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Variation in Sensitivity of Aquatic Species to Toxicants: Practical Consequences for Effect Assessment of Chemical Substances

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ABSTRACT / This study addresses the relation between the sensitivity of aquatic species and mode of action of different classes of organic chemicals. We analyzed large data sets of ecotoxicological information to reveal the interspecies variation in sensitivity, to relate this variation to the compounds' mode of action, and to explain the observed patterns using general biological information. Here we present a general framework and recommendations for risk assessment procedures.

We recommend the use of toxicologically based classification schemes at an early stage of the risk assessment procedure. Screening programs are most efficiently run when only one species per compound is tested to prioritize substances. The toxicity of compounds belonging to the class of nonpolar narcotics is highly predictable and shows little interspecies variation. For these compounds quantitative structure–activity relationships (QSARs) can be used to estimate effect levels. Most effort should be put into testing reactive compounds and compounds with a specific mode of action as toxicity to some species can be 10^5–10^6 times higher compared with less sensitive species. The use of assessment factors in effect assessment procedures may lead to an underestimation of effects on the more sensitive species.

For many priority pollutants there is little information on their ecotoxicity. Predictive techniques are needed to compensate for this lack of data. Knowledge of the relation between modes of action of compounds and interspecies variation in sensitivity should be integrated in risk assessment procedures in order to make more efficient use of the limited financial resources available.

For many priority pollutants there is little information on their ecotoxicity in the aquatic environment. There are even few data on the so-called high production volume chemicals: chronic toxicity data for fish are available for only 6% of these chemicals. For the soil environment, the situation is much worse (Van Leeuwen and others 1996). Likewise, there is a sharp contrast between the number of species used in ecotoxicological studies and the total number of species, which is estimated to be close to 10^8 (Ehrlich and Wilson 1991). It is evident that we are forced to rely on predictive techniques and extrapolation in risk assessment procedures. There are simply too many species–compound combinations to be tested individually.

KEY WORDS: quantitative structure-activity relationship; Ecotoxicological effect assessment; Mode of action; Interspecies variation; sensitivity

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In effect assessment procedures, toxicity data for just a few laboratory species, i.e., the base set comprising a green alga, *Daphnia,* and fish, are used to derive an estimate of a no-effect concentration (PNEC: predicted no-effect concentration) based on application factors. This limited taxonomic coverage is then assumed to be representative of the theoretical set of all species with regard to their protection (Van Leeuwen and others 1996). The interspecies variation in sensitivity is, among other factors, one of the uncertainties in effect assessment procedures (Suter 1993). Insight into the patterns of the sensitivity of species informs our decisions when choices have to be made in pursuit of more efficient use of the limited resources available and to prioritize data needs. Compounds associated with the highest risks or uncertainties need our special attention.

During the last few years we have been studying the patterns in the sensitivity of species to toxic substances in order to: (1) reveal patterns in the interspecies variation in sensitivity, (2) relate these patterns to the compounds' mode of toxic action, and (3) explain

these patterns using general biological characteristics of the species. The studies were based on existing ecotoxicological data.

We emphasize that this research addresses the more general patterns in interspecies variation in sensitivity based on data sets with large numbers of aquatic species. This approach is complementary to detailed research into the mechanisms behind differences in sensitivity (e.g., metabolic pathways) which is, because of its nature, limited to just few species. The patterns observed in our generalist approach facilitate the formulation of hypotheses about causal relationships that can be studied in detail at a mechanistic level. Our research was initiated by the work performed by Slooff and others (1983) and Slooff and Canton (1983), who were among the first to address the enormous diversity of ecotoxicological responses. Their data formed the basis of our data sets.

We have analyzed thousands of toxicity data. To our knowledge there has never been such a large-scale study of patterns in the sensitivity of species. The extensive analysis by Mayer and Ellersieck (1986) focused mainly on individual interspecies correlations in sensitivity and the contribution of experimental factors such as temperature, pH, and hardness on the outcome of the toxicity experiment. Other comparative studies were limited to a few species or one taxonomic group (e.g., Blanck 1984, Lipnick 1985, Wängberg and Blanck 1988, Vittozzi and DeAngelis 1991, Cronin and others 1991, Calleja and others 1994) or to a few compounds or one mode of action (e.g., LeBlanc 1984, Williams and others 1985, Vaishraiv and Korthals 1990, Staples and others 1997). In this paper, the detailed results of the research project (Hoekstra and others 1994, Notenboom and others 1995, Van der Wal and others 1995, Legierse and others 1996, Vaal and others 1997a,b) are put into a more general conceptual framework. Recommendations and relevant implications for the effect assessment of environmental pollutants are given.

Background

Models based upon patterns of species sensitivities to toxic chemicals can be formulated such that they predict the sensitivity of species to a compound or group(s) of compounds. We call these models quantitative species sensitivity relationships (QSSRs) (Hoekstra and others 1994). These models are the counterparts to models predicting the toxicity of compounds based on chemical information: quantitative structure–activity relationships (QSARs). QSARs describe the toxicity of a range of compounds to one (group of) species, whereas QSSRs predict the sensitivity of a range of species to one (group of) chemical(s). QSARs are used as an accepted predictive technique for the assessment of the ecotoxicological effects of various compounds. QSARs can also be applied in reverse when experimental data fit specific QSAR points at a certain mode of toxic action. In this way, QSARs can be used to assign a certain mechanism to a particular chemical. Important in this respect is that the structural rules (chemical domain) of a model are well understood and strictly defined (Hermens 1995, Hermens and others 1995). At present, methods are being developed that enable the classification of compounds on the basis of their structural characteristics and knowledge of their mode of action. This facilitates the prediction of ecotoxicity (Verhaar and others 1992, Russom and others 1997).

Structure–activity relationships have been developed for the prediction of the toxicity of a large number of chemicals, but they do not include any information on the differences in the sensitivity of species. This is because the QSARs applied predict toxicity levels for a single species only. More information on interspecies variation in sensitivity will be of great value for the further development and application of predictive techniques in assessment procedures. It is known that this variation can be substantial: the ratio between the no-effect levels of the most sensitive and the least sensitive species can be as large as 10,000 (Slooff and Canton 1983). The development of predictive techniques will require more knowledge of the relationship between the toxic mode of action and the variation in sensitivity among species.

Outline of the Research

Principal component analysis, a statistical pattern recognition technique, was used to investigate the relation between taxonomy and sensitivity of species (Vaal and others 1997a). A data set containing toxicity data of as many species and taxonomic classes per compound as possible and, in addition, a similar set for each compound under study were needed. The pattern analysis identifies factors that determine the variation in the data set. In addition to the pattern analysis, we investigated the relation between interspecies variation in sensitivity and the chemical's mode of toxic action (Vaal and others 1997b). For this purpose a second data set was constructed with as many species data per compound as possible.

The number of appropriate and available data fell short of our expectations; it was not possible to construct large data sets for soil or terrestrial organisms containing various taxonomic classes and groups of

compounds with different modes of action. Sufficient data were available only for aquatic species. It appeared that many compounds have been tested on only a few species, which made the construction of a matrix with a large array of species, similar for each compound under study, more complicated. Both analyses were performed on LC_{50} data from short-term (two to four days) toxicity experiments, for reasons of data availability and comparability of test methods. A preliminary pattern analysis has been performed on a small set of chronic toxicity data for aquatic species (Van der Wal and others 1995). All data were primarily retrieved from the Aquire Database (1993) and analyzed on a molar basis.

In all the analyses, compounds are classified according to the method of Verhaar and others (1992). Four major classes are distinguished: nonpolar narcotic compounds (I), polar narcotic compounds (II), reactive compounds (III), and compounds with a specific mode of action (IV). Class IV can be further subdivided into several subclasses, such as acetylcholinesterase (AChE) inhibitors, oxidative phosphorylation uncouplers, and other neurotoxicants. Narcotic toxicity is considered to correspond to a baseline level of toxicity exerted by any chemical. When compounds consist of functional groups that are reactive or can interact with specific target sites (receptor mediated toxicity) effect concentrations are between 10 and 10^4 times lower (Verhaar and others 1992).

Patterns in Species Sensitivity

An acute toxicity data matrix of 21 compounds by 26 aquatic species was analyzed using principal component analysis as a pattern recognition technique (Vaal and others 1997a). Compounds included five narcotic compounds, six polar narcotics, two reactive compounds, five compounds with a specific mode of action, and three heavy metals. Species included 8 fishes, 2 amphibians, 12 arthropods, and 1 mollusk, 1 annelid, 1 planarian, and 1 coelenterate. The PCA technique observes the matrix as rows of objects and columns of variables. In our case, the species are the objects and the chemical compounds the variables. This corresponds with an analysis of the patterns in species sensitivity. The opposite case, compounds as objects and species as variables, describes the patterns in toxicity of compounds. The first case is used in a short explanation of the PCA method in Appendix 1. Figure 1 shows the relationships among the species. The first two axes (PC1 and PC2) describing the patterns in species sensitivity explained only about 50% of the variance in the data matrix. Compounds that determined the species sensitivity pattern in the analysis are plotted into the same figure. These compounds have a high overall toxicity and a large standard deviation when averaged over all species. Species are relatively sensitive to compounds situated at the same side of the axis.

Species very sensitive to one (group of) chemical(s) may be among the least sensitive to other compounds. The most sensitive species group depends on the mode of toxic action, confirming that there is no ''most sensitive'' species (Slooff and others 1983, Cairns 1986). The patterns in Figure 1 show that there is a tendency for species belonging to the same taxonomic group (either phylum or class) to be more alike. Daphnids and insect larvae were among the most sensitive to heavy metals and organophosphorus insecticides (AChE inhibitors), while fishes, and probably also amphibian larvae, appeared to be the most sensitive groups for other neurotoxic insecticides such as dieldrin and lindane.

The factor explaining most of the variation in the matrix was the toxicity of the compounds rather than the differences in the sensitivity of the species. The first axis describing the patterns in toxicity of the compounds explained 82% of the matrix variance (not shown) and could be attributed to the average toxicity of the compounds to all species. All species were of similar weight in listing the chemicals according to their toxicity. This indicates that the difference in toxicity of the compounds was much larger than the difference in the levels of species sensitivity. As a consequence, compounds can be unambiguously arranged according to their level of toxicity, no matter which species is used. Patterns in species sensitivity are more diffuse.

In an additional study we focused on the patterns in species sensitivity to 14 organophosphates (Legierse and others 1996). The purpose was to analyze factors determining the underlying mechanisms of species-selective toxicity. The organophosphates investigated in this study belong to either the phosphorothionates or phosphates group. Phosphorothionates are poor anticholinesterases (Fukuto 1990, Chambers 1992), and they need to be activated prior to AChE inhibition; phosphates are direct inhibitors of AChE. Species included five crustaceans, an insect, and nine fish species. The first principal component explained 89% of the total variation and expresses the relative sensitivities of the species, i.e., patterns in species sensitivities to organophosphates do indeed exist and appear to be related to phyla systematics. Arthropods, especially daphnids, were most sensitive, and fish species were relatively insensitive. The loadings of the compounds were about equal for different organophosphates, indicating that compound-specific differences in toxicity

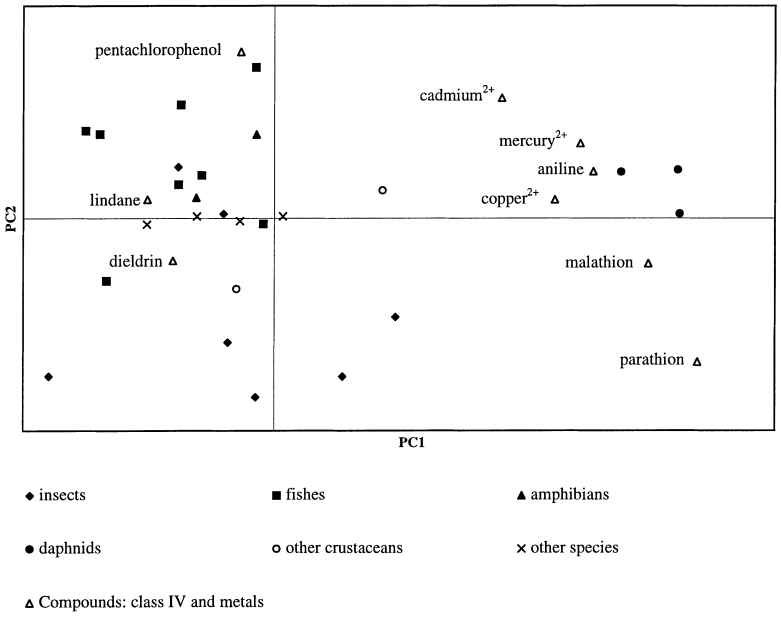

Figure 1. Patterns in interspecies variation in sensitivity; result of the statistical pattern analysis is shown schematically on the two principal component axes. Only names of compounds that strongly determine the species patterns are given. Species are relatively sensitive to compounds situated on the same side of the axis; a species is very sensitive when situated close to a compound.

are not of great importance in the principal component model.

In order to investigate if the observed patterns can be extended to a greater variety of aquatic species, a second data set was constructed using acute toxicity data of as many aquatic species per compound as possible. Per compound at least ten species were included belonging to at least four taxonomic classes among which one fish, one daphnid and one insect. The average phosphate toxicities were, contrary to expectations, not consistently higher than the toxicities of phosphorothionates. Sensitivity distribution ranges were not consistently smaller for phosphates, suggesting that the impact of activation rate differences on the selective toxicity of different organophosphates is negligible for species belonging to different taxonomic groups. It is suggested that differences in whole-body background AChE activity play an important role in the intrinsic sensitivity differences among aquatic species (Legierse and others 1998). Other dynamic differences, such as the sensitivity of the nervous system or the response to AChE inhibition, seem to play a minor role.

The availability of chronic toxicity data (no-observed-effect concentrations) was a serious limitation and restricted pattern analysis to a few taxonomic classes and compounds. However, analysis of the data set on 15 aquatic species and 22 compounds confirmed the predominant role of compound toxicity in explaining the variation (Van der Wal and others 1995). No clear patterns in interspecies variation in sensitivity could be distinguished, which was mainly due to the influence of the experimental design on the outcome of the chronic toxicity experiments. Experimental conditions in long-term toxicity experiments are of a more diverse nature due to differences in exposed life-cycle stages, exposure time, effect parameters and statistics, which appeared to disguise the differences in sensitivity between species and make the data less comparable from a toxicological point of view.

Toxicity in Relation to Compound Hydrophobicity

For the investigation of the relation between sensitivity and mode of action, we constructed a data set of acute aquatic toxicity data for as many species data per compound as possible that met our quality criteria (Vaal and others 1997b). The data set included toxicity data for 32 chemicals (4 nonpolar narcotics, 5 polar narcotics, 3 reactive compounds, and 20 specifically acting compounds) and 237 aquatic species, ranging from 12 to 52 species per compound.

The average toxicities of compounds in relation to their log K_{ow} values are shown in Figure 2. The line indicates baseline toxicity calculated from the levels of the average toxicity of the nonpolar narcotic compounds to all species. From our multispecies analysis it appears that log K_{ow} is a good predictor of average baseline toxicity for compounds with a nonpolar narcotic mode of action. The QSAR in Figure 2 is quite similar to the QSARs for narcotic toxicity mentioned for individual species (e.g., Könemann 1981, Russom and others 1997).

Maximum acute LC_{50}s of compounds that act by

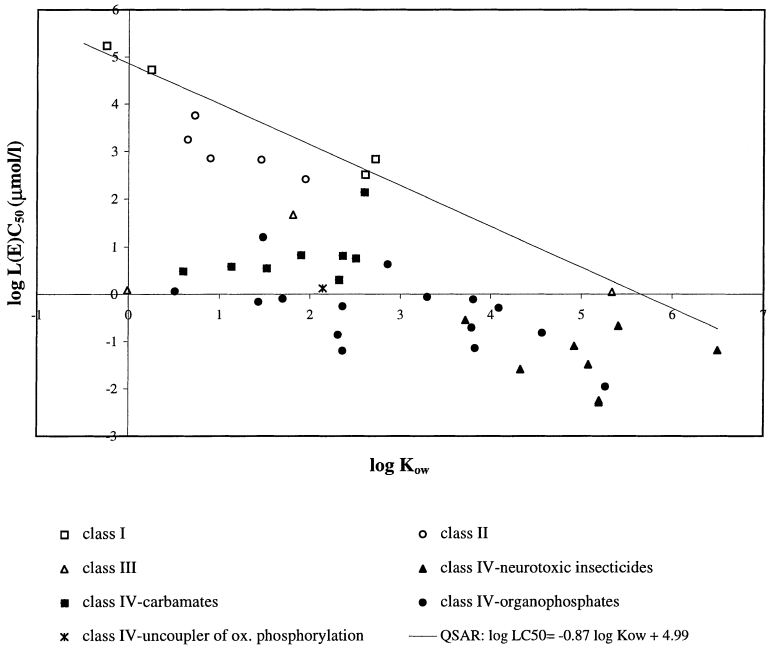

Figure 2. Average toxicities for compounds with different modes of actions versus log K_{ow}-values. The line represents the QSAR for average baseline toxicity to all species, calculated for the non-polar narcotic compounds.

polar narcosis are a factor of 10 lower than the log K_{ow} predicted values (represented by the line), which agrees with QSAR studies for individual organisms (Verhaar and others 1992). Figure 2 also includes data from the additional study on organophosphates. Reactive compounds and compounds with a specific mode of action are a factor of 10 to 100,000 more toxic than predicted, and the average toxicities seem to be independent of log K_{ow} values. The toxicities of the more hydrophobic reactive and specific acting chemicals tend to decrease towards baseline toxicity levels. This trend has been observed before (Deneer and others 1988) and may be related to the narcotic effect being caused by a relatively high concentration of the more hydrophobic chemicals in the cell membranes compared with the concentration in the aqueous phase within the organism where specific interactions may take place. The narcotic effect of the more hydrophobic chemicals may then overwhelm the specific effects.

Sensitivity Distributions Depend on Mode of Action

Figure 3 shows the sensitivity frequency distributions per chemical group derived from the toxicity data of the 237 species and 32 chemicals mentioned above. Each distribution curve is based on acute toxicity data for at least ten species from different taxonomic classes (among which were at least one fish, one *Daphnia,* one insect, and one additional taxonomic class). Toxicity is expressed as logarithms of the toxic ratio, the ratio between the average measured toxicity of the compound and its predicted level of baseline toxicity, calculated with the QSAR in Figure 2. The vertical line at zero denotes this baseline toxicity. The peak breadth indicates the predictability of the level of toxicity.

Figure 3 clearly shows the relation between the compounds' modes of action and the shape of sensitivity distributions. The variation in species sensitivity to chemicals with a nonpolar mode of action is the smallest, whereas this variation can be as large as 10^5–10^6 for compounds with a reactive or specific mode of action. Normality of the distribution appeared to be an appropriate assumption for most compounds in the study (Vaal and others 1997b). Reactive and specifically acting compounds cause large interspecies differences in effects. This means that, in order to derive equally precise estimates of safe environmental concentrations (PNECs), these compounds will need more testing than nonpolar and polar narcotic compounds. The toxicity of compounds with a narcotic mode of action is highly predictable, and there is little interspecies variation. Thus, prediction of PNEC values for these compounds can be derived from QSARs based on hydrophobicity, as pointed out by Van Leeuwen and others (1992).

Recommendations for Effect Assessment Procedures

Expert systems that classify chemicals on the basis of their structural characteristics and knowledge of mode of toxic action have been developed by Verhaar and

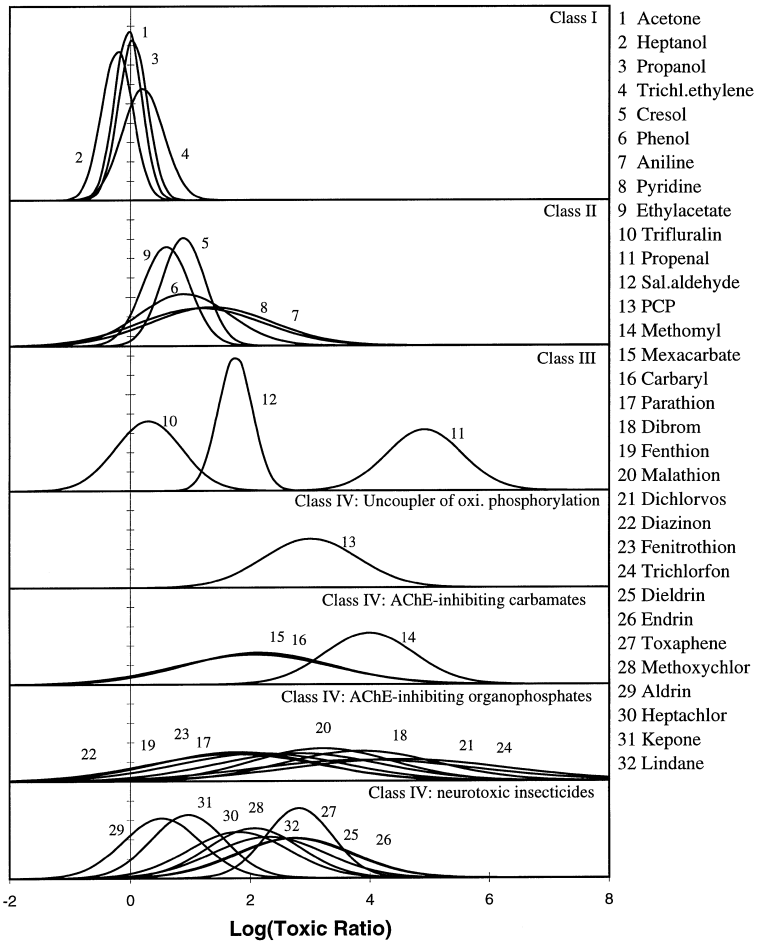

Figure 3. Sensitivity distributions of 32 compounds, grouped per type of mode of action. The number of species per distribution ranges from 12 to 52. Toxicity is scaled to hydrophobicity and expressed as the Toxic Ratio: the ratio between the mean measured toxicity and the toxicity predicted on the basis of the QSAR from Figure 3. Higher Toxic Ratios indicate greater levels of toxicity shown by the compound with respect to the predicted level (after Vaal and others (1997b) with permission).

others (1992) and Russom and others (1997). These toxicologically based classifications demonstrate that compounds of the same chemical classes may have different modes of toxic action. The use of such classification systems can be of great value for chemicals that have not yet been tested. The reliability of the predicted toxicity strongly depends on the accuracy with which a compound can be assigned to one of the classes.

We recommend the use of these toxicologically based classification schemes in effect assessment procedures. It would allow a more efficient use of the limited resources available to estimate the risks associated with the large number of compounds for which no data are yet available. In our research, we have demonstrated that, from a species protection point of view, some classes deserve special attention. Compounds that have a specific mode of action show the largest interspecies variation in toxicity. The sensitive species are at risk. For effect assessment, more toxicological information is needed for these compounds than for the compounds with small interspecies variation in sensitivity.

It is shown in this paper, as in Van Leeuwen and others (1992), that the narcotic toxicity of class I compounds can be predicted well on the basis of log K_{ow} and that the differences between species are rather small. Thus, for this particular group of compounds, PNEC values can be calculated on the basis of QSARs. Chronic QSARs are available for many species, or can be derived from acute QSARs by the application of an acute–chronic ratio (Van Leeuwen and others 1992). A large proportion of the organic aquatic toxicants can be classified according to the method of Verhaar and others (1992). About 240 of the 2000 so-called high production volume chemicals (HPVCs) can be unambiguously classified as belonging to class I, the nonpolar narcosis-type chemicals. The use of QSARs for these compounds to set priorities will speed up the risk assessment process. About 375 chemicals of these HPVCs can be assigned to class III and IV. Only a general indication of acute aquatic toxicity can be given for these chemicals (Verhaar and others 1994).

More information is needed to perform an effect assessment on class II–IV compounds. Screening pro-

grams are most efficiently run when they are aimed at detecting the level of toxicity exerted by the compound. Effort should be put into discriminating between compounds. It has been demonstrated in our pattern analysis that the level of toxicity is the dominant factor determining interspecies differences in sensitivity. For prioritization one species would suffice to discriminate among compounds. The most toxic compounds have priority because they pose a greater risk to ecosystems.

Only a few acute toxicity data are usually available in a preliminary effect assessment. An assessment factor is then applied to derive PNEC values. For class III and IV compounds these factors may considerably underestimate the effects on the more sensitive species (Van Leeuwen 1995). Most effort should be put into testing these classes because the LC_{50}s of some species can be 10^5–10^6 times lower than for less sensitive species. Although the toxicity of class II compounds is within two orders of magnitude of the predicted minimum toxicity (Figure 3), some compounds (aniline, pyridine) show considerable interspecies variation in sensitivity, making the use of predicted PNEC much less reliable than for class I compounds. Further research is needed to clearly define the domain of class II.

Even greater interspecies variation is expected for the sublethal end points of toxicity. Refined effect assessment is then more appropriate because it demands more input data and more sensitive, sublethal end points (Van Leeuwen 1995). In the Netherlands these data are used to generate statistical extrapolation models to calculate PNECs. Hence, all information on species from different taxonomic or functional groups is taken into account and a concentration is calculated that is regarded as safe for most species present in an ecosystem. This extrapolation method can be applied if at least five, but preferably more, chronic values are available for representative species (OECD 1995).

The working method followed in this study demanded toxicity data on a great number of species per compound meeting certain quality criteria. At present, the scarcity of such data in the literature is limiting our research. Nevertheless, this approach has led to conclusions and generalizations that are of importance for refining and increasing the efficiency of effect assessment procedures.

The analyses of interspecies variation in sensitivity in this study reveal that the differences in intrinsic properties of species determine their sensitivity to various chemicals. However, ecological effects of environmental pollutants do not depend only on these properties. The ecological vulnerability of a species is determined by more than its toxicological sensitivity alone. The extent to which an organism is exposed to the substance and the ability of a population to recover after exposure to a toxic compound are other factors determining whether an organism will be affected by a certain toxicant (Van Straalen 1994).

The analysis of ecological vulnerability must include population level effects, such as the analysis of the toxicant disturbance on life-history attributes that determine the growth rate (Calow and others 1997). The lack of sufficient data on species' life-history attributes and the influence of toxicants upon them for large groups of species will significantly restrict the possibility of adapting a generalistic approach as described above.

In summary, we recommend the following:

- Test one species in screening programs in order to prioritize substances.
- Classify compounds for the effect assessment procedure as early as possible using toxicologically-based schemes (Verhaar and others 1992, Russom and others 1997).
- If a chemical belongs to class I, use QSARs for baseline toxicity to estimate PNEC.
- If a chemical belongs to class II, III, or IV, use at least three acute toxicity data for preliminary effect assessment, and be careful in applying assessment factors for class III and IV.
- If a chemical belongs to class II, III or IV, use at least five chronic toxicity data in a refined effect assessment.

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Appendix 1: Short Description of Principal Component Analysis

The PCA technique observes the toxicity matrix as rows of objects and columns of variables. In our case, the species are the objects and the chemical compounds the variables. This corresponds with an analysis of the patterns in species sensitivity. The opposite case, compounds as objects and species as variables, describes the patterns in toxicity of compounds. The first case is used in a short explanation of the PCA method. More detailed information can be found in Kowalski (1983).

The toxicity of a compound is one axis in a space the dimension of which is determined by the number of compounds in the analysis. The toxicity of each of these compounds to a species situates this species as a point in this space. Species that have similar sensitivity to each of

the compounds will be situated close to each other. Thus the data of all species in the matrix are represented by a point swarm in this space. PC analysis searches for new axes describing the positions of the species relative to each other, losing as little information as possible. The new axes are linear combinations of the original axes. The reduction is successful when only a few components have to be used to describe most of the variation in the data matrix. PCA provides an efficient way to convert a data matrix into a few informative pictures showing the relationship between the species as measured by the compounds.

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