increases (up to a certain limit) with an increasing number of toxicity data; it decreases with an increasing number of species in the community. The method was developed to set priorities in scientific research and not as a ready-made recipe for the protection of ecosystems.

The Van Straalen method (Van Straalen and Denneman 1989) was originally developed for the assessment of the effects of chemicals in the terrestrial environment and was derived from the procedure developed by Kooyman (1987). Because of its general concept, the method is applicable to other environmental compartments than soil if analogous assumptions are used. The method estimates the HCp, i.e. a hazardous concentration for p percent of species in a community. The method offers the possibility of choosing different protection levels. In Van Straalen and Denneman (1989) as well as in Premises for Risk Management (1989), this percentage p has been arbitrarily set to 5, i.e. the HC5 is defined as the concentration at which 5% of the species in the community may be adversely affected in the sense that the NOEC is exceeded for 5% of the species. The HC5 is the quotient of the geometric mean of a number of chronic NOEC values and the application factor. For calculations with the Van Straalen method, the Health Council of the Netherlands (1989) recommended that at least three standard chronic tests have to be carried out. The species used in these standard tests must be chosen on the basis of their ecological function (trophic level), their anatomical design (morphology) and their route of exposure.

Apart from the assumption of a log-logistic distribution of sensitivities of species in both the Kooyman and Van Straalen method, which has already been discussed, several other remarks can be made:

1. In both methods interactions between species are not taken into account.
2. The toxicity data used are exclusively based on values obtained by laboratory research.
3. Both methods are used to derive "safe" concentrations for single compounds. No attention is given to exposure to mixtures of chemicals. This also holds for the other methods.
4. The Kooyman method is somewhat arbitrary because the results depend on the number of species (N) in the community. The Van Straalen method estimates a 95% protection level for all species.
5. The assumption made in the Van Straalen method that an ecosystem is protected if the NOEC is exceeded for only 5% of the species is no well founded and is arbitrary. From an ecological point of view, it should be added that ecosystems will not be protected if this 5% contains key species.
6. In the Van Straalen method NOECs are used. NOECs are ecologically more relevant but the estimation of NOECs is still underdeveloped compared with LC_{50} tests. In addition, a greater variation is expected in NOECs than in LC_{50} values.
7. The nature of the chosen distribution function greatly influences the results of the method. This holds especially for the Kooyman method because the HCS largely depends on the behavior of the distribution function in the tails. The results of the Van Straalen method are less dependent on the behavior of the distribution function in the tails.
8. The principle of the Kooyman method, the chosen level of protection (protection of the most sensitive species) and, especially, the very strong influence of the variance of the data, in the sense

that a larger variance leads to a larger application factor (see comments under point 2 and Appendix E in the Advice of the Health Council), often lead to very low and unrealistic values.

9. Both the Kooyman and Van Straalen methods can be improved statistically in order to obtain realistic error estimates in HCS and HCp values. At the moment this work is carried out at the National Institute of Public Health and Environmental Hygiene (Aldenberg, personal communication).

In conclusion it can be stated that it is very easy to criticize the Van Straalen method on several aspects. Nevertheless, it seems to be the most realistic extrapolation method available today. Calculations can be done easily, protection levels can be chosen arbitrarily, and realistic figures are obtained. However, the Health Council urged the need for further evaluation of this and other methods. A part of this evaluation is presented below.

Follow-up of Advice of the Health Council: Further Evaluation of the Van Straalen Method

Methodology

In the Van Straalen method (Van Straalen and Denneman 1989) the *HCp* is calculated from the geometric mean of a number of chronic NOEC values by:

$$Hcp = \frac{\exp(x_m)}{T}$$

$$T = \exp\left[\frac{3d_m S_m}{\pi^2} \ln \frac{1 - \delta_1}{\delta_1}\right]$$

where *HCp* is the hazardous concentration for percentage of the species; p is the percentage of species not protected by the *HCp* value; X_m is the sample mean of ln NOEC values for m test species; S_m is the sample standard deviation of ln NOEC values for m test species; m is the number of test species; δ_1 is the fraction of the ecosystem that is not protected (recommended value $\delta_1 = 0.05$); δ_2 is the probability of overestimating the *HCp* (recommended value 0.05); d_m is the value such that the probability of $(S_m > d_m) = \delta_2$; and T is the application factor between *HCp* and $\exp(X_m)$.

The method is evaluated by means of two data sets consisting of results of (semi)chronic and acute toxicity tests with 11 aquatic species and eight chemicals. No comparisons are made with field studies as adequate data from field experiments were not available for these chemicals.

Chemicals

(Semi)chronic NOEC values were obtained from Slooff and Canton (1983) for the following eight compounds: potassium bichromate ($K_2Cr_2O_7$), sodium bromide (NaBr), tetrapropylene benzene sulfonate (TPBS), 2,4-dichloroaniline (2,4-DCA), *p*-nitrotoluene (*p*-NT), dinitro-*o*-cresol (DNOC), dimethoate, and pentachlorophenol (PCP).

Test Species

The (semi)chronic tests were carred out on 11 species: bacteria (B) (*Pseudomonas fluorescens* and *Microcystis aeruginosa*), algae (A) (*Scenedesmus pannonicus*), plants (P) (*Lemna minor*), crustaceans (D) (*Daphnia magna*), insects (I) (*Culex pipiens*), hydrozoans (H) (*Hydra oligactis*), mollusks (M) (*Lymnaea stagnalis*), viviparous and oviparous fish (F) (*Poecilia reticulata* and *Oryzias latipes*), and amphibians (Am) (*Xenopus laevis*). Criteria were survival, growth, and reproduction; also 48-h and 96-h ($L(E)C_{50}$ values were used, with growth and mortality as criteria. Part of these data were derived from Adema and others (1981), Canton and others (1980, 1983, 1985), and Slooff and others (1983). Other data were derived from the range-finding tests for the (semi)chronic toxicity experiments. These data are given in Tables 4 and 5.

Calculations

For the calculation of *HCp* values the lowest (semi)chronic NOEC values were used. Two factors were varied: (1) the level of protection; for the calculation 90, 95, 97.5, and 99% were chosen as the species protection levels by varying δ_1, and (2) the number of test species used for the calculation of the *HCp*. For the computation of *HCp* three, five, seven, nine, and 11 test species were used. *S. pannonicus, D. magna,* and *O. latipes* were chosen as the primary set of standard organisms, since OECD test guidelines are available for these species. For the calculation of *HCp* values with five test species, this standard set was complemented with *L. stagnalis* and *C. pipiens*. These species were chosen because this combination yielded the most realistic *HCp* values for the eight test compounds. For the calculation with seven test species, the test battery was complemented with *L. minor* and *M. aeruginosa*. For the calculation with nine species representatives of all taxonomic groups were used.

Results

The results are given in Table 6 and Figure 3. The general trend is that higher *HCp* values for ecosystems are obtained with more information and lower protec-

Table 4. (Semi)chronic no-observed-effect concentrations (mg/liter) of eight compounds[a] for 11 different test species[b] (derived from Slooff and Canton 1983)

Test species		Criteria	Test compounds							
			$K_2Cr_2O_7$	NaBr	TPBS	2,4-DCA	*p*-NT	DNOC	Dimethoate	PCP
B	*P. fluorescens*	specific growth rate	0.32	3,200	32	10	10	10	320	1
B	*M. aeruginosa*	specific growth rate	1	3,200	32	1	3.2	3.2	32	1
A	*S. pannonicus*	growth (biomass)	0.32	3,200	1	3.2	10	10	100	0.1
P	*L. minor*	specific growth rate	0.32	3,200	1	1	10	0.32	32	1
D	*D. magna*	mortality	0.1	3,200	10	0.032	3.2	1	0.032	0.1
D		reproduction	0.1	10	3.2	0.032	1	1	0.1	0.1
I	*C. pipiens*	mortality	3.2	100	10	10	3.2	10	0.32	3.2
I		development	3.2	100	10	10	3.2	10	0.32	3.2
H	*H. oligactis*	specific growth rate	3.2	1,000	1	3.2	10	0.32	100	0.032
M	*L. stagnalis*	mortality	10	3,200	3.2	3.2	10	1	32	0.1
M		reproduction	0.32	10	0.32	1	0.32	0.032	10	0.01
M		hatching	1	3,200	3.2	3.2	10	1	32	0.0032
F	*P. reticulata*	mortality	10	100	10	3.2	10	1	32	0.32
F		mortality + behavior	10	32	10	1	10	1	0.1	0.32
F		growth	10	320	10	1	10	1	10	0.1
F	*O. latipes*	mortality	10	3,200	3.2	0.32	1	0.1	0.32	0.032
F		mortality + behavior	10	320	3.2	0.32	1	0.1	0.32	0.032
F		hatching + growth	100	10,000	10	3.2	32	1	100	0.32
Am	*X. laevis*	mortality	1	32	3.2	1	10	0.32	1	0.032
Am		development	3.2	320	10	0.32	3.2	0.32	32	0.032
Am		growth	3.2	320	10	1	32	0.32	32	0.032

[a]Potassium bichromate ($k_2Cr_2O_7$), sodium bromide (NaBr), tetrapropylene benzene sulfonate (TPBS), 2,4-dichloroaniline (2,4-DCA), *p*-nitrotoluene (*p*-NT), dinitro-*o*-cresol (DNOC) and pentachlorophenol (PCP).

[b]Bacteria (B) (*Pseudomonas fluorescens* and *Microcystis aeruginosa*), algae (A) (*Scenedesmus pannonicus*), plants (P) (*Lemna minor*), crustaceans (D) (*Daphnia magna*), insects (I) (*Culex pipiens*), hydrozoans (H) (*Hydra oligactis*), mollusks (M) (*Lymnaea stagnalis*), viviparous and oviparous fish (F) (*Poecilia reticulata* and *Oryzias latipes*), and amphibians (Am) (*Xenopus laevis*).

Table 5. Acute EC_{50} (population growth or immobility) and LC_{50} values (mg/liter) of eight compounds for 11 species[a]

Test species		Test compounds							
		$K_2Cr_2O_7$	NaBr	TPBS	2,4-DCA	*p*-NT	DNOC	Dimethoate	PCP
B	*P. fluorescens*	—[b]	—	—	—	—	—	—	—
B	*M. aeruginosa*	2.2	6,500[c]	61	0.69	22[c]	37[c]	400[c]	20[c]
A	*S. pannonicus*	2.6[c]	10,000	42[c]	11[c]	15	34[c]	470[c]	0.47[c]
P	*L. minor*	1.6[c]	11,000[c]	20[c]	44[c]	51[c]	2.5[c]	1,900[c]	7.5[c]
D	*D. magna*	1	11,000	17	1.3	7.5	3.4	2.9	0.48
I	*C. pipiens*	—	—	—	—	—	—	—	34
H	*H. oligactis*	66[c]	5,600	11[c]	—	23	4.4[c]	—	0.73
M	*L. stagnalis*	33	—	26	23	21	7.4	—	0.56
F	*P. reticulata*	230	16,000	24	22	49	1.8	560	0.85
F	*O. latipes*	140	24,000	16	20	51	3.5	108	1.1
Am	*X. laevis*	120	—	24	17.8	15	—	—	0.26

[a]See Table 4 for abbreviations. Source: data were derived from Adema and others (1981), Canton and others (1980, 1983, 1985) and Slooff and others (1983).

[b]No data available.

[c]Unpublished data from the National Institute of Public Health and Environmental Hygiene.

tion levels. From these data it also appears that stringent *HCp* values (based on a 95% protection level, i.e., HC5 values) are obtained in cases where few test data are available. For example, when a standard set of three species is used, the HC5 differs by an order of 2.5 to 1200 from HC5 values based on seven species or more. This holds for all compounds, except TPBS and PCP. The differences are less pronounced when extrapolations are based on five or seven species.

Ratios were calculated with acute and chronic tox-

Table 6. Hazardous concentrations (mg/liter) for 5% of species in a community (HC5) calculated according to Van Straalen and Denneman (1989) for *m* species

Parameter	Test compounds							
	$K_2Cr_2O_7$	NaBr	TPBS	2,4-DCA	*p*-NT	DNOC	Dimethoate	PCP
HC5 (m = 3)[a]	0.00047	0.032	0.28	0.00029	0.038	0.00098	0.0000034	0.0092
HC5 (m = 5)	0.0045	0.12	0.056	0.0019	0.044	0.00061	0.00020	0.000086
HC5 (m = 7)	0.013	0.37	0.054	0.0089	0.091	0.0030	0.0015	0.00040
HC5 (m = 9)	0.028	0.77	0.092	0.017	0.16	0.0061	0.0041	0.00064
HC5 (m = 11)	0.029	1.0	0.11	0.025	0.23	0.010	0.0030	0.0012

[a]m represents the number of test species; m = 3: *S. pannonicus*, *D. magna* and *O. latipes*; m = 5: ditto plus *L. stagnalis* and *C. pipiens*; m = 7: ditto plus *L. minor* and *M. aeruginosa*, m = 9: ditto plus *H. oligactis* and *X. laevis*, m = 11: all species.

icity data for different numbers of species and HC5 values based on data for three and five different species. The HC5 calculated for nine taxa was used as denominator. The results are summarized in Table 7. The geometric mean ratio when only one fish LC_{50} is available is nearly 2000, but the variation among these ratios is very high. For *D. magna*, this ratio is about 320. Approximately the same ratio is obtained if the lowest LC_{50} for three standard acute toxicity tests is used.

The geometric mean ratio calculated from the lowest of three chronic tests on algae, crustaceans, and fish is about 10. The ratio calculated from the lowest NOEC of five and seven different species is about 5. For preliminary hazard assessment, it implies that if chronic data are available for algae, crustaceans, and fish, and a safety factor of 10 is applied on the lowest NOEC value, a realistic estimate of the HC5 is obtained.

Optimization of Number and Type of Test Species Needed

Uncertainty in the procedure of Van Straalen decreases when more ecotoxicological information becomes available. This is shown by the maximum and minimum values for the ratios depicted in Table 7. For optimization of the number of species that should be used for the calculation of HC5 values with this procedure, an uncertainty factor is introduced (Table 8). Uncertainty reaches a minimum when the uncertainty factor becomes 1. This is nearly achieved when using HC5 values based on seven test species (uncertainty factor = 1.7).

Despite several comments that can be made on this limited evaluation for only eight chemicals and only 11 species, this conclusion goes into the direction of the current choice of tests made by the US EPA (Stephan and others 1985) for the calculation of ecotoxicological quality criteria: more than three species are needed (Table 3). Ethical and financial considerations, however, demand minimization of the number of tests. Although the uncertainty for data on five species is within an order of magnitude higher than for seven species, reasonably adequate estimates for HC5 values are obtained when these values are calculated using only five NOECs. Further research has to be carried out to obtain more information on error estimates of HC5 values.

At the moment OECD test guidelines for (semi)chronic single-species tests are available for green algae, daphnids, and fish. Internationally accepted test guidelines should be developed for at least two more test species. These species must be selected on the basis of their ecological function, their morphology, and the route of exposure. From the results presented, it is propsed that the standard test battery should be complemented by internationally accepted test guidelines on a mollusk and an insect, as these species fulfill the above-mentioned requirements. For the terrestrial environment, especially the soil, preliminary effects assessment is not possible because an adequate number of ecotoxicological tests has not been developed.

Conclusions

The three chronic tests on gree algae, daphnids, and fish suffice for preliminary hazard assessment for the aquatic environment. Realistic estimates for HC5 values are obtained when a safety factor of 10 is applied on the lowest NOEC value obtained in these tests. If only one acute fish or *Daphnia* LC_{50} value is available, safety factors of, respectively, 2000 and 300, may be applied (Table 7).

For refined hazard assessment more tests are needed. Reasonably adequate estimates for "safe" concentrations for ecosystems are obtained when HC5 values are calculated on the basis of data on ≥ 5 test species.

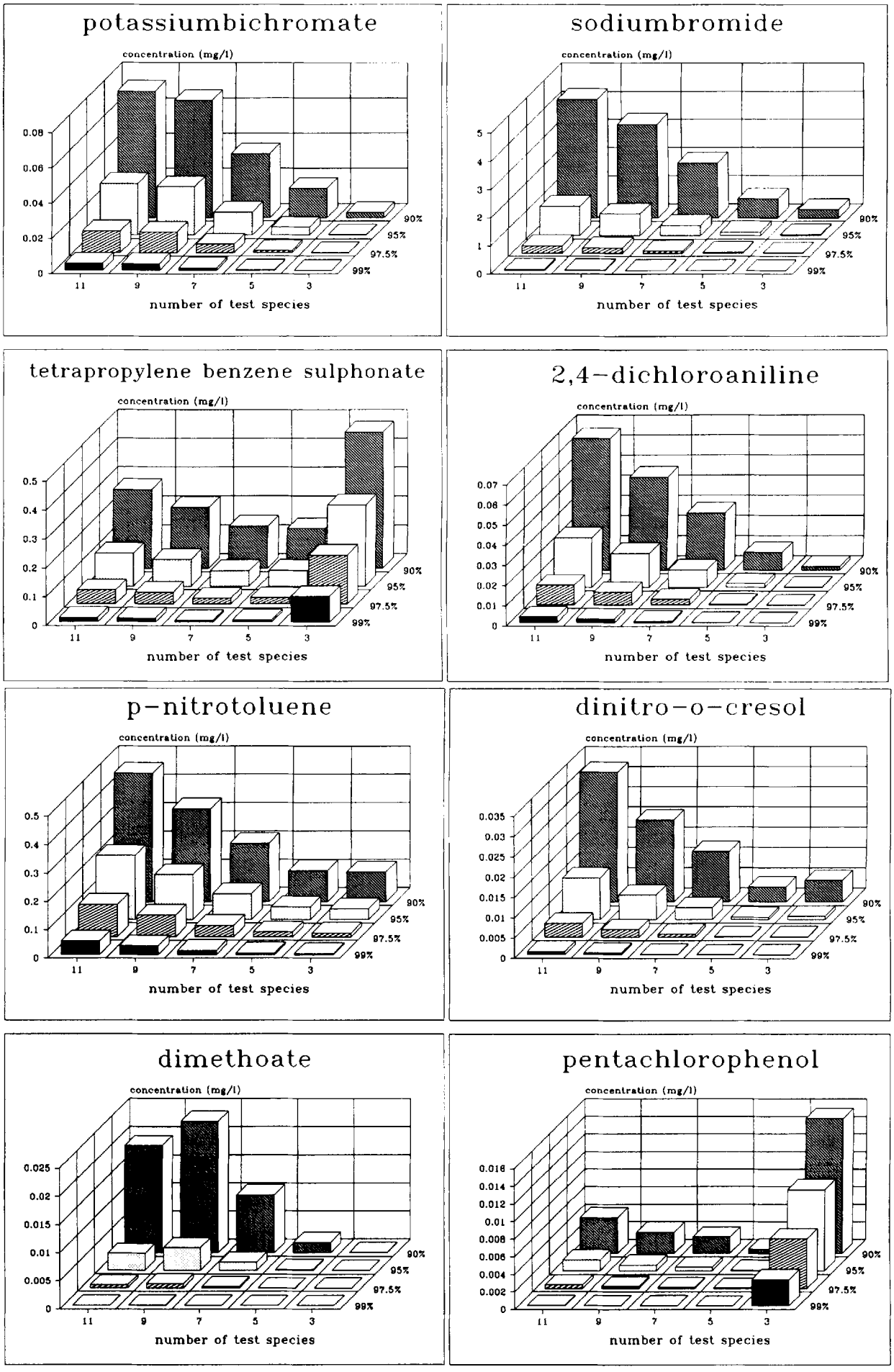

Figure 3. *HCp* values for ecosystems for eight compounds calculated according to Van Straalen and Denneman (1989) for different numbers of test species and protection levels.

Table 7. Ratios between different combinations of acute and chronic toxicity data[a] and HC5 values calculated for three, five, or seven taxonomic groups[b]

	Acute LC_{50}			Chronic NOEC			HC5		
Compound	F	D	A,D,F	A,D,F	(m = 5)	(m = 7)	A,D,F	(m = 5)	(m = 7)
$K_2CR_2O_7$	5,100	36	36	3.6	3.6	3.6	0.017	0.16	0.48
NaBr	31,000	14,000	13,000	13	13	13	0.041	0.16	0.47
TPBS	170	180	170	11	3.5	3.5	3.0	0.60	0.59
2,4-DCA	1,200	77	77	1.9	1.9	1.9	0.017	0.11	0.53
p-NT	320	47	47	6.3	2.0	2.0	0.24	0.28	0.57
DNOC	570	560	560	16	5.2	5.2	0.15	0.10	0.48
Dimethoate	27,000	720	720	7.9	7.9	7.9	0.00083	0.05	0.36
PCP	1,700	750	730	50	5.0	5.0	14	0.13	0.63
Geometric mean	2,000	320	310	8.9	4.4	4.4	0.11	0.15	0.51
Max.	31,000	14,000	13,000	50	13	13	14	0.60	0.63
Min.	170	36	36	1.9	1.9	1.9	0.00083	0.05	0.36

[a]The lowest LC_{50} or NOEC values were used.

[b]The HC5 value based on data for nine taxonomic groups is used as denominator. A = *S. pannonicus*, D = *D. magna* and F = *O. latipes*; m = number of species tested; m = 3: A, D, and F; m = 5: ditto plus *L. stagnalis* and *C. pipiens*; m = 7 ditto plus *L. minor* and *M. aeruginosa*; m = 9: ditto plus *H. oligactis* and *X. laevis*.

Table 8. Uncertainty in the procedure of Van Straalen and Denneman (1989) when tests on three, five, or seven taxonomic groups are used for the calculation of the HC5

	Uncertainty factor (max. ratio/min. ratio)[a]
HC5 (m = 3)[b]	17,000
HC5 (m = 5)[c]	12
HC5 (m = 7)[d]	1.7

[a]The maximum and minimum ratio represent the quotient of the HC5 based on, respectively, three, five, and seven test species and the HC5 based on data for nine taxonomic groups for eight chemicals; m represents the number of test species.

[b]*S. pannonicus*, *D. magna* and *O. latipes*.

[c]As b plus *L. stagnalis* and *C. pipiens*.

[d]As c plus *L. minor* and *M. aeruginosa*.

It is proposed that the standard test battery for the aquatic environment should be complemented by internationally accepted test guidelines on at least a mollusk and an insect species.

The extrapolation procedure of Van Straalen and Denneman (1989) is a useful tool in ecotoxicological effects assessment but should only be used if more than three (semi)chronic test data are available. Further statistical improvements for error estimates of HC5 values are necessary.

For the terrestrial environment, especially the soil, preliminary effects assessment is not possible because an adequate number of ecotoxicological tests has not been developed (OECD 1989b).

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