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Ordering chemical compounds by their chronic toxicity to aquatic species. A principal component analysis.

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SUMMARY

This research is part of a project where the objective is to gain insight in the variation of the sensitivity of species to toxicants.

Patterns in the chronic toxicity of chemical compounds to aquatic species are studied using a multivariate statistical technique, principal componant analysis. It is a follow-up of a similar study using acute toxicity data.

In this study a dataset with chronic toxicity data (NOECs) on growth for 15 aquatic species and 22 compounds is analysed. The species belong to different taxonomic classes: algae, plants, protozoans, crustaceans, fishes and amphibians. The organic chemicals belong to several toxicological classes: nonpolar narcotics, polar narcotics, reactive compounds and compounds with a specific mode of action. Three heavy metals, a halogen and a detergent are also included.

Toxicity data was obtained from the EPA-database Aquire and additional literature surveys.

Principal component analysis is used to find structure in the variation of chemical toxicity to species. The ratio of acute over chronic toxicity is calculated for each compound. Also a comparison is made between the mean toxicity of a compound and its octanol-water partitioning coefficient.

The major conclusions from this study are:

1. The major part of the variation in species sensitivity is determined by the toxicity of compounds and not by intrinsic differences between the species.
2. The compounds can be ordered according to chronic toxicity in an unambiguous way; the ordering is valid for practically all species considered and similar to the ordering according to acute toxicity.
3. No clear patterns in the sensitivity of the species are observed.
4. Ratio of mean LC_{50} and $NOEC_{growth}$ is very similar for all compounds. The ratio has a mean value of 4. For the acute to chronic ratio a 95% confidence interval stretching from 1 to 50 has been calculated for the 13 compounds in this study where both acute and chronic toxicity data were available.
5. Mean toxicities of chemicals with a nonspecific mode of action show a strong relation with their octanol-water partition coefficient (K_{ow}) and, as expected, chemicals with more specific modes of action are more toxic than predicted by their K_{ow}.

SAMENVATTING

Dit onderzoek maakt deel uit van een project dat inzicht wil verschaffen in de variatie in de gevoeligheid van soorten voor toxicanten.

Patronen in de chronische toxiciteit van chemische stoffen voor aquatische soorten worden daartoe onderzocht met een multivariate statistische techniek, principale componenten analyse. Het is een vervolg op een vergelijkbare studie met acute toxiciteitsgegevens.

In deze studie is een gegevensset geanalyseerd met chronische toxiciteitsgegevens (NOECs) voor groei voor 15 aquatische soorten en 22 chemische stoffen. De soorten behoren tot verschillende taxonomische klassen: algen, vaatplanten, protozoën, kreeftachtigen, vissen en amfibieën. De organische verbindingen zijn in te delen in verschillende toxicologische groepen: niet-polair narcotische, polair narcotische of reactieve verbindingen en verbindingen met een specifiek werkingsmechanisme. Drie zware metalen, een halogeen en een detergent zijn ook in de gegevensset opgenomen.

De toxiciteitsgegevens zijn verzameld uit de Aquire-database van de EPA en uit aanvullend literatuuronderzoek.

Principale componenten analyse is toegepast om patronen te onderkennen in de variatie van toxiciteit voor soorten. De ratio van acute over chronische toxiciteit is berekend voor elke toxicant. Ook wordt een vergelijking gemaakt tussen de gemiddelde toxiciteit van een stof en de octanol-water partitie-coëfficiënt.

De belangrijkste conclusies van deze studie zijn:

1. Het grootste gedeelte van de variatie in soortsgevoeligheid wordt veroorzaakt door de toxiciteit van de verbindingen en niet door intrinsieke verschillen tussen de soorten.
2. De stoffen kunnen op eenduidige wijze geordend worden naar hun chronische toxiciteit. Deze ordening is hetzelfde voor bijna alle soorten in de analyse en vergelijkbaar met de ordening gebaseerd op acute toxiciteit.
3. Er zijn geen duidelijke patronen in de soortsgevoeligheid gevonden.
4. De ratio van LC_{50} over $NOEC_{groei}$ is vergelijkbaar voor alle verbindingen. Deze acuut/chronisch-ratio heeft een gemiddelde waarde van 4. Voor deze ratio is een 95% betrouwbaarheidsinterval berekend dat loopt van 1 tot 50 voor de 13 stoffen in deze studie waarvoor zowel acute als chronische toxiciteitsgegevens beschikbaar waren.
5. De gemiddelde toxiciteit van verbindingen met een niet-specifiek werkingsmechanisme vertoont een sterke relatie met de octanol-water partitie-coëfficiënt (K_{ow}) en, zoals verwacht, zijn verbindingen met meer specifieke werkingsmechanismen toxischer dan voorspeld op basis van hun K_{ow}.

1 INTRODUCTION

Differences in sensitivity of species are an important issue in environmental toxicology. These differences play an important role in the calculation of maximum tolerable concentrations (MTRs) via extrapolation methods.
For risk assessment it is desirable to have data on many species, but it is not feasible to test more than a few different species. Insight into the patterns of differences in sensitivity will be very useful in the selection of relevant species to be used for testing with a particular chemical or class of chemicals. Moreover, the development of quantitative species sensitivity relationships (QSSRs) may be used to predict the toxicity for species that are not tested for economical, practical or ethical reasons. With a QSSR the toxicity of a chemical will be related to certain biological characteristics of the species (Hoekstra et al., 1994; Vaal et al., 1994).

The research is focused on detecting patterns in differences in sensitivity. Patterns can only be detected using large data sets and multivariate techniques are useful to analyse such large data sets. Within this study it was chosen to use Principal Component Analysis (PCA). The advantage of such a type of analysis is that general trends in sensitivity patterns become visible.
In addition to the multivariate analysis other calculations and comparisons have been made to help understand the structure of the relationship between species sensitivity and compound toxicity. For each compound the sensitivity ratio was calculated and if possible the ratio of acute to chronic toxicity was calculated. A comparison was made between the calculated mean toxicity of each compound and the octanol-water partition coefficient.

In a previous study, a LC_{50} data matrix was analysed in a similar way using principal component analysis (Vaal et al., 1994). Because no-observed effect concentrations are more relevant in risk assessment, this study is repeated here with $NOEC_{growth}$. Another reason for performing this analysis was to see whether patterns detected in the acute toxicity data were reproducible with chronic toxicity data.

2 METHODS

2.1 Selection of data

A search of the database Aquire (US-EPA, Duluth, MN, version June 1994) was performed to get the data for this study. Using this data, a matrix was filled with aquatic toxicity data of as many species and compounds as possible. A species was included when the resulting matrix contained toxicity data for at least 40% of the compounds. Compounds were selected similarly. The Aquire search included only $NOECs_{growth}$ and strongly related endpoints like e.g. EC_{10}s. The articles of Slooff & Canton (1983) and Slooff et al. (1983) formed the major part of the dataset. Scarcity of data prevented the use of stringent criteria to selecting data for the dataset.
If more data per species-compound combination were available the geometric mean was taken. If a result was given as a range within which the $NOEC_{growth}$ would fall, the geometric mean of the range limits was used. Ranges with an open end were not used. Exposure duration must be long enough for the results to be considered chronic. The following limits have been employed for bacteria, algae, blue-green algae and protozoans: six hours and longer; for the remaining animals and duckweed: longer than 96 hours.
Other literature sources were searched selectively to fill in gaps in the matrix.

Originally it was decided not to limit the chronic toxicity data to a single endpoint. However during the search for data it became clear that a dataset that was limited to only one endpoint would be almost as large as a dataset combining all possible endpoints. The endpoint allowing for the largest dataset was growth. Hence only growth and the closely related photosynthesis endpoints were used for the final analysis. An advantage gained by restricting the dataset to only one endpoint is that it makes interpretation of the analysis much easier.

2.2 Principal components analysis

Principal component analysis (PCA) is a multivariate statistical technique that can detect patterns in a (large) data matrix. A data matrix is built from rows of objects and columns of variables. A more extensive explanation of PCA is given in a previous report (Vaal et al., 1994) and more detailed information can be found in Kowalski (1983).
We used the software package SIMCA (Umetri, 1992) for calculating the components. Our data contains missing values and not all programs are suitable for analysing such data. SIMCA however works well with moderate amounts of missing values.

The data can be analysed in two different ways:

Species as objects
Here the objects are the species, and the variables are the chemical compounds. This corresponds with an analysis of the *patterns in species sensitivity.*

Compounds as objects.
This is the opposite case, in which the compounds are the objects and the species are the variables. This describes *patterns in toxicity of compounds.*

Only the second analysis, which produced the clearest results, is presented in the main text. The results of the first analysis can be found in Appendix 2.

2.3 Presentation of PCA results.

The results of a PCA are visualized by two kinds of plots:

a. the score plot, and

b. the loading plot.

The score plot gives information on the resemblance of the objects. This is indicated by proximity. The loading plot supplies information on which variables have the strongest influence on the principal components and the manner in which they act and counteract with each other. When evaluating a loading plot, it is helpful to think of the variable markers as heads of arrows originating in the origin. Arrows pointing in the same direction indicate that these variables act in a similar fashion. Arrows with opposite directions counteract and when arrows are at an angle of about 90 degrees, they are independent of each other.

2.4 Data transformation prior to the analysis

Data were ^{10}log transformed. Subsequently they were multiplied by -1 to facilitate the interpretation of the PC analyses. In this way toxic compounds and sensitive species coincide in the plots of loadings and scores. The principal component model in SIMCA is fitted to the data using a method of least squares.

To give each variable equal influence on the determination of a component, the data are centered and scaled to unit variance. In this way no species has more impact on the final analysis than any other species.

3 RESULTS

3.1 Data, descriptive statistics

The resulting matrix consisted of 15 species and 22 compounds. The species are listed in Table 1. Nine species are micro-organisms (bacteria, algae and protozoans). Duckweed, a waterplant is included and some animals: a hydra, a waterflea, two fishes and an amphibian. The chemicals included in this study are listed in Table 2, including an indication of their toxicological mode of action. Organic compounds can be classified using a classification system published by Verhaar et al. (1992). This classification identifies organic compounds as belonging to one of four groups: nonpolar narcotics (class I), polar narcotics (II), reactive compounds (III) and compounds with a specific mode of action (IV). Of the final 15 chemicals, three belong to class I, seven chemicals to class II, two chemicals to class III and three to class IV. The heavy metals (three), the halogen and the surfactant cannot be classified using this system.

In Table 3 the matrix with the log NOEC-values for growth per compound-species combination is presented. Toxicity data are expressed as μmol/L, the metals as μmol/L metal ion. The final matrix contained toxicity data for nearly 60% of the species-compound combinations. Of this data about 60% was obtained from the Aquire-database. Only four percent of the data are averages of several log $NOEC_{growth}$-values.

Table 3 shows that sensitivity of species, averaged over all compounds, ranged from -0.79 to 4.47 (log $NOEC_{growth}$-values, column marked Average in Table 3). Toxicity of compounds, averaged over all species, ranged from 1.03 to 2.61 (log $NOEC_{growth}$-values, row marked Average in Table 3). With the exceptions of the species *Scenedesmus pannonicus* and the compound Cr^{6+} and the combination of *Daphnia magna* and Cd^{2+}, all cells in the matrix are based on only one result. For *S. pannonicus* Sloof et al. (1983) gives two results with different exposure durations; standard deviations range from 0.00 to 0.364. For chromium two or three results are available in three cases. The standard deviations for this compound are 0.00; 0.165 and 0.456. For *D. magna* and cadmium Van Leeuwen et al. (1985) have two results giving a standard deviation of 0.357.
Appendix 1 links the data used in this study to the literature references.
An analysis of variance (Table 4) shows that both species and compounds contribute significantly to the overall variation. The contribution of the species is only halve that of the compounds based on the MS (mean squares). For greater understanding of the relative importance of species and compounds, variance components have been calculated (Graybill, 1961).

$\sigma_{species}$	=	0.73
$\sigma_{compounds}$	=	1.01
$\sigma_{error/interaction}$	=	1.30

Comparing these values with the variance components calculated for the LC_{50}-dataset (0.17; 1.48 and 0.67 respectively), it is evident that the part of the total variation associated with the species variation is larger in the present study. At the same time the larger σ for the combined error and interaction terms says that the patterns are more diffuse.

3.2 Principal component analysis

Only one principal component analyses will be presented here. The PCA aimed at detecting patterns in species sensitivity is not presented because no clear patterns could be detected. The analysis for patterns in compound toxicity is presented below.

3.3 Patterns in compound toxicity

The objects are compounds, and the variables are species. This corresponds with an analysis of *patterns in compound toxicity*, as measured by means of the fifteen species.
The results of the modelling components are presented in Table 5. Four components contributed significantly to the model. The total amount of explained variance by these four components is 84.6 %. The first two components explain 76.5% and 4.5% of the matrix variance respectively, together they account for 80.9%. This result is very comparable with that of the previously reported LC_{50}-data set (Vaal *et al.*, 1994), where the first two components explained 78.6% and 3.5%.

The loadings for the first two components assigned to the species are listed in Table 6 and are plotted in Figure 1. The first component calculates the average toxicity for the compounds. A contrast between the micro-organisms and duckweed (near zero and negative loadings) and the larger fauna (positive loadings) characterises the second component.
The compound scores are presented in Table 7, alongside with standard deviations and object leverages. The scores represent the average toxicity of a compound for all species in the dataset. The two most toxic compounds (mercury and cadmium) and the two least toxic compounds (acetone and bromine) take extreme positions on the first component. The high leverages of these compounds indicate that they are important in defining the model. Mercury is the most toxic compound in this set. Unfortunately no $NOEC_{growth}$ data for the higher organisms are available for this compound, but the results of this analysis suggest that mercury will be at least as toxic for these organisms as it is for the algae and the protozoans.
From Figure 2 and Table 7 an ordering along the first component from the least toxic compound to the most toxic compound can be constructed: bromine, acetone, propanol, ethyl acetate, ethyl propionate, pyridine, dimethoate, allylamine, nitrotoluene, cresol, heptanol, aniline, TPBS, salicyl aldehyde, DCA, DNOC, Cr^{6+}, PCP, Cd^{2+}, Hg^{2+}
The position of chromium with a large negative score on the second component is indicative of high toxicity for species with a large negative loading. The algae *Chlorella pyrenoidosa* and *Scenedesmus pannonicus*, the protozoan *Chilomonas paramecium* and the bacteria *Pseudomonas fluorescens* are the most pronounced examples of this case. It also means that species with a positive loading are relatively insensitive for chromium. These species include the fishes *Oryzias latipes* and *Poecilia reticulata*, the daphnid *Daphnia magna* and the coelenterate *Hydra oligactis*. This group formed by the more complex animals is less sensitive for chromium toxicity then the micro-organisms in general. The exception to this rule being *Entosiphon sulcatum*, a protozoan, which suffers least of all species from chromium toxicity. Other observations of compounds showing high toxicity for particular species are dimethoate and mercury in Figures 1 and 2. Their position suggests specific toxicity for the fish *Poecilia reticulata* and the protozoan *Entosiphon sulcatum*. In the case of dimethoate and *Poecilia reticulata* this is supported by available data.

Summarizing the first component in the PCA explains the major part (>75%) of the variation present in the data. This first component orders the compounds according to their mean toxicity for all species considered. Most toxic are the heavy metals and PCP; least toxic are the nonpolar narcotics. For chromium it is observed that this heavy metal is generally more toxic to micro-organisms than to more complex animals.

3.4 Sensitivity Ratio's$_{95:5}$ and Mean toxicities.

The first component of the Principal Component Analysis for patterns in compound toxicity is used as a model for estimating missing values in the dataset. This is defendable when keeping in mind that this component alone explains more than 75% of total variance. After estimation for each compound a mean log NOEC$_{growth}$ is calculated. The resulting values are directly comparable since all of them include data on all species. The same matrix with the blanks filled with estimates is used to calculate Sensitivity Ratio's 95%:5% ($SR_{95:5}$s) (Hoekstra et al., 1994). The method used assumes that de data are lognormally distributed. Table 8 lists the compounds with their respective mean log NOECs$_{growth}$ and $SR_{95:5}$s. Also presented in Table 8 are the compounds that were present in the LC_{50}-matrix.
Table 8 is sorted, so that the toxicity increases from top to bottom. The least toxic compounds are bromine and then the nonpolar narcotics propanol and acetone and the ester narcotics ethylpropionate and ethylacetate. Next comes a group of compounds with low toxicity, consisting mainly of polar and nonpolar narcotics. The odd one out in this group is dimethoate, which is an organophosphorus pesticide. Next come TPBS, salicylaldehyde and DCA, classified as detergent, reactive and polar narcotic respectively, which are also relatively toxic compounds. The most toxic compounds are the uncouplers of oxidation (PCP and DNOC) and the heavy metals (Hg2+, Cd2+ and Cr6+).
Unfortunately there is no apparent link between the mean NOECs$_{growth}$ on the one hand and the $SR_{95:5}$ on the other hand.
For most compounds estimation based on the lognormal distribution of the $SR_{95:5}$ is fully justified, since the distribution of species sensitivities does not show significant deviation from the assumed log-normal distribution. For four compounds there is however a significant deviation. Also in these four cases the same parametric estimate is given, because a reliable nonparametric estimation needs 20 or more species. All four compounds have distributions that are skewed to the left indicating that some species are considerably more sensitive than most.

3.5 Relation toxicity versus log K_{ow}

A plot of mean NOECs$_{growth}$ for the compounds plotted against their log K_{ow}s is presented in Figure 3. The drawn line was calculated for chemicals acting by a nonspecific mode of action (acetone, propanol, nitrotoluene and heptanol). This line indicates baseline chronic toxicity. The ester narcotics ethylacetate and ethylpropionate are also situated on this line. Other compounds are situated below this line, they are more toxic than predicted by their hydrophobicity only. The most toxic compounds relative to baseline toxicity are DNOC and PCP.
Similar to the same graph based on LC_{50}s, there is a strong relation between log K_{ow} and toxicity.

3.6 Acute to chronic ratios

Mean values for $NOEC_{growth}$ from this study were compared with mean LC_{50} values from the previous study (Vaal et al., 1994). The result of this comparison is visualized in Figure 4. The actual values are listed in Table 8. Clearly the correlation (r^2=0.96) between these parameters is good.
A straight line fitted well to the available data. The slope of the fitted line was almost equal to 1 (one). Based on this finding the following hypothesis was tested : no relation exists between a compound's toxicity and the mean acute to chronic ratio. Put in mathematical form this gives:
$ACR_i = LC_{50\,i} / NOEC_{growth\,i}$ = constant · ε_i, where ε_i is a random contribution by compound i and the toxicity values are expressed as µmol/l..
In Figure 4 the data is log-transformed and here this relation takes the form:
$\log ACR_i = \log LC_{50} - \log NOEC_{growth} = 0.58 + \log \varepsilon_i$
This gives a value of 0.58 for log constant or a mean ACR of $10^{0.58} = 3.76$, with a 95% confidence interval of 2.12 to 6.68. For observations of ACRs on single compounds a 95% confidence interval has the following boundaries: 1 and 53. All actual ACRs lie within this range, with the exception of Ethyl propionate (ACR= 0.70).

4 DISCUSSION

4.1 Problems associated with the use of chronic toxicity data

Chronic toxicity date are considered more relevant in environmental protection than acute toxicity data. $NOECs_{growth}$ have therefore been collected to build the dataset under study in this report. This study has shown that using $NOECs_{growth}$ for this purpose poses some problems.
One of these is that with NOECs the differences between the species are smaller than with acute toxicity data like EC_{50}s, making it harder to detect patterns, at least with the present dataset. Smaller differences do not imply that these differences are of not worth studying.
Another problem is that not enough $NOECs_{growth}$ are available when the aim is to build a matrix with data on twenty or more species, about the same number of compounds and with as few as possible missing values. This is illustrated in the present dataset by the fact that taxonomically it is unbalanced. Most species are micro-organisms, like bacteria, algae and protozoans. For a better comparison with a previous analysis and in order to have a better chance of detecting a pattern, a more representative sample of the taxonomical spectrum would have been preferred. Unfortunately this data is not (readily) available, if it exists at all. For good comparability, unfortunately the compounds in the $NOEC_{growth}$ dataset are not the same as in the LC_{50} dataset. Especially Class IV organic compounds, those with a specific mode of action, are lacking. As a result in the present dataset is also chemically unbalanced. Both imbalances have possible influences on observations based on this dataset, like e.g. underestimating the differences that exist between species when using chronic toxicity data.
Finally there are the disadvantages of the NOEC and its statistic properties. The major drawback of the NOEC is its critical dependance on size (number of organisms used) and variability of the experiment (Hoekstra & Van Ewijk, 1993). This makes the data less comparable from a toxicological point of view.

4.2 Patterns in compound toxicity

When evaluating the analysis on compound toxicity it is clear that the intrinsic toxicity of a compound is a major feature. The first component reflects this and more than 75% of matrix variance is explained. The second component although significant explains only 4.5% of the matrix variance. When trying to describe this component care needs to be taken in not giving more importance to this component than is warranted. A possible description of the second component is a differentiation between compounds more toxic to algea and bacteria (bottom half of Figures 1 and 2) and compounds more toxic to animals (top half of Figures 1 and 2).
Based on the first component, compounds can be ranked based on their toxicity. The resulting ranking is as given in Table 8. The ranking found here is very similar to the ranking found in Vaal et al. (1994) using acute toxicity data, which is also given in Table 8. Bromine and the low molecular narcotics have low toxicity. High molecular narcoticals (mostly polar) are more toxic. Next come some more specific working compounds like DCA (polar narcotic), salicylaldehyde (carbonyl reactivity), DNOC and PCP (uncouplers of oxidation) and TPBS (detergent). Heavy metals (especially cadmium and mercury) are the most toxic. This ranking of chemicals based on their overall toxicity is valid for many species.

4.3 Sensitivity Ratios

Presented in Table 8 are mean log $NOEC_{growth}$ values and sensitivity ratios, which are calculated using data on all species. Values for the sensitivity ratios vary between 37 and 7678. There appears to be no relation between either the $NOEC_{growth}$ or the toxicological mode of action and the variation in sensitivity. In contrast there was evidence of a relation with LC_{50} and toxicological mode of action, with the most toxic substances also showing the largest differences between species.

Using $NOECs_{growth}$ to calculate the $SR_{95:5}$ results in quite different values then using LC_{50}s. In most cases the values calculated in this report are larger for compounds with low toxicity (e.g. acetone and propanol in Table 8) and smaller for compounds with high toxicity (e.g. PCP, cadmium and mercury in Table 8). One possible explanation for this can be a result of the way the NOEC is determined. It is usually an experimental concentration and not the result of a calculated concentration-effect relationship and therefore carries more noise. This may cause compounds with little variation in toxicity to appear more variable among species than they really are. The fact that only fifteen species are present also gives a reason. If a thorough search of literature provides five or more additional species, preferably from taxonomic classes not represented in the present dataset, the following is expected. For compounds like the nonpolar narcotics and the polar narcotics the added species are likely to give more data around the center of the distribution. This would result in a smaller $SR_{95:5}$. Compounds with higher toxicity like the heavy metals and organics with a specific mode of action are likely to have some species added to the extremes of their distributions. In this case the $SR_{95:5}$ is likely to be higher.

For compounds with a narcotic mode of action (nonpolar and polar) the $SR_{95:5}$ is larger when calculated with $NOECs_{growth}$ than with LC_{50}s.

In a previous study using acute toxicity data aniline was noticed because of a relatively large $SR_{95:5}$ compared to compounds with a similar mode of action. In the present study the calculated $SR_{95:5}$ is much lower. The most likely explanation for this difference is the lack of a *Daphnia magna* $NOEC_{growth}$, as a representative of the Cladocerans. Cladocerans as a group were the most sensitive for aniline in the LC_{50}-study. Aniline, however, still has a larger $SR_{95:5}$ than the other polar narcotis (Class II).

Dimethoate has a large $SR_{95:5}$, a result that is in agreement with findings from the acute toxicity study and also with other ongoing research. The compounds like dimethoate that inhibit acetylcholine-esterase typically have large $SR_{95:5}$s. But also in this case experimental data for *Daphia magna* is lacking. Since in the LC_{50}-study Cladocerans were among the most sensitive species for organo-phosphate insecticides a low $NOEC_{growth}$ is expected.

4.4 Relation toxicity versus K_{ow}

Figure 3 demonstrates the relationship between the chronic toxicity of the compounds (using the estimated averages from Table 8) and their hydrophobicity as expressed by the log K_{ow}. As also shown in the study with LC_{50} data (Vaal et al., 1994), toxicity increases with increasing log K_{ow}. This relationship is especially valid for compounds with minimum toxicity: nonpolar narcotics. The line drawn in the figure is based on the nonpolar narcotic compounds. The toxicity of the nonpolar narcotics correlates well (r^2=0.99) with the log K_{ow}.

The equation for this regression line is:

$$\log NOEC_{growth}\ (\mu mol/l) = -0.69 * \log Kow + 3.93$$

Most other chemicals, with the exception of the two ester narcotics ethyl acetate and ethyl propionate, are clearly more toxic than the base-line toxicity chemicals due to their reactive nature or specific mode of action. The compounds with the largest deviation from baseline toxicity are allylamine (reactive), aniline (polar narcotic), and DNOC and PCP (uncouplers of oxidation). The vertical bars in Figure 3 show the interval within which 90% of species is expected to fall with their sensitivities (cf. $SR_{95:5}$).

4.5 Acute to chronic ratios

One finding of this study is the strong relationship between the $NOEC_{growth}$ and LC_{50}. This leads to a model stating that the acute to chronic ratio (ACR) has no systematic relation with the level of toxicity or mode of action. The mean ACR is 3.76. For the individual compounds in the dataset the 95% confidence interval for the ACR is from 1 to 50. It was expected that chemicals with different toxicological mechanisms have dissimilar ACRs, while chemicals acting through the same mechanism have similar ACRs.
In a study on predicting chronic lethality to fishes from acute toxicity by Mayer et al. (1994) it is concluded that their linear regression approach is useful for the preliminary assessment of chronic toxicity of chemicals and effluents. They also conclude that predicting chronic no-effect concentrations for survival and growth is possible with their approach for fish species that are difficult to culture under chronic testing conditions. Because of the likelihood of different modes of action between lethal and reproductive effects Mayer et al. (1994) do not recommend estimating reproductive effects using linear regression techniques.
In a report, based on the ECETOC Aquatic Toxicity database which holds data on 368 substances and 122 aquatic test species, ECETOC (1993) gives values for acute to chronic ratios. For 19 substances, other than the ones in this study, they give a range for ACRs of 1 to 28. All of them within the limits of the 95% confidence interval calculated in this study.

4.6 Multivariate statistics in ecotoxicology.

Multivariate statistical techniques help in identifying clusters or groups within the data. This helps by identifying which compounds or species can be treated as a group. For such a group research on one of the members may apply to other group members as well. These groups may be broader or smaller than would be the case on simple criteria. This approach is taken here, but is also tested by Devillers et al. (1988).
Using this multivariate technique may also lead to hypotheses. Belfroid (1994)investigated possible causes for the extreme sensitivity of Daphnids for organophosphorus insecticides; this study was prompted by findings of Vaal et al. (1994) using PCA. Belfroid suggests that this sensitivity could be caused by formation of active metabolites via oxidation in this group of organisms. Since the organophosphorus insecticides from Vaal et al. are not present in this study and no actual NOEC-data on dimethoate is available, the present study can not add new evidence. The same mechanism may account for the high sensitivity of *Daphnids* for aniline, which is also activated by oxydation. Unfortunately also for this compound no $NOEC_{growth}$ is available.
Multivariate statistics can help in generating new ideas for directing research in the field of ecotoxicology and as such is useful. The large amount of data needed is a problem however as is demonstrated in this study, where data on both more compounds and species is greatly missed.

5 CONCLUSIONS

The present study is part of a project investigating the variation in species sensitivity in relation to the toxicity of chemicals. The aim here is to see whether the multivariate statistical approach first used with acute toxicity data in Vaal et al. (1994) also works with chronic toxicity data. There are two reasons to try this. Firstly chronic toxicity data are more relevant in risk assessment. Secondly to see whether the patterns detected with acute toxicity data (Vaal et al., 1994) are reproducible with chronic toxicity data. The multivariate technique used is principal component analysis (PCA) (Kowalski, 1983).

The major conclusions from this study are:

1. The major part of the variation in species sensitivity is determined by the toxicity of compounds and not by intrinsic differences between the species.
2. The compounds can be ordered according to chronic toxicity in an unambiguous way; the ordering is valid for practically all species considered and similar to the ordering according to acute toxicity. It is given in Table 8.
3. No clear *patterns in the sensitivity of the species* are observed.
4. Ratio of mean LC_{50} and $NOEC_{growth}$ is very similar for all compounds. The ratio has a mean value of 4. For the acute to chronic ratio a 95% confidence interval stretching from 1 to 50 has been calculated for the 22 compounds in this study.
5. Mean toxicities of chemicals with a nonspecific mode of action show a strong relation with their octanol-water partition coefficient (K_{ow}) and, as expected, chemicals with more specific modes of action have a higher mean chronic toxicity.

Tables and Figures

Table 1 Species and taxonomic groups used in the analyses.

Species Name	Common Name	Phylum	Class	Abbreviation
Pseudomonas fluorescens	Bacteria	Bacteria	Bacteria	Pseu flu
Pseudomonas putida	Bacteria	Bacteria	Bacteria	Pseu put
Microcystis aeruginosa	Blue-green Algae	Cyanobacteria	Cyanophyceae	Micr aer
Chlorella pyrenoïdosa	Green Algae	Chlorophyta	Chlorophycyae	Chlo pyr
Scenedesmus pannonicus	Green Algae	Chlorophyta	Chlorophycyae	Scen pan
Selenastrum capricornutum	Green Algae	Chlorophyta	Chlorophycyae	Sele cap
Lemna minor	Duckweed	Spermatophyta	Liliatae	Lemn min
Chilomonas paramecium	Cryptomonad	Protozoa	Mastigophora	Chil par
Entosiphon sulcatum	Flagellate Euglenoid	Protozoa	Mastigophora	Ento sul
Uronema parduzci	Protozoan	Protozoa	Ciliata	Uron par
Hydra oligactis	Hydra	Coelenterata	Hydrozoa	Hydr oli
Daphnia magna	Water flea	Arthropoda	Phyllopoda	Daph mag
Oryzias latipes	Medaka, high-eyes	Chordata	Osteichtyes	Oryz lat
Poecilia reticulata	Guppy	Chordata	Osteichtyes	Poec ret
Xenopus laevis	Clawed toad	Chordata	Amphibia	Xeno lae

Table 2 Compounds and chemical classes used in the analyses.

Compound Name	CAS #	Chem. class [1]	Toxicological Mechanism	Abbreviation	Log Kow [2]	MW [3]
Acetone	67641	1	non-polar narc.	Acetone	-0.24	58.1
Heptanol, 1-	111706	1	non-polar narc.	Heptanol	2.72	116.2
Propanol, 1-	71238	1	non-polar narc.	Propanol	0.25	60.1
Ethyl acetate	141786	2	ester narcosis	Ethace	0.73	88.1
Ethyl propionate	105373	2	ester narcosis	Ethpro	1.21	102.1
Aniline	62533	2	polar narcosis	Aniline	0.90	93.1
Cresol, o-	95487	2	polar narcosis	Cresol	1.95	108.1
Dichloroaniline, 2,4-	554007	2	polar narcosis	DCA	2.91	162.0
Nitrotoluene, p-	99990	2	polar narcosis	NiTolu	2.37	137.1
Pyridine	110861	2	polar narcosis	Pyridine	0.65	79.1
Allylamine	107119	3	reactive	Allylam	0.07	57.1
Salicylaldehyde	90028	3	Carbonyl react.	Saliald	1.81	122.1
Dimethoate	60515	4	AChE inh. OP [4]	Dimeth	0.78	229.2
Dinitrocresol, 4,6-,o-	534521	4	Unc. of Oxid. [5]	DNOC	2.13	198.1
Pentachlorophenol	87865	4	Unc. of Oxid. [5]	PCP	2.14	266.3
Tetrapropylene Benzenesulfonate	11067815	D		TPBS		325.1
Na Br	7647156	H		Br^-		79.9
$CdCl_2$	10108642	M		Cd^{2+}		112.4
$HgCl_2$	7487947	M		Hg^{2+}		200.6
$Na_2Cr_2O_7$	7778509	M		Cr^{6+}		52.0

[1] Chemical classification of organic compounds according to Verhaar et al. (1992):
1 = inert chemicals, 2 = less inert chemicals,
3 = reactive chemicals, 4 = specifically acting chemicals;
D = detergens/surfactant, H = halogen, M = Heavy metal.

[2] Experimental Log Kow values retrieved from Thor Database (Leo and Weininger, 1989).

[3] Molecular Weight in g/mole

[4] Acethyl Choline Esterase Inhibitor, Organophosphorus-type

[5] Uncoupler of Oxidation.

Table 3 Matrix of data, mean log $NOEC_{growth}$ (μmol/l) per species-compound combination, overall mean, standard deviation and percentage of matrix filled per species and per compound.

Compounds	Pseu flu	Pseu put	Micr aer	Chlo pyr	Scen pan	Sele cap	Lemn min	Chil par	Ento sul	Tiron par	Hydr oli	Daph mag	Oryz lat	Poec ret	Xeno lae	Average	St.dev.	n = 15	% avail. data
Acetone	-	4.47	3.96	4.77	5.01	5.08	4.78	4.78	3.09	4.47	-	4.24	-	-	-	4.47	0.59	10	66.7%
Heptanol	-	2.76	1.54	2.19	2.38	2.48	-	3.00	2.68	2.17	-	-	-	-	-	2.40	0.45	8	53.3%
Propanol	-	4.65	3.63	4.28	4.70	4.52	-	3.46	3.18	3.98	-	-	-	-	-	4.05	0.58	8	53.3%
Ethace	-	3.87	3.80	-	2.23	4.36	-	4.57	4.00	3.85	-	-	-	-	-	3.81	0.75	7	46.7%
Ethpro	-	3.42	2.14	3.50	-	3.14	-	4.08	4.05	3.81	-	-	-	-	-	3.45	0.67	7	46.7%
Aniline	3.10	3.14	0.24	2.07	2.08	2.03	-	3.43	2.41	2.99	-	-	-	-	-	2.39	0.97	9	60.0%
Cresol	-	2.48	1.81	2.50	2.27	2.78	-	3.09	2.50	2.46	-	-	-	-	-	2.48	0.37	8	53.3%
DCA	1.79	-	0.79	-	1.30	-	0.79	-	-	-	1.30	-0.43	1.30	0.79	0.79	0.93	0.62	9	60.0%
NiTolu	1.86	-	1.37	-	1.86	-	1.86	-	-	-	1.86	-	2.37	1.86	2.37	1.93	0.32	8	53.3%
Pyridine	-	3.63	2.55	3.28	3.36	2.80	-	1.69	2.14	3.36	-	-	-	-	-	2.85	0.68	8	53.3%
Allylam	-	4.09	0.79	2.45	-	2.36	-	2.13	2.61	4.74	-	-	-	-	-	2.74	1.31	7	46.7%
Saliald	-	1.91	1.12	1.91	1.60	1.65	-	1.43	1.06	1.65	-	-	-	-	-	1.54	0.32	8	53.3%
Dimeth	3.14	-	2.14	-	2.64	-	2.14	-	-	-	2.64	-0.04	2.64	-0.36	2.14	1.90	1.24	9	60.0%
DNOC	1.70	-	1.21	-	1.70	-	0.21	1.44	1.44	-1.22	0.21	0.93	0.70	0.70	0.21	0.77	0.84	12	80.0%
PCP	-	-	0.57	-	-0.43	-0.12	0.57	-	-	-	-0.92	0.08	0.08	0.08	-0.92	-0.11	0.55	9	60.0%
TPBS	1.99	-	1.99	-	0.49	-	0.49	-	-	-	0.49	-	1.49	1.49	1.49	1.24	0.66	8	53.3%
Br	4.49	-	4.49	-	4.49	-	4.49	4.17	3.16	4.76	3.99	5.18	4.99	3.49	3.49	4.27	0.63	12	80.0%
Cd^{2+}	-	-0.02	-0.09	1.10	0.56	0.45	-	-0.19	0.45	-0.55	-	-1.80	-	-	-	-0.01	0.83	9	60.0%
Hg^{2+}	-	-1.35	-1.66	0.68	-	-0.53	-	-1.26	-0.96	-0.48	-	-	-	-	-	-0.79	0.78	7	46.7%
Cr^{6+}	0.79	0.86	0.65	0.09	0.34	0.45	0.34	-2.64	2.59	1.29	1.34	0.06	1.83	1.83	1.34	0.74	1.18	15	100.0%
Average	2.36	2.61	1.65	2.40	2.15	2.25	1.74	2.21	2.29	2.48	1.36	1.03	1.92	1.24	1.36	2.01		178	
St.dev.	1.15	1.82	1.51	1.40	1.55	1.74	1.77	2.18	1.33	1.98	1.52	2.41	1.49	1.21	1.37		1.67		
n = 20	8	13	20	12	17	14	9	15	15	15	8	8	8	8	8	178		300	
% avail. data	40.0%	65.0%	100.0%	60.0%	85.0%	70.0%	45.0%	75.0%	75.0%	75.0%	40.0%	40.0%	40.0%	40.0%	40.0%				59.3%

Table 4 Analysis of variance, species and compounds as sources of variation.

Source of Variation	SS	df	MS	F	P-value	F crit, $\alpha = 0.05$
Species	249.22	19	13.12	7.71	<0.001	1.63
Compounds	87.28	14	6.23	3.67	<0.001	1.73
Error	452.43	266	1.70			
Total	788.93	299				

Table 5 Analysis of patterns in toxicity of compounds, description of principal components.

Component	SS Expl. (%) [1]	Total SS Expl. (%)	Variance expl. (%)	Total Variance expl. (%)	Normalized eigenvalue	Residual matrix variance	Sign. [2]
1	81.2	81.2	76.5	76.5	12.2	0.24	Yes
2	7.2	88.4	4.5	80.9	1.1	0.19	Yes
3	4.5	92.9	2.5	83.5	0.7	0.17	Yes
4	3.0	95.9	1.1	84.6	0.5	0.15	Yes

[1] Sum of squares explained

[2] Significance of the principal components at the 5% confidence level based on SIMCA cross validation rules (Umetri, 1992).

Table 6 Analysis of patterns in toxicity of compounds, loadings of species as variables for the first two components (PC1 and PC2).

Variable	Loading PC1	Loading PC2
Pseu flu	0.25	-0.27
Pseu put	0.27	0.02
Micr aer	0.27	0.00
Chlo pyr	0.26	-0.37
Scen pan	0.29	-0.27
Sele cap	0.27	-0.15
Lemn min	0.27	-0.06
Chil par	0.23	-0.34
Ento sul	0.23	0.44
Uron par	0.24	0.12
Hydr oli	0.28	0.07
Daph mag	0.22	0.13
Oryz lat	0.29	0.15
Poec ret	0.20	0.51
Xeno lae	0.28	0.21

Table 7 Analysis of patterns in toxicity of compounds, scores of compounds as object for the first two components (PC1 and PC2).

Object name		PC 1			PC 2		
	Initial SDO [1]	Score	OLEV [2]	SDO [3]	Score	OLEV [2]	SDO [3]
Acetone	19.76	-5.35	0.36	0.98	0.89	0.35	0.51
Heptanol	0.31	-0.28	0.02	0.27	-0.05	0.02	0.27
NiTolu	1.27	-0.51	0.03	1.12	-1.27	0.09	0.32
Propanol	10.53	-4.31	0.29	0.59	0.45	0.28	0.48
Ethace	7.25	-3.47	0.23	1.62	-0.74	0.23	1.39
Ethpro	4.10	-2.69	0.18	0.85	-0.46	0.18	0.75
Aniline	1.83	-0.14	0.01	1.82	0.21	0.02	1.79
Cresol	0.30	-0.48	0.03	0.18	0.15	0.03	0.17
DCA	2.02	1.67	0.11	0.28	-0.09	0.11	0.28
Pyridine	2.04	-1.52	0.10	0.79	0.28	0.10	0.75
Allylam	2.35	-0.91	0.06	1.97	-0.65	0.07	1.76
Saliald	1.80	1.65	0.11	0.35	0.58	0.11	0.15
Dimeth	3.92	-0.95	0.06	3.36	1.68	0.13	1.93
DNOC	7.43	2.55	0.17	2.33	0.39	0.17	2.20
PCP	13.22	4.33	0.29	1.30	-0.03	0.28	1.30
TPBS	2.27	1.22	0.08	1.41	-1.10	0.11	0.83
Br^-	30.25	-6.01	0.41	1.82	-0.23	0.39	1.77
Cd^{2+}	13.26	4.67	0.32	0.58	0.73	0.31	0.27
Hg^{2+}	24.32	7.15	0.48	1.28	1.26	0.47	0.50
Cr^{6+}	14.77	2.66	0.18	7.71	-2.50	0.24	1.46

[1] Initial SDO: Standard deviation of object after centering

[2] OLEV: Object leverage

[3] SDO: Residual standard deviation of object.

Table 8 Ordering of compounds by their average toxicity to species, Sensitivity Ratio's and Acute to Chronic Ratio's.

Object name	log NOEC		(µmol/l) [1]	log LC50		(µmol/l) [4]	ACR
	mean	st.dev.	SR95:5 [2]	mean	st.dev.	SR95:5 [4]	
Br^-	4.43	0.68	167				
Acetone	4.16	0.74	278	5.13	0.51	4	9.35
Propanol	3.69	0.67	163	4.58	0.62	50	7.77
Ethyl acetate	3.40	0.74	262	3.58	0.62	106	1.53
Ethyl propionate	3.06	0.68	168	2.91	0.56	66	0.70
Pyridine	2.54	0.66	150	3.53	0.76	252	9.90
Allylamine	2.35	0.99	1813	2.61	0.61	65	1.82
Phenol				2.92	0.47	22	
Benzene				3.05	0.51	39	
Dimethoate	2.25	1.04	2694 [3]				
Nitrotoluene	2.15	0.48	37				
Cresol	2.15	0.54	60	2.41	0.38	16	1.84
Heptanol	2.07	0.57	74	2.67	0.45	26	3.98
Aniline	2.03	0.88	789	2.81	1.00	2398	6.01
TPBS	1.42	0.63	118				
Salialdehyde	1.25	0.53	54	1.58	0.45	15	2.14
Cu^{2+}				1.24	0.91	1200	
DCA	1.22	0.60	96 [3]				
DNOC	0.87	0.78	367 [3]				
Cr^{6+}	0.74	1.18	7678 [3]				
PCP	0.19	0.62	107	0.44	0.85	468	1.76
Malathion				0.22	1.07	4349	
Lindane				0.19	0.76	169	
Parathion				-0.24	1.19	3429	
Cd^{2+}	-0.04	0.73	245	1.18	1.03	1010	16.62
Hg^{2+}	-1.03	0.85	612	-0.14	0.95	3240	7.86
Dieldrin				-1.15	0.87	433	

[1] Missing species-compound combinations estimated with first component of Compounds x Species PC model (Data from tables 6, 7)

[2] Sensitivity ratio 95% percentile : 5% percentile of the distribution of toxicity values over all species.

[3] The distribution of available plus estimated data differs significantly from the normal distribution, based on tests on both skewness and kurtosis (D'Agostino and Stephens, 1986) two-sided, α=0.05.
Parametric estimation assumes that data are normally distributed.
The number of species is insufficient for a reliable non-parametric estimate.

[4] Data from Vaal et al, 1994

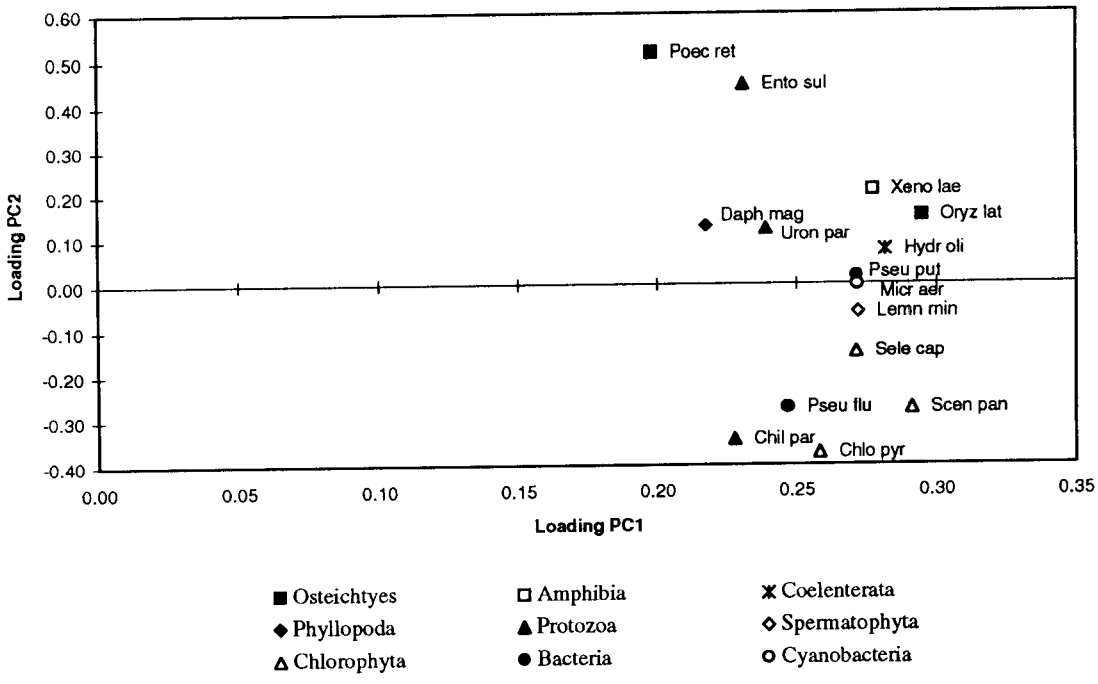

Figure 1 Loadings of species that coincide with patterns in compound toxicity.

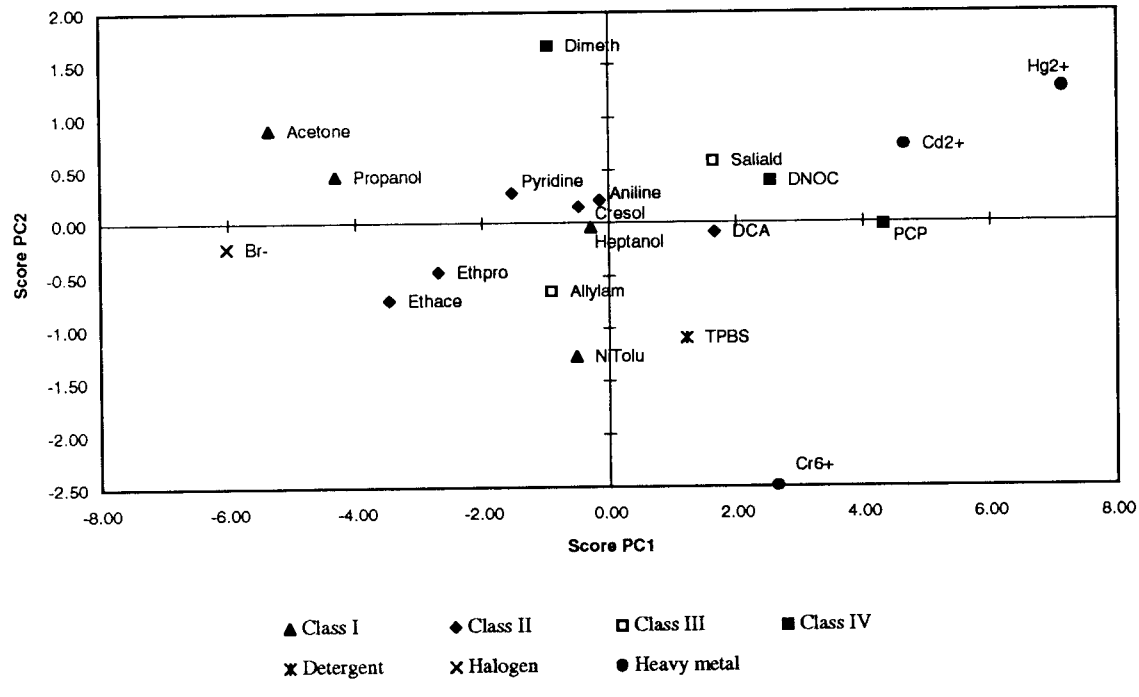

Figure 2 Patterns in compound toxicity, plot of scores of compounds.

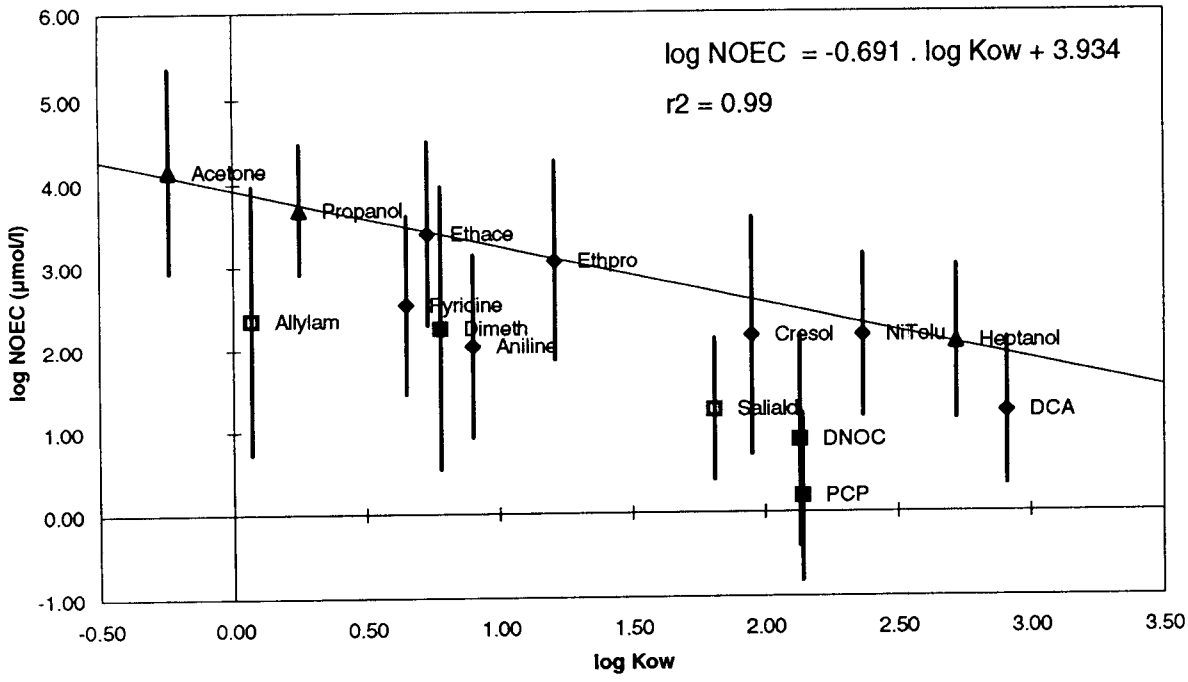

Figure 3 Average toxicity of compounds in relation to their hydrophobicity (log K_{ow}). Symbols as in Figure 2. Vertical bars indicate 90% confidence interval for observations on species (cf. $SR_{95:5}$).

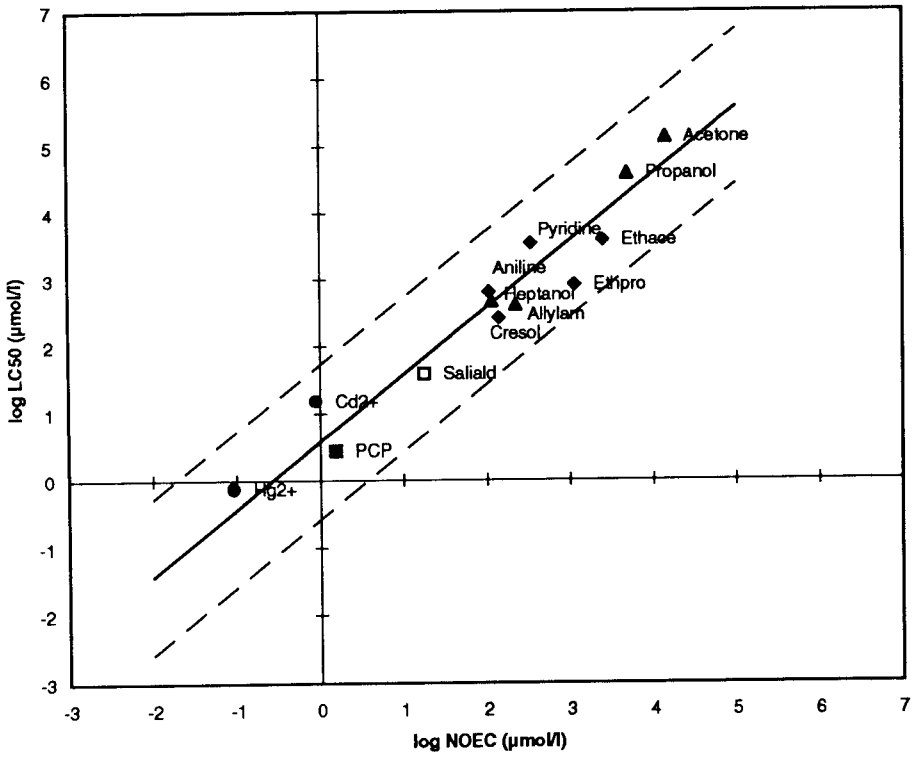

Figure 4 Relationship between acute and chronic toxicity. Symbols as in Figure 2. Dashed lines indicate 95% confidence interval for observations on acute to chronic ratio for individual compounds..

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Appendices

1: Matrix of data, origin of data (link to articles/reports). Full references are listed with the Literature Reference in the report.

2: Principal Component Analysis of Species sensitivity

Table a	Analyses of patterns in species sensitivity, description of principal components.
Figure a	Loadings of compounds that determine patterns in species sensitivity.
Figure b	Patterns in species sensitivity, plot of scores of species.

Appendix 1

Compounds	Pseu flu	Pseu put	Micr aer	Chlo pyr	Scen pan	Sele cap	Lemn min	Chil par	Ento sul	Tron par	Hydr oli	Daph mag	Oryz lat	Poec ret	Xeno lae
Species												h			
Acetone	-	n	n	n	n	n	c	n	n	n	-		-	-	-
Allylam	-	n	n	n	-	n	-	n	n	n	-	-	-	-	-
Heptanol	-	n	n	n	n	n	-	n	n	n	-	-	-	-	-
NiTolu	m	-	m	-	m	-	m	-	-	-	m	-	m	m	m
Propanol	-	n	n	n	n	n	-	n	n	n	-	-	-	-	-
Ethace	-	n	n	-	n	n	-	n	n	n	-	-	-	-	-
Ethpro	-	n	n	n	-	n	-	n	n	n	-	-	-	-	-
Aniline	o	n	n	n	n	n	-	n	n	n	-	-	-	-	-
Cresol	-	n	n	n	n	n	-	n	n	n	-	-	-	-	-
DCA	m	-	m	-	m	-	m	-	-	-	m	i	m	m	m
Pyridine	-	n	n	n	n	-	-	n	n	n	-	-	-	-	-
Saliald	-	n	n	n	n	-	-	n	n	n	-	-	-	-	-
Dimeth	m	-	-	-	m	-	m	-	-	-	m	i	m	m	m
DNOC	m	-	m	-	m	-	m	e	b	c	m	i	m	m	m
PCP	-	-	m	-	m	l	m	-	-	-	m	p	m	m	m
TPBS	m	-	m	-	m	-	m	-	-	-	m	-	m	m	m
Br-	m	-	m	-	m	-	m	e	b	c	m	i	m	m	m
Cd2+	-	n	n	n	n	n	-	n	n	n	-	q	-	-	-
Hg2+	-	n	n	n	-	n	-	n	n	n	-	-	-	-	-
Cr6+	f, m	d	a, j, m	a	a, m	a, k	g, m	e	b	c	m	p	a	m	m

a: Adema et al., 1981
b: Bringmann, 1978
c: Bringmann & Kühn, 1980a
d: Bringmann & Kühn, 1980b
e: Bringmann et al., 1980
f: Codina et al., 1993
g: Cowgill et al., 1991
h: De Wolf et al., 1988
i: Deneer et al., 1988
j: Hanstveit et al., 1985
k: Nyholm, 1991
l: Shigeoka, 1988
m: Slooff & Canton, 1983
n: Slooff et al., 1983
o: Tørsløv, 1993
p: Van Leeuwen et al., 1987
q: Van Leeuwen et al., 1985

Appendix 2

Table a Analyses of patterns in species sensitivity, description of principal components.

Component	SS Expl. (%) [1]	Total SS Expl. (%)	Variance expl. (%)	Total Variance expl. (%)	Normalized eigenvalue	Residual matrix variance	Sign. [2]
1	33.4	33.4	15.8	15.8	5.0	0.52	Yes
2	24.8	58.2	14.0	29.7	3.7	0.44	Yes
3	17.4	75.6	11.0	40.7	2.6	0.37	Yes
4	11.8	87.4	7.0	47.7	1.8	0.32	Yes

[1] Sum of squares explained

[2] Significance of the principal components at the 5% confidence level based on SIMCA cross validation rules (Umetri, 1992).

Appendix 2

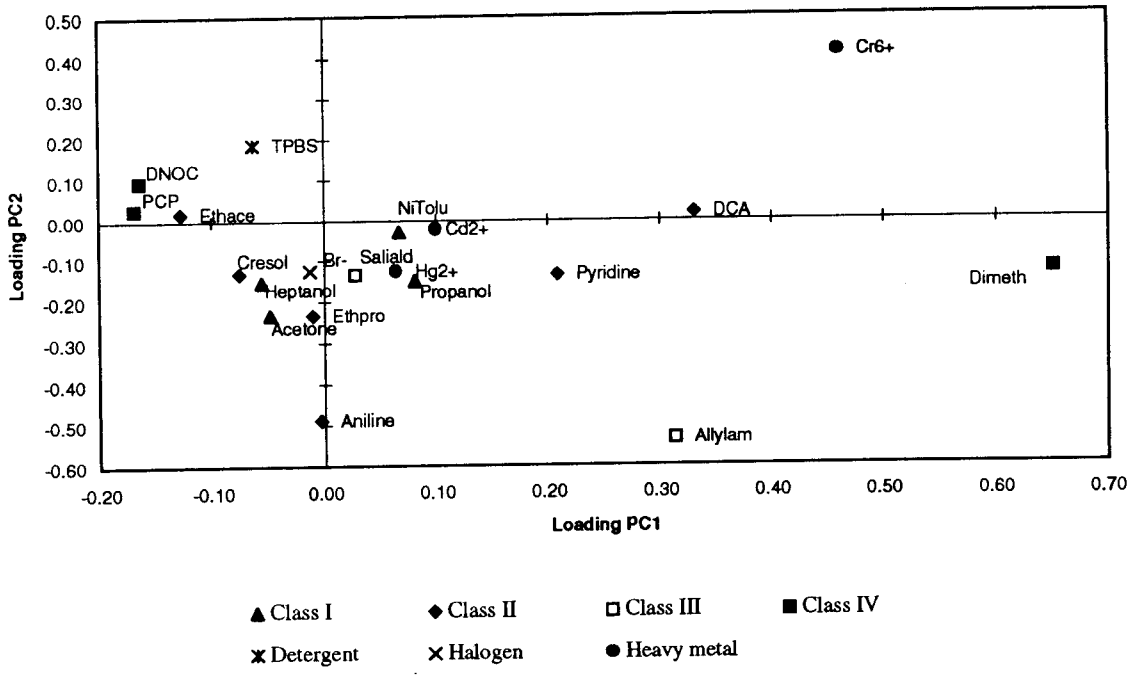

Figure a Loadings of compounds that determine patterns in species sensitivity.

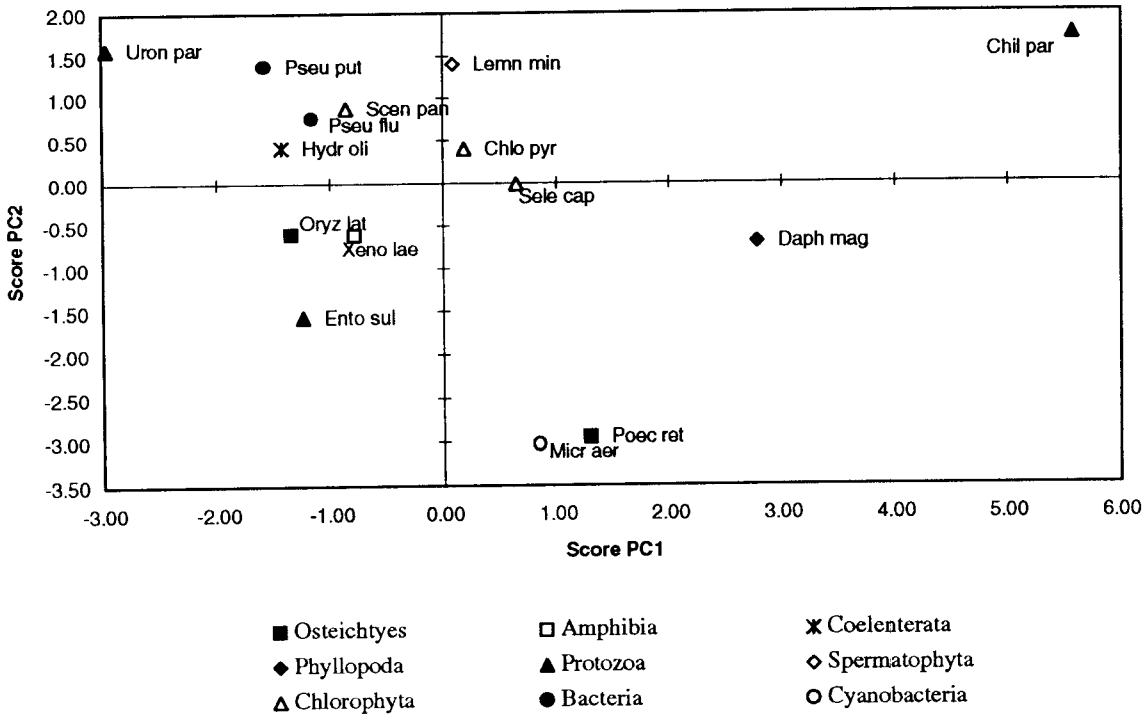

Figure b Patterns in species sensitivity, plot of scores of species.