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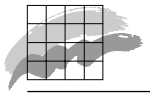
Differences in Species Sensitivity
and Acute – Chronic Relations

NERI Technical Report, No. 325



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2000

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Data sheet

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Abstract: The variation in sensitivity to a toxicant between species is described by the mean Sensitivity Ratio, SR95:5, (the median of the ratio between the 95th. and 5th percentile of the sensitivity distribution) and the safety factor SF95 (the left 95% confidence limit of the median of all tests). The sensitivity measures were calculated for birds, fish, invertebrates, terrestrial and aquatic plants. The values varied from 30 to 600 in the different groups. Cumulative frequency plots revealed that these figures do not protect more than half of the species given a test value for one insensitive species.

The relation between acute and chronic/long term toxicity measure was also studied for mammals, birds, fish, and invertebrates. Only for aquatic invertebrates and fish a correlation coefficient of any significance was observed. For these organisms acute mortality was compared with long term mortality, i.e. identical endpoints. The uncertainty of predicting long term mortality from acute mortality is, however, very uncertain calling for safety factors between 100 and 800 for aquatic invertebrates. Safety factors of this size make the extrapolation of little importance in legislation and general risk assessment.

Keywords:	Safety factor, Sensitivity Ratio, Sensitivity distribution, birds, fish, invertebrates, plants, acute to chronic ratio, extrapolation from single species test
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Foreword

It has become common practice to protect the environment from hazardous chemicals by use of risk assessment to establish environmental concentration at which only limited damage to the ecosystem can be expected. The methods and tools applied in the risk assessment need constant evaluation to secure that the methodology is adequate. As new knowledge surfaces the risk assessment procedures develops. The present report is a contribution to the development of safety factors used to account for the uncertainty when

- extrapolating from the results of test with a single species in the laboratory to many species in real ecosystems
- extrapolating from acute to chronic or long term effects.

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The authors would like to thank Dr. C. Boutin, Canadian Wildlife Service for access to plant data; Drs. S. E. Larsen and C. Damgaard, NERI, for statistical advice; Cand scient C. Hansen and Cand scient N. Seidelin, EPA, for comments on the manus; Mrs. B. Thestrup, L. Bødskov, K. Møgelvang and J. Jacobsen for word processing and drawings.

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Summary

In this report two factors are studied which have implications for the size of safety factors used in pesticide risk assessment: the variability in species sensitivities, and the relationship between acute LC50's and chronic NOEC's.

Variability in species sensitivities

In risk assessment of new pesticides and in many other cases, the toxicity of the chemical towards various groups of organisms is estimated from laboratory testing with one or a few species. The species tested are supposed to represent all species of a group of organisms or all the species of that particular group in the ecosystem(s), which is the scope of the assessment. In real ecosystems there is a number of species, sometimes many species, Which have different sensitivities to any given toxicant.

The variation in sensitivity of various species presents a challenge to risk assessors because it is essential to estimate how many species are protected at a certain environmental concentration. To estimate a safe concentration for the majority of all species, a safety or extrapolation factor is applied to the safe concentration derived from a single species test. It can be said that the safety factor should take account of the variation in sensitivity between species because we do not know whether the test species is the most sensitive one.

So why don't we use the most sensitive species as test species? Because there is no such species for all compounds. In the report it is illustrated that the relative sensitivity of the most frequently used test species changes considerably from one chemical to another for all the groups of organisms we have investigated i.e. aquatic and terrestrial plants, invertebrates, fish, and birds. However studies of birds have revealed that although there is no 'most sensitive' species, the sensitivity of a species does not vary randomly between chemicals. Some species on average are more sensitive than other species.

If we have to carry out the extrapolation from test species to all the species it represents in the real ecosystems, what safety factor should then be applied? From a scientific point of view it depends on the difference between the most and the least sensitive species. The expression most and least is however difficult to handle because they cannot be calculated. The point expressing the concentration where for example only 5% of all species are more sensitive is called the 5th percentile and the point where 95% of all species are more sensitive is the 95th percentile and they can generally be estimated. The distance between these two concentrations represents the variation in sensitivity between species towards that chemical or equivalently the width of the sensitivity distribution. It is often expressed as the factor or ratio between the 95th and the 5th percentile and called the Sensitivy Ratio, SR95:5.

For every chemical a SR95:5 can be established if a sufficient number of tests are available. But this is exactly the problem in many cases as only one or two species usually have been tested. To establish a general safety factor then it is necessary to look for a general picture among the compounds for which several species have been tested. What size do SR95:5 for pesticides have? This can be illustrated in a graph showing the cumulative frequency distribution of SR95:5, that is the percentage of pesticides having a SR95:5 lower or equal to x.

In algae (Figure 0.1) 20% of the pesticides have a SR95:5 which extrapolated from the figure is greater than 2.000.

In terrestrial plants, aquatic invertebrates and fish, 20% of the pesticides (or other reactive chemicals) have a SR95:5 greater than 5.000, 800 and 150 respectively. A similar graph for birds is presented in the Ecoframe Report (EPA 1999, Figure 4.5-1.). From that graph it is estimated that approximately 20-25% of pesticides have a SR95:5 greater than 40 and it would take more than the twice that number (ca. 100) to cover 95% of all pesticides tested against birds. From these figures it can be seen that the cumulative frequency curve of sensitivity ratios flattens at frequencies above c. 80%. This feature of the frequency curves implies that it takes relatively very high safety factors to cover the species sensitivity distribution for the 20% pesticides with the widest sensitivity distribution.

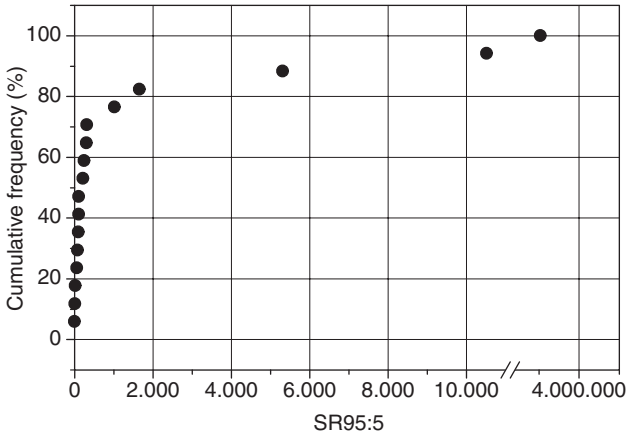

Figure 0.1. Cumulative frequency of SR95:5 for reactive substances in algae tests.

SR95:5 is proportional to the safety factor needed to protect a certain percentage of species. The SR95:5 indicates the distance between a very insensitive species and very sensitive species and consequently gives the safety factor necessary to apply to protect a sensitive species when the test is performed with a insensitive species.

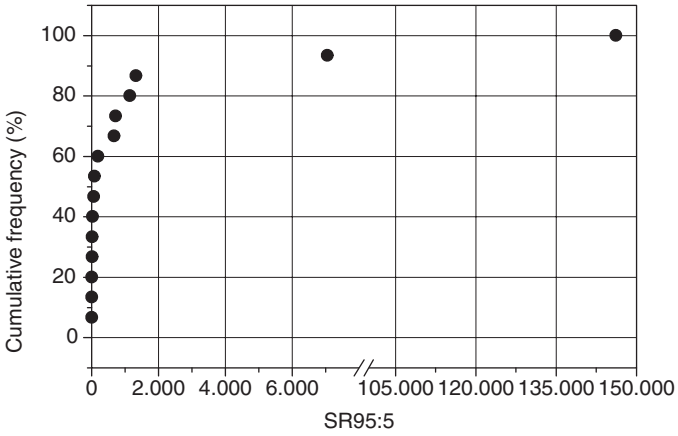

Figure 0.2. Cumulative frequency (%) of SR95:5 for reactive substances in aquatic invertebrate tests.

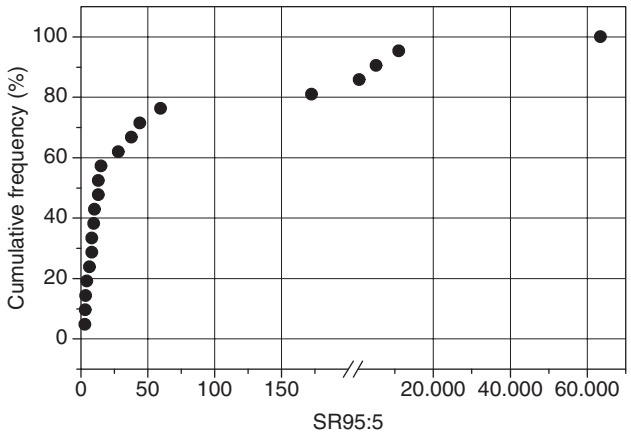

Figure 0.3. Cumulative frequency (%) of SR95:5 for reactive substances in fish tests.

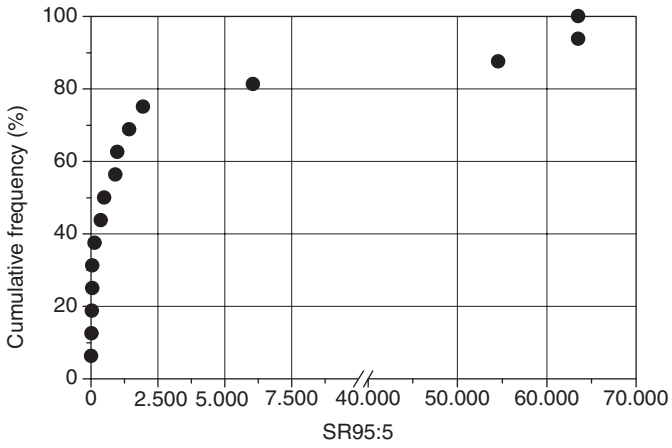

Figure 0.4. Cumulative frequency (%) of SR95:5 for reactive substances in terrestrial plant tests.

Another way of calculating safety factors accounting for the difference in species sensitivity has been published by Luttik and Aldenberg (1997). They suggested the use of a general sensitivity distribution for all species towards all tested compounds. An average safety factor protecting 95% of all species with 95% certainty against

an average compound can then be generated as the median LC50 divided by the left 95% confidence limit of the 5th percentile (the SF95). In the data set presented in the present report, the SF95 varied from 593 for aquatic plants, via 473 for aquatic invertebrates, 280 for terrestrial plants, 78 for fish to 33 for birds, Table 0.1.

Table 0.1. Inter-taxon comparison of variation in sensitivity to pesticides based on the sensitivity ratio (SR95:5) and the safety factor (SF95). The SR95:5 is the ratio between the 95th and the 5th percentile. The SF95 is the geometric mean of all data divided by the left 95% confidence limit of the 5th percentile.

Taxon	Biotope	SR95:5	SF95
Invertebrates	aquatic	355	473
Invertebrates	terrestrial	437	-
Plants	terrestrial	245	280
Birds	terrestrial	32	33
Plants	aquatic	501	593
Fish	aquatic	71	78

Actually the SF95 is numerically similar to the average SR95:5, which also is presented in Table 0.1. The average safety factor is not based on a worst case approach because the safety factor is estimated for an average compound indicating that half of the compounds have a greater variance exactly as it is for the average SR95:5 which is equal to the 50% cumulative frequency in Figure 0.1 – 0.4.

Relations between acute and chronic toxicity measures

The relationship between acute and chronic measures was analysed by use of linear regression for aquatic organisms, mammals and birds. The aim was to answer the question: Is there such a strong correlation between acute and chronic or long term toxicity that low acute toxicity values guaranties that values in chronic tests also will be insignificant and the chronic test therefore is not worth the effort?

In data from the pesticide legislation process no relation between five-day feeding LC50 and NOEC reproduction of birds was established. A weak relation between NOEC of reproduction and acute LD50 was seen for birds with a coefficient of determination, $r^2 = 0.35$.

For mammals a r^2 at 0.32 was found for the regression between acute toxicity and NOEC measured as NOEL or NOAEL (Figure 0.5c). The effect measures applied in the analysis of mammalian chronic tests contained a mixture of different endpoints, as the result of the most sensitive parameter is included in the database. The theoretical basis of a correlation between acute and chronic measures is not obvious when it involves different toxicological mechanisms.

In aquatic animals a conversion of acute values to long-term (mortality) values was more apparent. A r^2 of 0.73 was found for acute LC50 (48 h) and LC50 (21 d) mortality in *Daphnia* (0.5b). Similar values are reported in the literature from studies of aquatic invertebrates and

fish. A better correlation between acute and long-term values is expected when similar tests are used and only the duration of the test varies. This is often the case in aquatic animals.

In principle, the conversion from acute LC50 to chronic NOEC depends on three aspects:

1) The conversion from acute LC50 to acute NOEC; 2) the conversion from acute NOEC to chronic NOEC; 3) the inclusion of a safety margin accounting for x% of the species variability. Typically x is taken to be 95%.

When using regression equations, the first two factors are combined and quantified for different LC50 values by the intercept and slope of the regression equation.

The third factor, the uncertainty margin, can be calculated from the variation in the regression data. Depending on the amount of variation in different data set, the uncertainty factor ranged from 7.9 to 100.7 for aquatic animals. In combination with the first safety factor, an inclusive, regression based acute to chronic ratio (RACR) can be calculated accounting for 95% of the variation in the data. For a large data set on acute to chronic relations in fish and daphnids for different compounds presented in Sloof *et al.*. (1986), we estimated the RACR at approximately 500. In another data set (Clausen 1998) containing only results from tests with pesticides and crustaceans, the RACR was estimated at 807.

The uncertainty factor is not fixed but varies with the relative toxicity of the compound. The figures given above refer to the average LC50. For crustaceans in the Clausen data set the RACR varies between 694 and 1765 depending on the position in relation to the mean effect concentration, lowest at low effect concentrations (high toxicity).

For birds an analyses of a small data set relating acute LD50 to reproductive NOEC was carried out, revealing an RACR of approximately 100.

It is concluded that significant relation between acute and chronic (long-term) effect occurred in some organisms and for some types of data. In the data sets where such relations exists there was a considerable uncertainty attached to predictions of chronic effects from acute effects. This uncertainty calls for safety factors of magnitude 100 for birds and 500 - 1700 for crustaceans to cover 95% of the variation. Safety factors of this size make predictions of chronic effects from acute tests of little value in most practical situations.

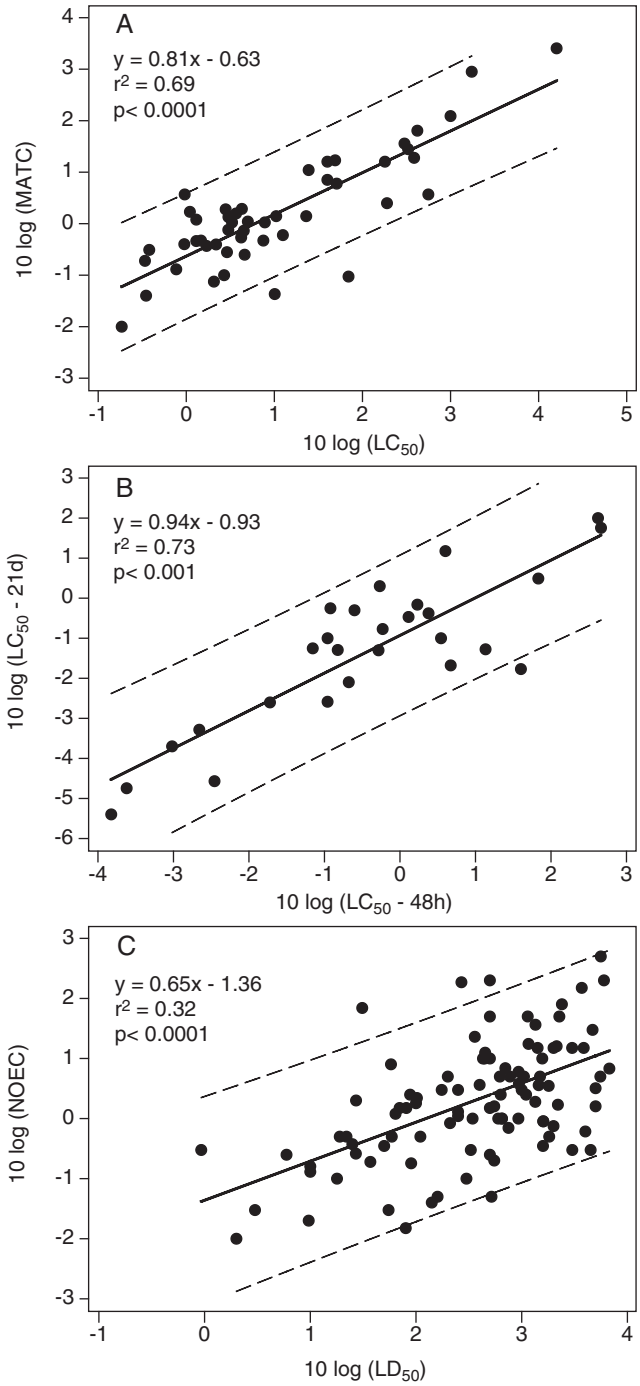

Figure 0.5. Regressions with prediction limits of log(long term or chronic values) against log(acute values). A. Fish and crustaceans exposed to pesticides, B. Crustacean data for acute and long-term LC50 values, C. Mammalian data (NOEC measured as NOEL or NOAEL. Estimates of regression coefficients and of prediction intervals are given in the text.

1 Introduction

In order to protect the ecosystem against adverse effects of pesticides and other chemicals, different risk assessment procedures have been developed. These enable the calculation of environmental concern levels on the basis of laboratory toxicity data and the application of 'safety factors', 'assessment factors' or 'uncertainty factors'. These factors account for the uncertainties in the extrapolation from limited laboratory data to the species rich and variable environment of the field. They are important scaling parameters in risk assessment and have a marked impact on the quality of regulations and the level of environmental protection.

A primary goal of risk assessment for a pesticide is to determine whether the predicted environmental concentration will have any toxic effects on species in nature. The calculation in the assessment is typically based on results from laboratory standard toxicity tests with a few species, e.g. one daphnid, one fish, one alga, one earthworm, one bird and one mammal. Each test species is assumed to represent a species assemblage in nature such as freshwater invertebrates, freshwater fish etc. The assessment is straightforward if the test species can be assumed to be the most sensitive within the group it represents, - or one of the most sensitive, - towards all toxicants. Then all or most species would be protected if the environmental concentration is below NOEC of the test species.

The variation between species may differ between environments and toxicant groups. Only limited attention has been paid to comparative analysis of the variation in species sensitivity between environments, so this subject forms an important part of the present study. The variation in species sensitivity between toxicant groups has obtained more attention in the literature. These relationships have been studied by e.g. Vaal *et al.*. (1997a, b), who found that for aquatic animals, the non-reactive organic molecules, so called narcotics, show the least variation in sensitivities between species. High variation was found for specifically acting chemicals, such as pesticides.

The use of variable safety values has been proposed on various occasions in the literature (Kooijman 1987, Van Straalen and Denneman 1989, Wagner and Løkke 1991, Aldenberg and Slob 1991, Jagoe and Newman 1997). The general approach is to start with a description of the distribution of the data. This is then used to calculate a concentration at which, for example, only 5% of the species can be expected to be affected. Subsequently, a correction is calculated which takes into account how many measurements have been used for estimating the parameters of the distribution. If few data are available, this results in large safety factor. If more data are available, the safety factor becomes smaller. It should be mentioned that a protection level of 95% of all species might not always be sufficient if there are species of high conservation value among the 5% affected species.

The present directives for risk assessment of chemicals of the European Union, as described in an OECD report (OECD 1992) use a

tiered approach with a stepwise calculation of safety values (Table 1.1.).

In this approach it is reflected that the endpoint of the test is of importance for the extrapolation to "safe environmental concentrations. Long term tests with sublethal endpoints such as reproduction, tumours etc., may be more sensitive than acute lethal tests. To extrapolate from acute EC50-values to no effect levels, a factor 10 is applied. Furthermore, the assumption of a correlation between acute and chronic effects is some times used to decide whether chronic test is needed. The relationship between acute and chronic (long-term) effects is dealt with in the present report.

Table 1.1. Assessment factors applied according to the modified EPA method (OECD 1992, Emans *et al.* 1993)

Available information		**Assessment factor**
1.	Lowest acute L(E)C50 value or QSAR estimate for acute toxicity	1000
2.	Lowest acute L(E)C50 value or QSAR estimate for minimal algae/crustaceans/fish	100
3.	Lowest NOEC value or QSAR estimate for chronic toxicity	10^x
4.	Lowest NOEC value or QSAR estimate for chronic toxicity for minimal algae/crustaceans/fish	10

x = after comparison of this value with the lowest acute L(E)C50 the lowest value should be selected.

1.1 Aims and outline of the report

First aim: inventory of sensitivity differences

The first aim of the study is to create an overview of the literature data about variation in sensitivity of species as has been observed for different environments, for different organism groups and in relation to different chemicals. This overview is furthermore used to illustrate the variation in relative sensitivity of one species when exposed to different compounds.

Due to scarcity of data for other groups, the inventory had to be limited to the following taxa/environment combinations:

a) aquatic and terrestrial invertebrates,

b) fish,

c) birds,

d) aquatic algae and plants,

e) terrestrial plants.

For each of these groups, the variability of the sensitivity data was analysed per toxicant, and per major toxicant group.

Second aim: inventory of distances between acute and chronic measures and calculation of safety limits

The second aim was to investigate the correlation between acute measures and chronic measures. This aim was partly pursued by reviewing literature data on LC50-NOEC conversions and partly by analyses of data by means of regression. The acute to chronic relationship was investigated for aquatic crustaceans, fish, birds and mammals.

2 Species differences in sensitivity

In this chapter the variation in sensitivity when different species are exposed to the same toxicant, is analysed. An inventory is presented of sensitivity data, measured as EC50 values, for different groups of organisms. The selected groups include aquatic and terrestrial invertebrates, fish, aquatic algae, aquatic plants, terrestrial plants and birds.

The references used were selected for containing LC50 values for preferably four or more species per compound, the measurement of the sensitivity data in comparable bioassays and exposure times, and the 'inclusiveness' and 'recency' of the references. The latter criteria were used, because the most recent reviews generally include the data of preceding reviews. In this way it was tried to prevent that the inclusion of both the recent and preceding studies would lead to the double use of the same data.

The sensitivity data were normalised by subtracting all values by the average sensitivity, calculated as the mean of the logarithmically transformed (10log) concentration data. In this way we focus strictly on the variation in sensitivity values and eliminated differences in the actual toxicity caused by either the toxic properties of the compound or the test procedure and circumstances (units, temperature etc.).

To illustrate the variation in sensitivity data, each value (species) is depicted as a separate point in a graph, hereby giving the reader a view of the sensitivity variation between species for every single compound. As the data are transformed by 10log, one unit on the ordinate axis corresponds to a factor 10 difference in effect concentration.

The sensitivity ratio and the standard deviation were calculated as the parameters allowing the most direct insight into the distribution of the data around the mean.

The pesticides are in general to be considered as highly reactive and specifically acting chemicals. High reactivity requires the presence of the right target site in the tested organism. A compound, which is highly toxic for some organisms, may be almost harmless to others that lack the sensitive target. An example hereof is the insecticide DDT, which is extremely toxic for many arthropods, but hardly toxic to algae. The reason is that algae lack the DDT sensitive sodium transport channels of nerve cells. DDT toxicity to algae, results mainly from the narcotic action of DDT as 'unreactive' organic molecule. As we will show below, it is not always easy to predict selectivity of a chemical. For a better recognition of narcotic action by some 'reactive' pesticides we have chosen to include some examples of the variation which is associated with narcotic action. The variation of narcotic chemicals can be regarded as a kind of 'baseline' variation, indicating the minimum variation that may be expected for the selected group of organisms, in combination with the application technique and the effect measure.

When regarding the sensitivity distribution one has to realise that the distribution is described by estimated parameters (the mean and the standard deviation) and the accuracy of these estimates depends on the sample size i.e. how many species are tested. To express this, confidence limits can be calculated for the estimates.

A line on the graphs indicates the position of the most frequently tested species. The line illustrates whether this species is among the most or the least sensitive species.

2.1 Analysing sensitivity data: a short resume

The variation in response of different species to the same toxicant can be analysed in different ways and illustrated by many different parameters.

Besides the graphical presentation, we have chosen two calculations to describe the variation in sensitivity between species.

A simple approach is to identify the lowest and the highest sensitivity value, which have been measured. This approach gives a direct, but delusive impression of the range of data. As discussed by Hoekstra *et al.* (1994) there is a serious disadvantage of using this measure because the extremes will continue to grow apart with increasing sample size. Another disadvantage is that the extremes offer no insight into the responses shown by the bulk of the data. The latter requires a shift in focus away from the extremes towards the distribution of the data around the average.

To analyse the distribution of the data, a histogram can be constructed, showing the frequency with which toxicity values were found in certain intervals (Figure 2.1A). A problem with this presentation is that the right end of the distribution stretches out to the far right.

A more balanced graph can be created by log-transformation of the sensitivity values before creating the distribution (Figure 2.1B). This graph represents the log-normal sensitivity distribution, characterised by a mean sensitivity and a standard deviation. A log-normal distribution may be expected when many different factors contribute in a multiplicative way to the sensitivities of the species (Hattis 1997). It has been found in several ecotoxicological studies that the log-normal distribution provides an adequate fit for many practical purposes.

The width of the distribution, which can be expressed as the sensitivity ratio (SR), has been introduced (see Hoekstra *et al.* 1992). The SR95:5 is the concentration at which 95% of the species are affected at a given level divided by the concentration at which only 5% are affected. Accordingly, the SR95:5 indicate the fraction in concentrations necessary to cover the central 90% of the data. In terms of safety factors the SR95:5 gives the divisor or factor necessary to protect a sensitive species (equal to the 5th percentile) if the test species is insensitive (equal to the 95th percentile).

Assuming normality of log-transformed sensitivity data, it is easy to use the mean and standard deviation in combination with normal probability tables to calculate the toxicant concentrations at which 5% or 10% of the species in the distribution are affected. Alternatively calculations of the protection level achieved by application of safety factors of 10 or 100 can be done assuming that the test species has a sensitivity corresponding to the 95th percentile.

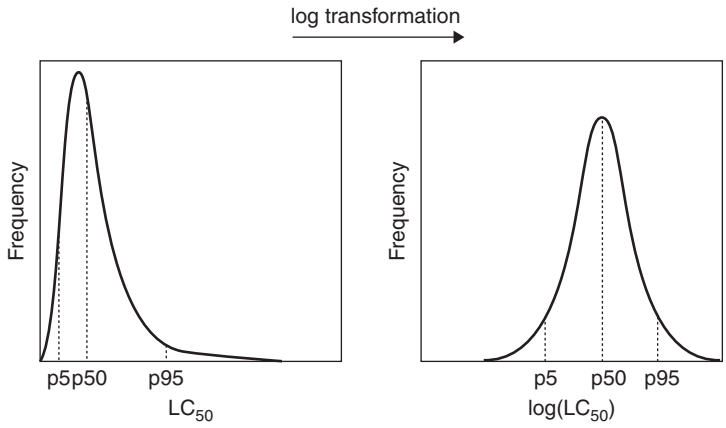

Figure 2.1. Using a sensitivity distribution to analyse variation in sensitivity data. A. A smoothed version of a histogram of sensitivity data based on actual observations. B. The same distribution after logarithmic transformation of the data to create a normal distribution. SR95:5 = p95/p5.

Ideally a sensitivity distribution should be constructed for every compound. This requires data from at least four different test species. Usually there is only data from one or two species available. To circumvent this problem two similar methods have been proposed (Baril *et al.* 1994, Luttik and Aldenberg 1997).

Baril *et al.* suggests an approach where the size of the safety factor covering the inter-species variability depends on the species, which has been tested, i.e. a species specific extrapolation factor. The calculation has been done for birds but not for other organisms at the moment. Further more the data for birds may be biased as most of the data stems from organophosphorous compounds. In the following we have used the procedure proposed by Luttik and Aldenberg for small samples of toxicity data, although it may not be as accurate as the Baril *et al.* method, but more generally applicable for the time being.

Luttik and Aldenberg suggested that the variation in sensitivity between species can be estimated from a general pesticide sensitivity distribution generated by lumping all available sensitivity distributions for individual pesticides weighed by the number of observations. Following Kooijman (1987) a median safety factor (SF95) can be calculated as the geometric mean of the original data divided by the left 95% confidence limit of the 5th percentile of the sensitivity distribution (for details of the Luttik and Aldenberg method see appendix). This estimates the concentration where at most 5% of all species with 95% certainty have a lower effect concentration (LD50, EC50). The method builds on the assumption that the new substance is a part of

the known distribution encompassing already tested substances. The method does not provide a safety factor that will protect 95% of all species against a new pesticide given a test result with one species, because it is estimated as a median compound, that is 50% of the compounds have a wider sensitivity distribution.

The distribution of the width of the sensitivity distributions for pesticides therefore can provide information of what proportion of all pesticides a given safety factor can be expected to cover. Using the SR95:5 as a indicator of the width of a pesticides sensitivity distribution, the cumulative frequency distribution of SR95:5 can illustrate what percentage of pesticide can be expected to have a SR95:5 smaller than a given number (x).

2.2 Variation in sensitivity between invertebrates: aquatic and terrestrial studies

Traditionally, aquatic animals, such as fish and invertebrates, have been used frequently in toxicity tests. Recent reviews, e.g. Vaal *et al.* (1997a, b) include the data of older studies, such as Sloof *et al.* (1983), Mayer (1986), Holcombe *et al.* (1987), Kooijman (1987), etc. Additional LC50 values were obtained from reviews by Staples *et al.* (1997) about phthalate esters, Morton *et al.* (1997) about azinphos-methyl, and van Wijngaarden *et al.* (1996) about chlorpyrifos.

The numbers of data for terrestrial invertebrates fitting our selection criteria were limited. Here we selected a database on edaphic arthropods exposed to dimethoate (Løkke and van Gestel 1998) and one about pyrethroids sprayed onto plants (Croft and Whalon 1982).

2.2.1 Results for aquatic and terrestrial invertebrates

Variation in pesticide sensitivity

The sensitivity of aquatic and terrestrial invertebrates to pesticides (compounds 8-18) had a considerable variability with average sensitivity ratios of 355 and 437 respectively (Table 2.1). The Luttik and Aldenbergs SF95 calculation gave 473. The large average variation is a result of several individual compounds having sensitivity ratio's spanning over more than three orders of magnitude (Figure 2.2).

Relative position of the same species

The small crustacean, Daphnia magna, was the species tested with most different compounds. As indicated by the line in Figure 2.3, its relative position in the distributions of the different compounds varies considerably.

Narcotics contra specifics

Figure 2.2 and Table 2.1 illustrates the lower variability of the sensitivity to narcotic compounds. The average SR95:5 is only 21.

The cumulative frequency distribution of SR95:5 for aquatic invertebrates (Figure 2.3) shows that approximately 20% of the pesticides have a SR95:5 greater than 1000.

Table 2.1. Variation in sensitivity data for invertebrates. Averages for all invertebrate species and chemicals in the indicated groups. All data are based on the normalised variation. Obs = the number of observations. Std(log(sens-avg)) = the standard deviation of the normalised data. SR95:5 = the sensitivity ratio defined as the 95th percentile divided by the 5th percentile. Further information about the shape of the distribution for the individual compounds can be found in Appendix .

Reactive group	**Obs**	**Std(log(sens-avg))**	**SR95:5**
Narcotics	28	0.36	21
Polar narcotics	47	0.59	25
Specifics aquatic	49	0.82	355
Specifics terrestrial	33	0.76	437

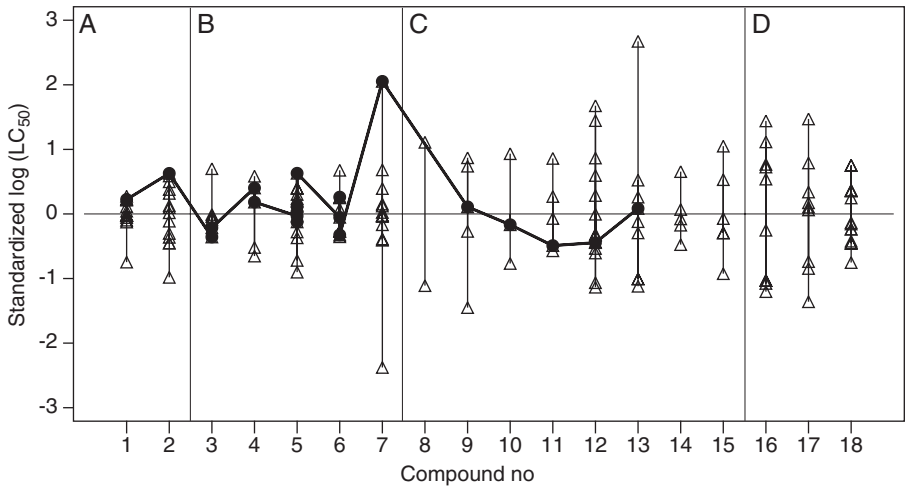

Figure 2.2. Differences in the sensitivity of aquatic and terrestrial invertebrates. Aquatic invertebrates(A) Narcotics: (1) acetone, (2) benzene. (B) Polar narcotics: (3) DMP, (4) DEP, (5) DBP, (6) BBP, (7) aniline. (C) Specifics aquatic: (8) dieldrin, (9) lindane, (10) malathion, (11) parathion, (12) pentachlorophenol, (13) azinphos-methyl, (14) and (15) chlorpyrifos. Terrestrial invertebrates (D) Specifics: (16) dimethoate, (17) cypermethrin, (18) fenvalerate. Plotted points represent the differences between the actual and the mean per compound of the logarithmically transformed sensitivities. The line indicates the position of Daphnia magna.

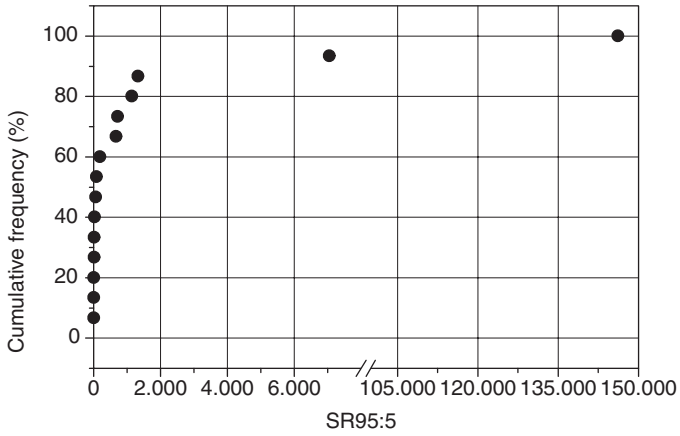

Figure 2.3. Cumulative frequency (%) of SR95:5 for reactive substances in aquatic invertebrate tests.

2.3 Variation in sensitivity between fish

There exist many toxicity data for fish. Especially trout species (*Oncorhychus*), fathead minnows (*Pimephales promelas*), the guppy (*Poecilia reticulata*) and the bluegill (*Lepomis macrochirus*) are frequently used test organisms. We have selected a number of studies, which either presents broad literature reviews including all these differences, or results of tests performed with different species but under similar test conditions.

2.3.1 Results for fish

Variation in pesticide sensitivity

A sensitivity ratio, SR95:5 of 71 was found and the SF95 was estimated at 78. Still for some compounds the sensitivity ranged over four orders of magnitude (Figure 2.4).

Relative position of the same species

The most frequently used test species in the present data set, is *Lepomis macrochirus*, the bluegill. The line connecting the position of this species suggests no apparent trend in sensitivity in relation to the different kinds of toxicants or in relation to their reactivity.

Variation between compounds

The variation between compounds in a group indicated a clear increase with the average reactivity of the compounds. Marked variation was observed in the group of reactive compounds, which was mainly caused by the very broad distributions of the cholinesterase inhibitors cuthion, malathion and azinphos-methyl.

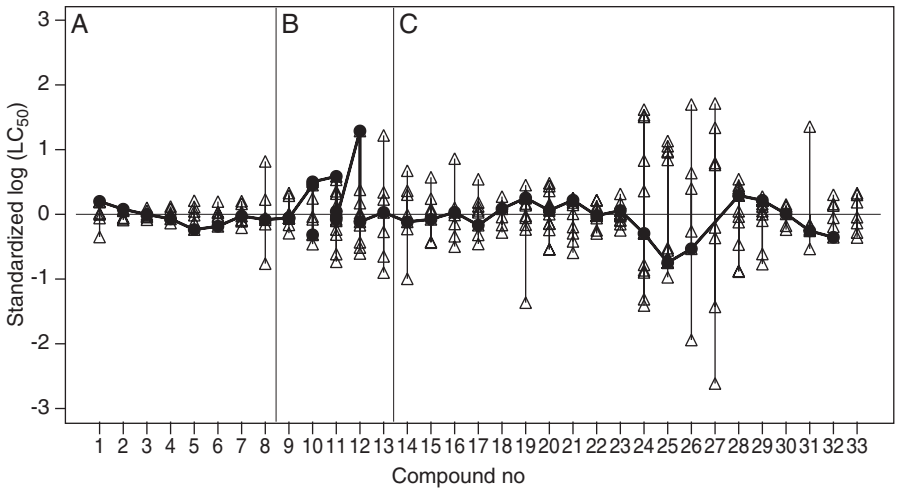

Figure 2.4. Differences in the sensitivity of fish. (A) Narcotics: (1) 2-(2-ethoxye-thoxy)-ethanol, (2) 2-methyl-2,4-pentanediol, (3) 2-methyl-1-propanol, (4) 2,2,2-trichloroethanol, (5) 2,4-pentanedione, (6) hexachloroethane, (7) acetone, (8) benzene. (B) Polar narcotics: (9) DMP, (10) DEP, (11) DBP, (12) BBP, (13) aniline. (C) Specifics: (14) parathion, (15) dieldrin, (16) lindane, (17) pentachlorophenol, (18) 2-chloroethanol,(19) lindane, (20) DDT, (21) toxaphene, (22) methyl parathion, (23) Baytex, (24) cuthion, (25) malathion, (26) malathion, (27) azinphos-methyl, (28) carbaryl, (29) zectram, (30) pentachlorophenol, (31) permethrin, (32) endrin, (33) 2,3,7,8-TCDD. Plotted points represent the differences between the actual and the mean per compound of the logarithmically transformed sensitivities. The line indicates the relative position of the blue gill.

Narcotics, polar narcotics and specifics

The variation in the response of fish to narcotic chemicals (1)-(8) is very small. Accordingly, the average standard deviation was 0.20 (Table 2.2), indicating that the basal conditions of fish tests contribute only moderately to the variation observed.

Variation between experiments with the same species

The experiments with phthalates (compounds 9 to 12) give an impression of the variation that may be observed between different studies with the same species.

A safety factor 10 applied to the 95th percentile protects 52% of all species. A factor 100 protects 96%.

Table 2.2. Average variation in all fish species for the chemical compounds in the indicated groups. All data are based on the normalised variation, For explanation of abbreviations see Table 2.1. Further information about the shape of the distribution for the individual compounds can be found in Appendix I.

Reactive group	Obs	Std(log(sens-avg))	SR95:5
Narcotics	50	0.20	3
Polar narcotics	48	0.43	17
Specifics	182	0.59	71

The cumulative frequency curve (Figure 2.5) reveals that c. 20% of the pesticides have a SR 95:5 greater than 100.

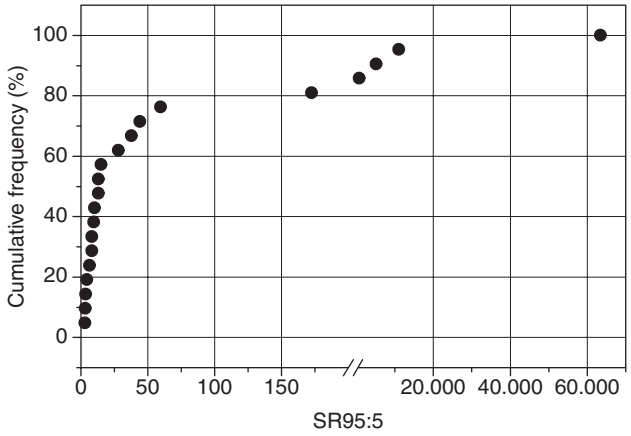

Figure 2.5. Cumulative frequency distribution of SR95:5 for fish.

2.4 Variation in sensitivity between birds

As is apparent from data compilations by Schafer (1972, 1983, 1994), Hill (1984), Joermann (1991), Baril *et al.* (1994), Luttik and Aldenberg (1997), and others, the majority of compounds tested for avian toxicity are organophosphates, acting as acetylcholinesterase inhibitors. These are followed in test-frequency by the carbamates and the organochlorine compounds. A few original compilations of data have been included in quite a few other studies.

The results from the Luttik and Aldenberg study was used as estimates of the variation in sensitivity between species as these calculations are based on the most extensive data available to us for this

purpose. To illustrate the variation in relative sensitivity of species, data from Tucker and Haegele (1971) and Schafer (1983) was used.

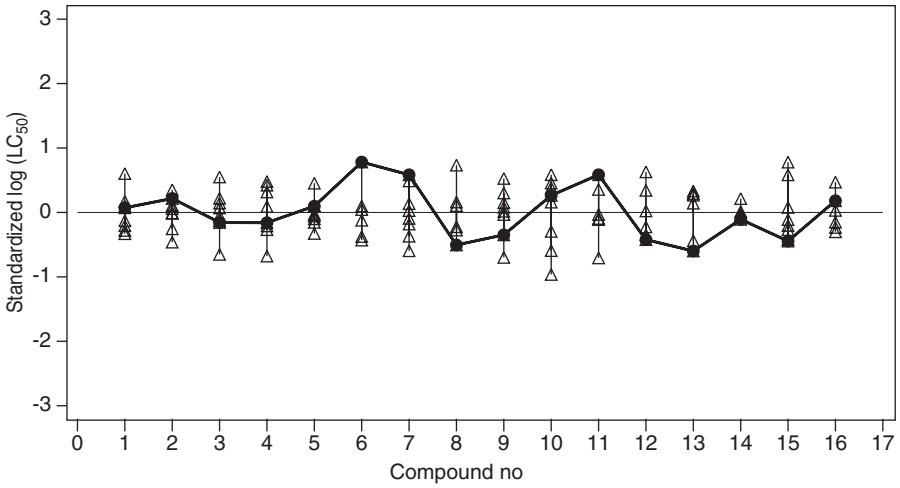

Figure 2.6. Variation in the effect of reactive chemicals on different bird species. All data in the figure originate from Tucker and Haegele (1971) combined with data from Schafer (1983). (1) abate, (2) azodrin, (3) baygon, (4) baytex, (5) bidrin, (6) dieldrin, (7) dursban, (8) epn, (9) landrin, (10) metasystox-r, (11) mobam, (12) parathion, (13) strychnine, (14) systox, (15) zectran, (16) 1080. Results stem from experiments in which gelatine capsules with the toxicants were applied to the stomach of the animals by means of a glass tube. Plotted points represent the differences between the actual and the mean per compound of the logarithmically transformed sensitivities.The line indicates the relative position of the mallard.

2.4.1 Results for birds

The Luttik and Aldenberg data had a standard deviation on a 10log scale of 0.46 corresponding to a sensitivity ratio of 32 and a safety factor of 33. The mean ratio between the lowest and the highest available toxicity data in the data set was 117 (range from 4-1280), which demonstrates that the SF-calculations are mean estimates and not worst cases.

Differences in variation between compounds were moderate in the data set presented in Figure 2.6. as the same active ingredient is part of several tests and the data are much more standardised than is the case for the other taxa in the present study.

Relative position of the same species

To indicate the variation in the relative position of the same species, we used the Mallard, *Anas platyrhynchos* as representative. There was no clear relationship between toxicity and specific modes of action of the different toxicants (Figure 2.6).

Narcotics, polar narcotics and specifics

As the data set used contain so few compounds a cumulative frequency curve was not constructed. However, in the Ecoframe report (US-EPA, 1999) such a diagram is presented and from the curve it is estimated that 20% of the pesticides have a SR95:5 for birds greater than 40.

20-25% and a SR95:5 at c. 100 is necessary to cover 95% of all pesticides

The present data set contained only reactive chemicals. Conclusions about differences with narcotic chemicals are therefore not possible.

2.5 Variation in sensitivity between aquatic algae and plants

A review by Lewis (1995) on the use of aquatic plant species in toxicity test shows that many algae species have been used in toxicity test, but only the species *Selenastrum capricornutum* and some *Scenedesmus*-species are used frequently. Of the vascular plants, especially duckweeds (*Lemna spp*.) have been popular. The above trends were reflected in the data, which were gathered for the present report. Studies were selected in which as many species as possible were exposed in a comparable way to one or more toxicants.

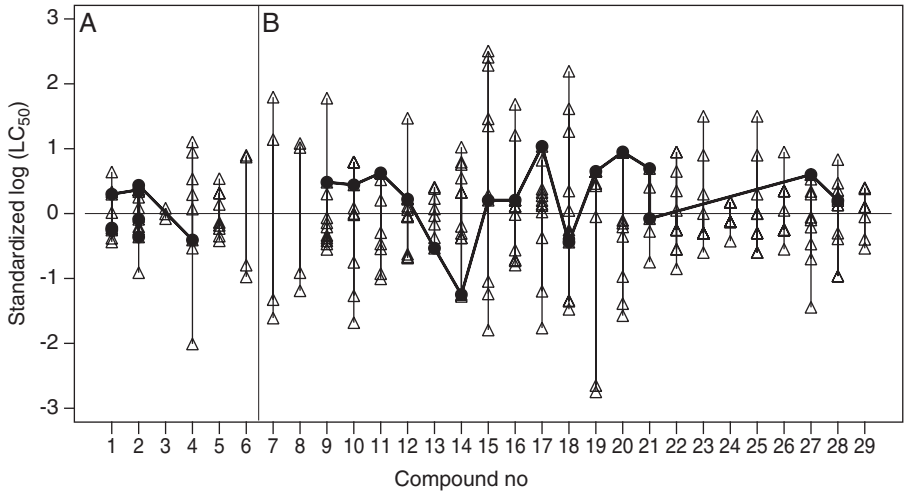

Figure 2.7. Variation in pesticide effects on different algae species and aquatic vascular plants: (A) polar narcotics: (1) DMP, (2) DEP, (3) DAP, (4) DBP, (5) BBP. (B) reactives: (6) simetryn, (7) pretilachlor, (8) thiobencarb, (9) metribuzin, (10) alachlor, (11) metolachlor, (12)atrazine, (13) atrazine, (14) atrazine, (15) 2,4-D acid, (16) diuron, (17) monuron, (18) simazine, (19) 1,4 p-naphtoquinone, (20) TCA, (21) paraquat, (22) paraquat, (23) diuron, (24) glyphosate, (25) norflurazon, (26) tributyltin oxide, (27) DDT, (28) methoxychlor, (29) fenitrothion. Plotted points represent the differences between the actual and the mean per compound of the logarithmically transformed sensitivities. The line indicates the position of *Scenedesmus quadricauda.*

2.5.1 Results for aquatic plants

Variation in pesticide sensitivity

A marked variation in species sensitivity was observed among algae for many compounds exceeding three orders of magnitude (Figure 2.7), as was indicated by the large sensitivity ratio of 501 and a safety factor (SF95) of 593.

Relative position of the same species

The most frequently tested species in the present data set, was the algae *Scenedesmus quadricauda* which is rather insensitive to most toxicants tested, with the exception of atrazine, simazine and paraquat. Besides this general trend, the relative position of the species varied without a clear pattern, also when the other species in the distribution were kept the same, such as in the study of Bednardz 1981 (no (14) to (20) in Figure 2.7).

Variation between compounds

Most compounds had a large variation in their sensitivity distributions. The compounds with the widest distributions were 2,4-D, simazin and pretilachlor.

Variation between experiments with the same selection of species

The present data on aquatic algae included four studies in which the same group of species was tested for all pesticides. This enabled a comparison of the variation observed for different compounds. The results of Kasai and Hatakeyama 1993 (6 to 8), of Fairchild *et al.* 1998 (9 to 12), Bednarz 1981 (14 to 20) and of Blanck *et al.* 1988 (22 to 26) all revealed a fairly similar variation for the selected compounds. The only real exception was glyphosate (24) in the study of Blanck et al 1988, who found a narrow range of variation for this compound.

Polar narcotics compared with specifics

The phthalates had moderate variation with a SR95:5 of 32 (Table 2.4). In the group of 'reactive' compounds, two compounds were found to have a narrow distribution comparable to narcotic compounds. One was glyphosate (24) which proved only toxic in concentrations around 13 mg/L, and the insecticide fenitrothion (29), a cholinesterase inhibitor, which may be expected to have no effects on algae (mean effect concentration as high as 5 mg/L). Yet, a prediction of the variation in effects of insecticides on algae remains difficult. DDT (27) and methoxychlor (28) which are not very toxic (averages of 24 mg/L, and 4.4 mg/L respectively) but nevertheless show rather broad sensitivity distributions, when compared with equally toxic narcotic compounds such as (1) to (4).

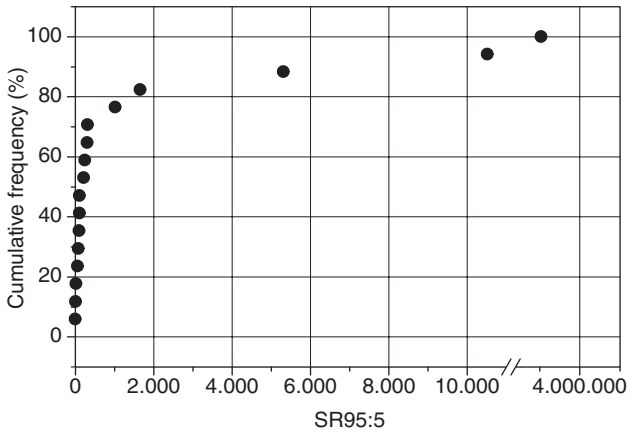

Figure 2.8. Cumulative frequency distribution of SR95:5 for algae.

Table 2.4. Average variation for all aquatic plant species for the chemical compounds in the indicated groups. All data are based on the normalised variation. For explanation of abbreviations see Table 2.1. Further information about the shape of the distribution for the individual compounds can be found in Appendix.

Reactive group	**Obs**	**Std(log(sens-avg))**	**SR95:5**
Narcotics	40	0.52	32
Specifics	244	0.85	501

The cumulative frequency distribution for algae (Figure 2.8) suggest that approximately 20% of the pesticide have a SR95:5 greater than 1000. For the calculation of cumulative frequency distribution only data on algae were used.

2.6 Variation in sensitivity between terrestrial plants

Data about pesticide effects on terrestrial plants are scattered throughout the literature. The selection of species with which tests have been performed has been based on several reasons, including: economic importance, geographical range, representation of plant kingdom, known sensitivity to pesticides, practicality of plants in test procedures, historical use in test procedures, requirements for registration procedures, etc. In an attempt to gather the scattered data to allow structured analysis, a large database has been constructed (the phytotox database, Royce *et al.* 1984), which contains the results of more than 3500 papers and more than 78000 dose-response records. Rankings, of which compounds and species have been investigated most, have been published in Fletcher *et al.* (1988). Fletcher *et al.* 1985 and 1990 has published extensive reviews of toxicity data based on the phytotox database. For the purpose of estimating variation in sensitivity the use of very different test methods is not ideal.

In addition to these data reviews, we got the kind allowance to use another comprehensive data compilation based on the test results for the Canadian registration procedures. Boutin (1993) gathered these data.

Variation between compounds

The terrestrial plants also had great variation in sensitivity (Figure 2.9). The calculations revealed a sensitivity ratio of 245 and a safety factor (SF95) of 280.

Relative position of the same species

The most frequently tested species was *Triticum aestivum*, winter wheat. This proved to be rather sensitive to one half of the compounds, and rather insensitive to the other half (Figure 2.9).

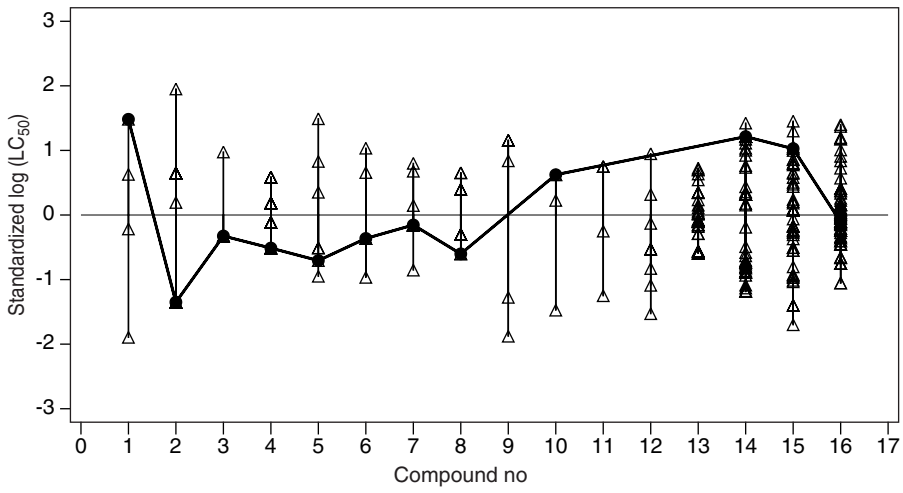

Figure 2.9. Variability in sensitivity of terrestrial plant species. (1) dalapon, (2) 2,4-D, (3) dicamba, (4) diphenamid, (5) trifluralin, (6) picloram (all in uMole), and (7) 2,4-D, (8) diphenamid, (9) dinoseb, (10), linuron, (11) terbacil (all in kg/ha). (12) glyphosate, (13) promethryn, (14) sulfonylurea, (15) dinitroanaline, (16) imidazolinone. Plotted points represent the differences between the actual and the mean per compound of the logarithmically transformed sensitivities. The line indicates the position of winter wheat.

Effects of the size of the date set

The data in column (14) to (16) represent very large data sets, with 46, 47 and 68 species respectively.

The cumulative frequency distribution (Figure 2.10) show that more than 20% of the pesticides have an SR95:5 greater than 5000.

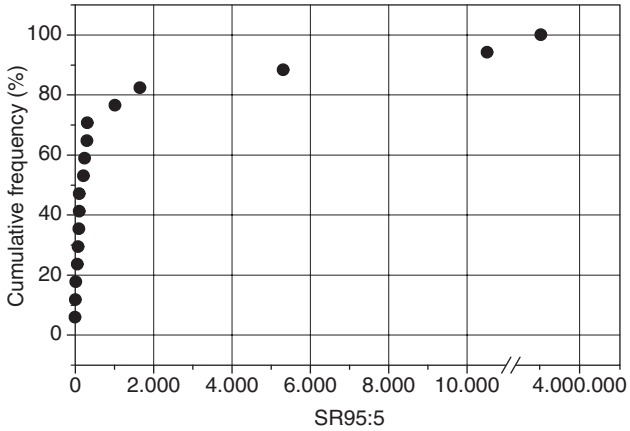

Fig 2.10. Cumulative frequency distribution of SR95:5 for terrestrial plants.

Table 2.5. Average variation for all terrestrial plant species for the chemical compounds in the indicated groups. All data are based on the normalised variation. For explanation of abbreviations see Table 2.1. Further information about the shape of the distribution for the individual compounds can be found in Appendix II.

Reactive group	Obs	Std(log(sens-avg))	SR95:5
Specifics	278	0.75	245

2.7 Variability in sensitivity data: general discussion

No 'most sensitive species'

There seems to be no simple rules to predict the relative sensitivity of a species between compounds. The ragged lines in Figures 2.3 to 2.7 form a graphical illustration hereof.

There is convincing evidence that the same species may, in an unpredictable way, have differences in sensitivity towards different compounds. Accordingly, a species which is one of the most sensitive to compound A may be amongst the least sensitive to compound B. For birds, this has been found, for example, by Joermann (1991), Wiemeyer and Sparling (1991), Tucker and Haegele (1971) and Hill (1984). However, within specific groups of chemicals, for example the cholinesterase inhibitors, a rather consistent ranking of bird species across compounds has been observed (Baril *et al.* 1994). Schafer (1984) has also reported this trend for carbamates and chlorinated hydrocarbons.

A lack of toxicant-response relationship has furthermore been shown for different species of aquatic algae and plants, as is illustrated by the data of Blanck *et al.* 1984, Fairchild *et al.* 1997, Fairchild *et al.* 1998, Bednarz 1981 and others. Also results on aquatic animals give no

clear trends. On the basis of a large database, Vaal *et al.* (1997a) state that 'As expected, the patterns in species sensitivity are more diffuse; species very sensitive to one (group of) compound(s) might be among the least sensitive to other compounds'. The analysis of another large database resulted in the same conclusion (Mark and Solbé 1998) when comparing the average toxicity of *D. magna* with that of other species. For acrylamide monomer and cadmium *D. magna* was the most sensitive. For atrazine and lindane it was the least sensitive species. Song *et al* (1997) also found that *D. magna* not always is the most sensitive aquatic species to pesticides. Similar data for other species/environments confirm that a most sensitive, or 'sentinel' species (Power and McCarty, 1997) does not exist.

Consequently, the use of a 'most sensitive species' must be considered a fallacy.

To protect all species it is therefore necessary to apply a safety factor, which accounts for the unknown relative sensitivity of the test species.

If toxicity data for a new compound are limited to a single species, there is a considerable uncertainty about the sensitivity of this species relative to the species that it is supposed to represent. A way to get an estimate of environmentally 'safe' concentrations is to find information about the average toxicity and the variation in toxicity between species around this average and then decide what fraction of species should be protected with what certainty, like 95% of all species with 95% certainty. This approach, however, describes an average (median) pesticide, half of the compounds having a wider sensitivity distribution. The safety factor therefore protects 95% of the species with at least 95% certainty for 50% of the pesticides.

Cumulative frequency distribution curves illustrates that many pesticides have sensitivity distributions much wider than the average. The median SR95:5 corresponds to the 50% cumulative frequency. From the cumulative frequency curves it can be seen that an SR95:5 >80% is 2-3 times greater than SR95:5>50%. It is evident from the figures that an SR95:5 covering 95% of all pesticides sensitivity distributions is considerably higher. The data set is, however, too small to allow extrapolations of the 95% values.

The presented variation measures include both the inter-species variation in sensitivity and the variation caused by the experimental uncertainties, inter-laboratory differences etc., which as mentioned earlier, may be considerable. This combined variation is, however, what has to be dealt with in a real risk assessment and it is therefore relevant.

The data used in the present report needs to be improved for plants and invertebrates. In these groups the tests that have generated the data are very inhomogeneous and in some cases not ideal for the purpose of this report. Consequently the calculations are preliminary. There is a need for compilation of better data set and for analyses of the importance of test conditions etc. for the calculation of safety factors.

In conclusion:

The sensitivity ratios and safety factors of the above-discussed groups indicate marked differences in how variable the data are within these groups. An overview of the observed differences is given in Table 2.7. For aquatic plants a SF95 of 593 was estimated. For birds a SF95 of 33 was found. This difference may be due to differences in taxonomic homogeneity within a group of organisms and heterogeneity of test conditions. Even within the most homogeneous group, the birds, the most sensitive species may be over 1000 times more sensitive than the most resistant species. Due to the characteristics of the sensitivity distribution with very long tails (Figure 2.1) very large safety factors are needed to protect 95% of all species when standard deviation of the sizes seen for invertebrates and plants are found.

The SR and SF calculated from a general sensitivity distribution relate to an average compound and half of the substances will have a larger variation.

The uncertainty of the estimates decreases dramatically if more species are tested. For birds the SF95 drops from 33 to 20 if two species are tested instead of one (see appendix). This is in many situations in contradiction to the presently used procedure where the lowest value is to be used if two or more species have been tested (Table 1.1). Such a procedure will often result in greater safety factors when more species are tested although the uncertainty decreases.

Table 2.7. Inter-taxon comparison of variation in sensitivity to pesticides based on the average sensitivity ratio (SR95:5) and average the safety factor (SF95). The SR95:5 is the average ratio between the 95th and the 5th percentile. The SF95 is the geometric mean of all data divided by the left 95% confidence limit of the 5th percentile.

Taxon	**Biotope**	**SR95:5**	**SF95**
Invertebrates	aquatic	355	473
Invertebrates	terrestrial	437	-
Plants	terrestrial	245	280
Birds	terrestrial	32	33
Plants	aquatic	501	593
Fish	aquatic	71	78

3 Relationship between acute and chronic long term toxicity measures

3.1 Introduction

The relationship between acute LC50 and chronic NOEC may be important in pesticide risk assessment. Two examples are considered here.

First, it is often assumed that a constant conversion factor of 10 relates the acute LC50's to chronic NOEC's (see point 2.5.2.2., Council Directive 97/57/EC (1997)).

Second, a low LC50 may trigger the decision to demand additional chronic NOEC tests in risk assessment procedures.

Which relationships will be discussed?

In accordance with risk assessment practice, we will not discuss the relationship between the *acute* LC50 and the *acute* NOEC, or that between the *chronic* LC50 and the *chronic* NOEC (for data on these comparisons see for example Elonen *et al.* 1998, Staples *et al.* 1997, Dorn *et al.* 1997). Instead we focuses on the conversion from the *acute* LC50 to the *chronic* NOEC, which implies both a change to a lower effect measure and to different modes of action. We have included analysis of a data set on birds, where acute LC50's are related to EC50's of reproduction.

Toxicological considerations

The acute LC50 to chronic NOEC conversion suffers from a weak theoretical basis. The reason is that the modes of action which act at high concentrations in the acute tests may not be correlated directly with the modes of action seen at low concentrations after longer time of exposure. This was also concluded at the OECD workshop on extrapolations from the laboratory to the field. We cite 'It was also pointed out in this context that delayed toxic effects, e.g. reproductive effects and tumours, cannot be predicted at all on the basis of acute tests'. Surprisingly the text continues with 'A factor 10 was felt to be supported by most data (especially for neutral organics) with some exceptions (e.g. anilines) where larger factors may be appropriate (Stay *et al.* 1990)' (OECD 1992, page 12). An impression of the small size of conversion factors for narcotic organic compounds can be obtained from a review of Call *et al.* (1985) who found acute to chronic ratio's for fish in the range from 2.8 to 27.6, with an average of 9.8.

Statistical considerations

The use of a constant ratio, for example an 'acute to chronic ratio' (ACR's e.g. Länge *et al.* 1998, Solbé *et al.* 1998) suffers from statistical drawbacks. A major problem is that ACR's are only constant when the data they are based on have a zero intercept on a normal scale, or a slope of one when expressed on a logarithmic scale. If these assumptions are not met they vary with the size of the LC50 values.

Due to these disadvantages of ACR's, the use of regression curves coupled to the calculation of the associated prediction intervals are preferred. After regression relationships have been calculated, a regression based ACR ('RACR') can be derived for any particular LC50-NOEC combination.

The use of regression equations results in the calculation of an average relationship between acute LC50 and chronic NOEC values with an intercept and a slope, for which confidence intervals can be calculated.

When using regressions, two 'safety factors' can be calculated.

- The first is based on the average relationship between LC50 and NOEC data, described by the intercept and slope of the regression curve.
- The second safety factor accounts for the distribution of the observations around the regression prediction, and is calculated as a confidence limit.

For simplicity reasons we have chosen to apply an ordinary least squares regression for all calculations, as did Sloof *et al.* (1986). Since this does not treat the LC50 variables as estimates, the safety margins are slightly underestimated compared to an errors-in-variables model (see Suter and Rosen, 1988).

So called 'uncertainty factors', UF's (Sloof *et al.* 1986) indicates the distance from the regression estimate to the lower 95% prediction limit. This can be considered as safety factors accounting only for the variability of the data in one point of the regression line estimate.

3.2 Inventory of acute LC50 to chronic NOEC conversion data

With the aim to analyse the correlation between acute and chronic toxicity measures, we gathered data covering aquatic and terrestrial life forms. Data were collected either from the literature or from a database developed by Henning Clausen at The National Environmental Research Institute in connection with a study of the development of the pesticide hazard profile over a decade (Clausen 1998) in collaboration with the Danish Environmental Protection Agency. The database consists of data provided for the registration process in the Agency, supplemented with values from other sources. Values indicates as greater than (>) was not used. Thus the compounds with toxicity below the concentration bounds used in the test (e.g. 5000 ppm) are not included in the regressions.

3.2.1 Aquatic bioassays

In the aquatic data all results are expressed as toxicant concentrations per volume water. This implies that both safety factors and calculated ratio's can be compared between tests (Table 3.1.).

Fish and daphnids

A comprehensive study on fish and daphnids published by Sloof *et al.* (1986), included a chronic to acute regression based on a large range of compounds including pesticides and non-pesticides, organic and inorganic compounds (N = 164). As a mean was not given in the paper, an approximate mean log(LC50) value of 0 was extrapolated by eye from the data. When inserted into the regression equation of logNOEC =-1.28 + 0.95logL(E)C50 it yields a log(NOEC) estimate of -1.28. Back-transforming we obtain an NOEC of 0.05 mg/L, which has to be divided by 25.6 (1.41 on log basis), i.e. the safety factor of the 95% prediction interval (referred to as uncertainty factors or UF by Sloof *et al.*, 1986). This results in an actual 95% safety limit of 0.002 mg/L implying an RACR of approximately 500.

Fish and crustaceans separated: all compounds

Based on LC50's and maximum acceptable toxicant concentrations (or MATC's) Suter and Rosen (1988) calculated regression equations linking acute to chronic effects for fish and crustaceans. The MATS represent the geometric means of the lowest concentration causing a statistically significant effect and the highest concentration not affecting any parameter in any life stage of the species studied given infinite exposure. Many different chemicals were included in the analysis. This resulted in the following regression equations for *fish* (N = 41): logMATC = -0.60 + 0.98logLC50 with a 95% prediction interval of 1.27 (18.6 on normal scale), and for *crustaceans* (N = 43): logMATC = -0.88 + 1.00logLC50, with a 95% prediction interval of 0.9 (7.9 on normal scale). The confidence limits are given by the predicted MATC $\pm$ the prediction interval.

For mean logLC50 values for fish of 1.8 and crustaceans of 1.58, the above equations yield logNOEC predictions, including the 95% prediction limits, of -0.106 and -0.2 mg/L, respectively. From these results RACR's can be calculated of $10^{(1.8+0.106)}$ = 81 for fish, and $10^{(1.58+0.2)}$ = 60 for crustaceans.

Fish and crustaceans: pesticides

The data of Suter and Rosen (1983) were screened for pesticides; 28 compounds and a total of 47 pairs of LC50-NOEC observations were found. Most tests were performed with the fish *Ciprinodon variegatus* and the crustacean *Mysidopsis bahia*. The regression calculations yielded the following result: Log(NOEC) = 0.81log(LC50) - 0.63 with 95% prediction limits (at the average LC50) of a factor 16.3. For the mean log(LC50) of 1.03 the log(NOEC) estimate is 0.20. This yields an NOEC of 1.6 mg/L, which divided by 16.3 yields 0.098. This implies a RACR of 109, which is somewhat larger than the RACR's based on all compounds. The data and regression results are presented graphically in Figure 3.1A.

Crustaceans: LC50 to LC50.

In addition to the acute LC50 to chronic NOEC comparisons, we also analysed a data set from the Clausen-database on crustacean data containing the 48-hour acute and 21-day mortality LC50's. Regression calculations on these data (N = 28) resulted in the following equation: log(LC50chronic) = 0.94log(LC50acute) -0.93. Accordingly, the log(NOEC) at the mean log(LC50) of -0.43 is -1.33, which is 0.046 on a normal scale. Divided by the 95% safety factor at the mean log(LC50) of 100.7, this becomes 0.00046. Compared to the median LC50 of 0.37 this yields an RACR of 807. Data are presented graphically in Figure 3.1B.

Aquatic analysis from the ECETOC database, ACR's and regression analysis

From analysis of the ECETOC database, it was concluded that it required an ACR of 84 to be certain that 90% of the ACR's for 26 pesticides were accounted for. A further analysis dealing specifically with *Daphnia*, resulted in mean ACR's for atrazine, endosulfan, lindane and tributyltin, of respectively: 49.3, 55.6, 44.1 and 180 (Länge *et al.* 1998).

Another analyse of the ECETOC database (Solbé *et al.* 1998) includes 2203 studies, 361 chemical and 121 aquatic species. This study presents a graph of a regression equation from acute LC50 to chronic NOEC based on all data, but without giving any details on the safety margins. An estimate of 1.7 log units (a factor of 50) was obtained by eye.

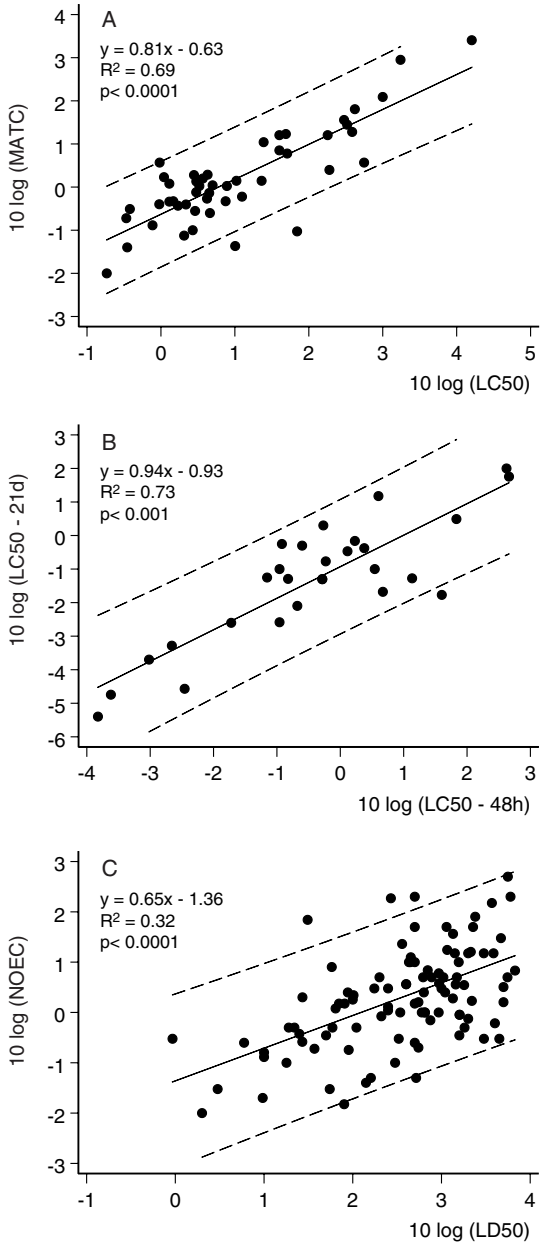

Figure 3.1. Regressions with prediction limits of log(long term or chronic NOEC)'s against log(acute LC50) values. A. Fish and crustaceans exposed to pesticides, B. Crustacean data for acute and long-term LC50 values, C. Mammalian data. Estimates of regression coefficients and of prediction intervals are given in the text.

3.2.2 Tests with birds and mammals

Calculations were performed on data from the Clausen-database on mammals and birds.

Note that in the data sets the units in which LC50 and NOEC are expressed are not always identical. Accordingly, an easy comparison with other organisms on the basis of conversion factors is not possible. Uncertainty/safety factors, however, remain comparable.

Acute to chronic regressions for mammals (Figure 3.1.C)

After selecting pesticides for which both LD50's and NOEC's or NOAEL's were available, the data set on mammals contained 95 pairs of observations (Figure 3.1C). For a preliminary analyses NOEC and NOAEL were assumed to be approximately equal. The following regression equation was calculated for all data: Log(NOEC) = 0.65Log(LC50)-1.36 and an uncertainty factor of 45.1, which is more than twice as large as observed in aquatic tests. For an exposure to 250 mg/kg the regression equation results in an NOEC of 1.6, which divided by 45.1 yields 0.035 mg/kg as the NOEC estimate at 95% certainty resulting in an ACR of 7136. This regression based ACR is expressed in different units than the aquatic tests and cannot be compared directly.

The endpoints in the mammalian NOAEL-tests differs substantially in sensitivity, some being with out any known significant impact on the performance of the whole animal, others being lethal. The comparison of NOAEL's should therefore only be carried out with data endpoints that have been assessed as comparable. The assumption that NOEC and NOAEL's are comparable is therefore also false.

Acute to chronic regressions for birds

The Clausen-database was used to calculate ACR's for birds. ACR's could be made for two correlations: 1. Between acute LD50 and reproductive NOEC; 2. Between five day feeding experiments and reproductive NOEC. The number of reproduction data limited the exercise.

Acute LD50's and reproductive NOEC's were available for 17 pairs of observations, resulting in the regression equation: log(NOEC) = 0.49log(LC50)+0.82, with a UF95 of 16.3 and a RACR of 102.

Five days feeding-based LC50's and reproductive NOEC's were available for 12 data pairs. The regression equation was: log(NOEC) = 1.55, the slope being not significantly different from zero. The results of the feeding tests are less accurate due to uncertainties of the methodology (Mineau *et al.* 1994).

3.3 Conclusions

3.3.1 The size of 'safe' conversion factors

The inventory of the aquatic data revealed marked variability in the results. Conversion factors based on regression relationships and including an uncertainty factor in relation to variability between test results (the 'RACR's'), range from 109 to 807 for pesticides.

Regression estimates

For aquatic organisms the acute LC50's and the chronic NOEC's or MATC's lay a factor 4 to 20 apart at the intercept, where the LC50 = 0, an accordingly the concentration is 1 mg/L.

The conversion factors for LC50(21 days) and NOEC based calculations were larger than for MATC based calculations. This may be related to the fact that the NOEC is the highest concentration at which no effects are observed, whilst the MATC includes also the lowest significant effect concentration. Both measures depend very much on the number of points on the dose-response curve and the number of replicates in the experiment. In tests with great variation between replicates the MATCH value may correspond to an effect concentration considerably above zero thus overestimating the no-effect-level. The opposite may be true for experiments revealing a NOEC.

The reproduction test for crustaceans revealing the more accurate LC50(21 days), however, gave the highest RACR of 807. A comparable value, an approximate RACR of 500, was estimated from the results presented in Sloof et. al. (1986) including both pesticides and non-pesticides.

Uncertainty factors

The uncertainty factors (95% confidence interval) for aquatic organisms varied from 7.9 to 100.7 or 16.3 -100.7 if only pesticides are included.

A value of 7.9 was found for a data set in which crustaceans were exposed to a large range of chemicals, while the chronic effect levels were based on MATC's. The value of 100.7 was found for crustaceans exposed selectively to modern pesticides, while the chronic effect levels were measured as LC50's for 21 days mortality. The later is assumed to be the best estimate for pesticides.

Terrestrial data

For birds, the values for the uncertainty factors ranged from 16 in acute LD50 to NOEC(reproduction) to 31 in LD50(five day feeding) to NOEC(reproduction), the later being based on a less precise test method. RACR values for birds cannot be compared with the aquatic organisms due to their expression in different units.

The endpoints used in birds and mammals differ between the acute and chronic tests, e.g. mortality and reproduction respectively.

The effect of non-average LC50 values on the calculation of RACR's

In the above examples, we have selectively used the average LC50 value to calculate the difference between observed LC50 and estimated NOEC. In general, this will over-estimate the RACR's at lower values, and under-estimate the RACR's at higher values. Precise differences are calculated for the selected pesticide data of Suter and Rosen (1988) (data set 3 in Table 3.1) and for the crustacean data from the Clausen-database (data set 5 in Table 3.1)) using the following equation:

log(RACR) = logLC50 - (logNOEC(est) - log(UF(LC50)))

in which RACR is the regression based acute to chronic ratio, LC50 the actual LC50 value for which the calculations are done, NOEC(est) the regression estimate of the NOEC at the LC50 value used, and

UF(LC50) the uncertainty factor at the LC50 value calculated according to the right part of equation (4).

For reasons of comparability, we selected values at one and two times the standard deviation below and above the average LC50.

At relatively low LC50 values the RACR's are generally lower than at the average LC50, whilst at LC50's above the average the RACR's are larger. The maximum differences between the RACR's are approximately 10 in the first data set, and 2.5 in the second (Table 3.2.).

Thus, large errors in safety factors will result from the use of a standard ACR for all LC50 values. Instead, RACR's should be used, which are based on regression calculations including confidence intervals.

Table 3.1 Overview of acute LC50 to chronic NOEC relationships. N = number of observations per study, UF95 is the safety factor required to account for variability in the sensitivity data (at a 95% confidence limit). RARC is the regression-based acute to chronic ratio. The UF95's are written in bold, because they are comparable between all species groups. Comparability of other variables depends on similarity of the units used to measure LC50 and NOEC. NOEC = NOEC for reproduction.

Aquatic	**Compounds**	**Reference**	**Conversion:**	**N**	**UF95**	**'RACR'**
1. fish	mixed	Suter	LC50-MATC	41	18.6	74
2. crustaceans	mixed	Suter	LC50-MATC	43	7.9	60
3. fish and crustaceans	pestic./ reactives	Suter	LC50-MATC	48	16.3	71
4. fish and Daphnids	mixed	Sloof	LC50-NOEC	164	25.6	500
5. crustaceans	pestic.	D-EPA	LC5048h-LC5021d	28	100.7	1000
Terrestrial						
6. birds	pestic.	D-EPA	LC50-NOECr	17	16.0	-
7. birds	pestic.	D-EPA	5d-LC50-NOECr	12	31.8	-

Table 3.2. Regression based acute to chronic ratio's (RACR) at different relative toxicity levels.

Source:	Suter and Rosen (1988)		Clausen	
Organisms:	Fish and crustaceans		Crustaceans	
Compounds:	Pesticides		Pesticides	
Distance from LC50	**log(LC50)**	**RACR**	**log(LC50)**	**RACR**
+2std	3,25	352	2,92	1765
+1std	2,14	183	1,25	1104
avg. Log(LC50)	1,04	101	-0,43	807
-1std	-0,07	59	-2,11	694
-2std	-1,18	36	-3,79	698

The value found for -2std in last column deviates from expected and reflects that data do not strictly follow a lognormal distribution at the extremes where also data points are scarce (Figure 3.1 B).

3.3.2 Using the acute measures as a trigger for chronic tests

The relationship between acute and chronic toxicity measures in the data presented for legislation is weak for birds and mammals. In the data presented on aquatic organisms a correlation exists between acute and chronic toxicity measures. Although the relationship may be very significant, it only accounts for a limited fraction of the variance (Figure 3.1). Consequently there is a considerable uncertainty attached to predictions of chronic toxicity from acute measures and safety factors of 100-1000 is necessary to cover 95% of the variation. The use of a relation with such safety factors associated, in the decision of when chronic tests are needed, is limited.

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Appendix 1

Appendix to section 2.2: Background information for the data in Figure 2.2. The numbers of the compounds in the figure are indicated in bold.

Narcotics: *Vaal et al. 1997:* acetone (**1**), benzene (**2**). Data are based for 70% on experiments by Sloof *et al.* 1983 and are supplemented with literature data. The values are presented as geometric means of all LC50 data found per species. Extreme values due to special life stages and/or extreme experimental conditions are excluded. **Polar narcotics:** *Staples et al. 1997:* DMP (**3**), DEP (**4**), DBP (**5**), BBP (**6**). The authors present an extensive database on the toxicity of phthalate esters. Phthalate esters are non-reactive plasticizers of high molecular weight polymers, such as PVC. All compounds (9)-(12) show polar narcosis, their toxicity increasing with decreasing water solubility from left to right in the figure. Data originate from many different sources and may include multiple measurements for the same species. All results selected for use in the present study are based on LC50 values, most at 96 h. Values include static and flow-through experiments. *Vaal et al. 1997:* Only invertebrates selected. aniline (**7**). **Specifics aquatic:** *Vaal et al. 1997:* dieldrin (**8**), lindane (**9**), malathion (**10**), parathion (**11**), pentachlorophenol (**12**). Explanation, see above. *Morton et al. 1997:* azinphos-methyl (**13**). The authors present an extensive literature review of effects of azinphos-methyl on salt and freshwater species, including 9 invertebrates. Toxicity reported as LC50 values in ug/L, and for most values after 96 h exposure. *van Wijngaarden et al. (1996, 5 species, (14)) and (1993, 6 species, (15))* have studied the toxicity of chlorpyrifos both in laboratory tests and in experimental ditches. The results originate from bioassays at their laboratory. The data represent LC50 values (ug/l), measured after 96 h exposure time in static or discontinuous flow bioassays. **Specifics terrestrial:** *Løkke and van Gestel 1998:* Dimethoate (**16**). *Croft and Whalon 1982:* Cypermethrin (**17**), fenvalerate (**18**). The authors present an extensive database for pyrethroid pesticides put together on the basis of literature data. The study deals with natural enemies of agricultural pests. All data are based on laboratory bioassays, but refer to different crops. The selected cypermethrin effects refer to hymenopterous wasps, the fenvalerate effects to coccinellid and carabid beetles.

Appendix to section 2.3: Background information for the data in Figure 2.4. The numbers of the compounds in the figure are indicated in bold.

Narcotics (A): *Thurston et al. 1985:* 2-(2-ethoxyethoxy)-ethanol (**1**), 2-methyl-2,4-pentanediol (**2**), 2-methyl-1-propanol (**3**), 2,2,2-trichloroethanol (**4**), 2,4-pentanedione (**5**), hexachloroethane (**6**). The authors report LC50 values in ug/L at 96 h after the start of the experiment. Values based on a flow-through experiment. Weights of the fishes varying between 0.1 and 7 grams. Compounds (**1**)-(**4**) are alcohols with differing toxicity, (**5**) is a narcotic compound with is supposedly neurotoxic, (**6**) is a narcotic compound showing rapid accumulation.

Vaal et al. 1997: acetone (**7**), benzene (**8**). **Polar narcotics (B):** *Staples et al. 1997:* DMP (**9**), DEP (**10**), DBP (**11**), BBP (**12**). *Vaal et al. 1997:* aniline (**13**) **Specifics (C):** *Vaal et al. 1997:* parathion (**14**), dieldrin (**15**), lindane (**16**), pentachlorophenol (**17**), malathion (**26**). *Thurston et al. 1985:* 2-chloroethanol (**18**), pentachlorophenol (**30**), permethrin (**31**), endrin (**32**),. Description of bioassays is given above**.** *Macek and McAllister 1970:* lindane (**19**), DDT (**20**), toxaphene (**21**), methyl parathion (**22**), Baytex (**23**), cuthion (**24**), malathion (**25**), carbaryl (**28**), zectram (**29**). Tests based on static bioassays, the fish weighing never more than 1.7 grams. LC50 values determined after 96 h. *Morton et al. 1997:* azinphos-methyl (**27**). An organophosphate. Two species tested using laboratory bioassays. Sensitivities of other species originate from a literature review presented in the same study. *Elonen et al. 1998:* 2,3,7,8-TCDD (**33**). Toxicity data on fish eggs. Reported as LC50-egg (at 32 days or more) based on known initial concentrations in the eggs. During the post-exposure period the eggs lay in uncontaminated water.

Appendix to section 2.5: Background information for the data in Figure 2.7. The numbers of the compounds in the figure are indicated in bold.

Polar narcotics (A): *Staples et al. 1997:* DMP (**1**), DEP (**2**), DAP (**3**), DBP (**4**), BBP (**5**). The data included in this literature review have been discussed in the text accompanying. **Specifics (B):** *Kasai and Hatakeyama 1993:* Simetryn (**6**), Pretilachlor (**7**), Thiobencarb (**8**). The effect of these herbicides on population development was measured for two different strains of two different algae species. *Fairchild et al. 1998:* Metribuzin (**9**), Alachlor (**10**), Metolachlor (**11**), Atrazine (**12**). Tests involved static exposure to the herbicide dilutions. Biomasses were determined 96 hours after the adding of the pesticide for algae and Lemna, and after 14 days for the submerged macrophyte species. *Tang 1997:* Atrazine (**13**). Studies performed as static exposure tests. The EC50 values after 28 days reported here were based on the chlorophyll *a* content of the cultures. *Bednarz 1981:* Atrazine (**14**), 2,4-D acid (**15**), Diuron (**16**), Monuron (**17**), Simazine (**18**), 1,4 p-naphtoquinone (**19**), TCA (**20**), DDT (**27**), Methoxychlor (**28**). The EC50 values were based on biomass development after 14 days. Exposure involved static bioassays. Compounds (14)-(18) are herbicides, (19) is a fungicide, and (27) and (28) are insecticides. *Sáenz et al. 1997:* paraquat (**21**). Tests were performed under static conditions. 96 EC50 values were based on cell counts. Data include a sensitive and a resistant strain of *Scenedesmus quadricauda*. *Blanck et al. 1988:* paraquat (**22**), diuron (**23**), glyphosate (**24**), norflurazon (**25**), tributyltin oxide (**26**). Tests performed under static conditions. EC100 values relate to visual observations of the concentration which causes no detectable growth after 14 days. *Kent and Currie 1995:* fenitrothion (**29**). The authors present toxicity data on growth rate and final biomass for a large number of algae. Only their EC50 values (in ug/L) for effects on growth rate (96 h) are used in the present study.

Appendix to section 2.6: Background information for the data in Figure 2.9. The numbers of the compounds in the figure are indicated in bold.

All compounds are specifics: *Fletcher et al. 1985:* dalapon (1), 2,4-D (2), dicamba (3), diphenamid (4), trifluralin (5), picloram (6), (all in uMole), and 2,4-D (7), diphenamid (8), dinoseb (9), linuron (10), terbacil (11), (all in kg/ha). uM data originate predominantly from glass-house studies. Kg/ha data from field studies. Data originate mainly from a database called 'Phytotox'. Values are based on different endpoints and moments of observation and include effects varying between 35 and 70% effect. *Løkke et al. 1995:* glyphosate (12). Data based on literature references and data sets from the Danish EPA. Measures expressed as NOEC (kg/ha) observed for various parameters and at different times after application. *Fletcher et al. 1990:* promethryn (13). Data based on a database called 'Phytotox'. EC50 values may be calculated by interpolation from raw literature data. Various effect measures are used. *Boutin 1999:* sulfonylurea (14), dinitroanaline (15), imidazolinone (16). Data from a large database on pesticide registration requirements for the Canadian Regulatory authority (134 species, 10 herbicides, 25 studies). Effect observations scaled from 1 to 10, relative to the control, are converted into effect percentages, which are used to calculate the ED25 (see also Boutin *et al.* 1993).

Birds

	Compound			Measure	Units	Time	Param	Sexe/ stage	Spec's /ob's	Avg(log (LC50))	STD(log (LC50))	P NORM	SK	CL5C	CL95C
1	Tucker and Haegele 1971 + Schafer 1983	abate	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	1.83	0.31	0.91	1.05	-0.33	0.60
2	Tucker and Haegele 1971 + Schafer 1983	azodrin	crotonamide	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	0.46	0.26	0.95	-0.67	-0.46	0.35
3	Tucker and Haegele 1971 + Schafer 1983	baygon	carbamate	Carb-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	1.23	0.35	0.96	-0.46	-0.65	0.55
4	Tucker and Haegele 1971 + Schafer 1983	baytex	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	0.93	0.40	0.94	-0.37	-0.68	0.48
5	Tucker and Haegele 1971 + Schafer 1983	bidrin	crotonamide	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	0.53	0.23	0.94	0.88	-0.33	0.45
6	Tucker and Haegele 1971 + Schafer 1983	dieldrin	PCH	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	6	1.80	0.44	0.89	1.23	-0.43	0.78
7	Tucker and Haegele 1971 + Schafer 1983	dursban	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	1.29	0.40	0.97	0.15	-0.60	0.58
8	Tucker and Haegele 1971 + Schafer 1983	epn	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	6	0.99	0.44	0.94	0.86	-0.51	0.73
9	Tucker and Haegele 1971 + Schafer 1983	landrin	carbamate	Carb-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	1.70	0.38	0.96	-0.71	-0.70	0.53
10	Tucker and Haegele 1971 + Schafer 1983	metasystex-r	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	1.47	0.56	0.90	-0.83	-0.96	0.59
11	Tucker and Haegele 1971 + Schafer 1983	mobam	carbamate	Carb-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	6	2.47	0.44	0.94	-0.37	-0.71	0.58
12	Tucker and Haegele 1971 + Schafer 1983	paration	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	6	0.75	0.42	0.92	0.67	-0.42	0.63
13	Tucker and Haegele 1971 + Schafer 1983	strychnine			LD50	mg/kg	14 d or more	mortality	separate /mixed	6	1.06	0.41	0.79	-0.94	-0.60	0.33
14	Tucker and Haegele 1971 + Schafer 1983	systox	phosphorothioate	OP-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	6	0.96	0.11	0.81	1.79	-0.11	0.21
15	Tucker and Haegele 1971 + Schafer 1983	zectran	carbamate	Carb-antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	8	0.92	0.46	0.87	0.98	-0.44	0.78
16	Tucker and Haegele 1971 + Schafer 1983	1080	fluoroacetate	antiChE	LD50	mg/kg	14 d or more	mortality	separate /mixed	6	0.78	0.29	0.93	0.82	-0.30	0.47
	Average(s) per reactive group								**reactives**	**114**		**0.36**	**0.98**	**0.00**	**-0.60**	**0.59**

Fish

		Compound		Measure	Units	Time	Param	Spec's /ob's	Avg(log (LC50))	STD(log (LC50))	P NORM	SK	CL5C	CL95C
1	Thurston et al. 1985	2-(2-ethoxyethoxy)-ethanol	narc	LC50	µmol/L	4d	survival, flow through	6	5.00	0.20	0.89	-1.04	-0.35	0.20
2	Thurston et al. 1985	2-methyl-2,4-penta-nediol	narc	LC50	µmol/L	4d	survival, flow through	6	4.95	0.07	0.84	-0.07	-0.09	0.08
3	Thurston et al. 1985	2-methyl-1-propanol	narc	LC50	µmol/L	4d	survival, flow through	6	4.34	0.07	0.97	0.54	-0.08	0.10
4	Thurston et al. 1985	2,2,2-tri-chloroethanol	narc	LC50	µmol/L	4d	survival, flow through	6	3.20	0.09	0.98	-0.17	-0.14	0.12
5	Thurston et al. 1985	2,4-pen-tanedione	narc	LC50	µmol/L	4d	survival, flow through	6	3.04	0.16	0.99	-0.28	-0.24	0.21
6	Thurston et al. 1985	hexachloroethane	narc	LC50	µmol/L	4d	survival, flow through	6	0.74	0.12	0.96	0.21	-0.18	0.20
7	Vaal et al. 1997	acetone	narc	mean LC50	µmol/L	variable	survival	8	5.19	0.14	0.97	0.17	-0.21	0.20
8	Vaal et al. 1997	benzene	narc	mean LC50	µmol/L	variable	survival	6	2.69	0.52	0.95	0.25	-0.76	0.82
9	Staples et al. 1997	DMP	pol-narc	LC50	µg/L	variable	survival/	9	4.75	0.20	0.90	0.63	-0.29	0.33
10	Staples et al. 1997	DEP	pol-narc	LC50	µg/L	4 d	survival	8	4.54	0.36	0.92	0.29	-0.46	0.50
11	Staples et al. 1997	DBP	pol-narc	LC50	µg/L	4 d	survival	14	3.28	0.39	0.97	-0.35	-0.73	0.59
12	Staples et al. 1997	BBP	pol-narc	LC50	µg/L	4 d	survival	10	3.35	0.55	0.87	1.49	-0.60	1.29
13	Vaal et al. 1997	aniline	pol-narc	mean LC50	µmol/L	variable	survival	7	2.69	0.70	0.97	0.57	-0.90	1.22
14	Vaal et al. 1997	parathion	reactive	mean LC50	µmol/L	variable	survival	7	0.33	0.54	0.94	-0.93	-1.00	0.67
15	Vaal et al. 1997	dieldrin	neurotoxin	mean LC50	µmol/L	variable	survival	7	-1.54	0.36	0.94	0.21	-0.44	0.57
16	Vaal et al. 1997	lindane	neurotoxin	mean LC50	µmol/L	variable	survival	7	-0.48	0.44	0.89	1.34	-0.50	0.86
17	Vaal et al. 1997	pentachlorophenol	uncoupler	mean LC50	µmol/L	variable	survival	8	-0.15	0.31	0.97	0.22	-0.46	0.54
18	Thurston et al. 1985	2-chloro-ethanol	neurotoxin	LC50	µmol/L	4d	survival, flow through	6	2.56	0.20	0.98	-0.11	-0.28	0.27
19	Macek and McAllister 1970	lindane	neurotoxin	LC50	µg ai/L	4 d	survival	12	1.67	0.48	0.72	-2.42	-1.36	0.45
20	Macek and McAllister 1970	DDT	neurotoxin	LC50	µg ai/L	4 d	survival	12	0.84	0.34	0.95	-0.25	-0.54	0.48
21	Macek and McAllister 1970	toxaphene	neurotoxin	LC50	µg ai/L	4 d	survival	12	0.89	0.30	0.82	-1.00	-0.59	0.25
22	Macek and McAllister 1970	methyl parathion	OP-anti-ChE	LC50	µg ai/L	4 d	survival	12	3.73	0.16	0.92	-0.51	-0.30	0.22
23	Macek and McAllister 1970	Baytex	OP-anti-ChE	LC50	µg ai/L	4 d	survival	12	3.22	0.15	0.96	0.58	-0.25	0.32
24	Macek and McAllister 1970	cuthion	OP-anti-ChE	LC50	µg ai/L	4 d	survival	12	2.01	1.13	0.89	0.37	-1.41	1.62
25	Macek and McAllister 1970	malathion	OP-anti-ChE	LC50	µg ai/L	4 d	survival	12	2.98	0.89	0.77	0.35	-0.97	1.13
26	Vaal et al. 1997	malathion	OP-anti-ChE	mean LC50	µmol/L	variable	survival	6	-0.04	1.23	0.98	-0.36	-1.94	1.70
27	Morton et al. 1997	azinphos-methyl	OP-anti-ChE		µg/L	most 4d	survival	8	2.17	1.46	0.94	-0.76	-2.61	1.71
28	Macek and McAllister 1970	carbaryl	carb-anti-ChE	LC50	µg ai/L	4 d	survival	12	3.76	0.50	0.87	-0.93	-0.89	0.54
29	Macek and McAllister 1970	zectram	carb-anti-ChE	LC50	µg ai/L	4 d	survival	12	4.01	0.34	0.74	-1.69	-0.77	0.28
30	Thurston et al. 1985	pentachlorophenol	uncoupler	LC50	µmol/L	4d	survival, flow through	6	-0.13	0.17	0.84	-0.70	-0.24	0.15
31	Thurston et al. 1985	permethrin	neurotoxic	LC50	µmol/L	4d	survival, flow through	6	-1.62	0.68	0.68	2.22	-0.53	1.36
32	Thurston et al. 1985	endrin	neurotoxic	LC50	µmol/L	4d	survival, flow through	6	-2.91	0.25	0.96	-0.35	-0.35	0.30
33	Elonen et al. 1998	2,3,7,8-TCDD	AhR interactie	LC50	pg/g ww	>> 10 d	survival of eggs following single exposure	7	3.09	0.28	0.91	-0.01	-0.36	0.33
	Average(s) per reactive group						**narcotics**	**50**		**0.20**	**0.86**	**0.22**	**-0.23**	**0.21**
							polar narcotics	**48**		**0.43**	**0.96**	**0.67**	**-0.65**	**0.59**
							reactive	**182**		**0.59**	**0.94**	**-0.27**	**-0.89**	**0.98**

Invertebrates

		Compound		Measure	Units	Time	Param	Spec's /ob's	Avg(log (LC50))	STD(log (LC50))	P NORM	SK	CL5C	CL95C
	Aquatic invertebrates													
1	Vaal et al. 1997	Acetone	narcotic	mean LC50	µmol/L	variable	survival	14	5.14	0.25	0.78	-2.02	-0.75	0.27
2	Vaal et al. 1997	Benzene	narcotic	mean LC50	µmol/L	variable	survival	14	3.09	0.47	0.96	-0.47	-0.98	0.63
3	Staples et al. 1997	DMP	pol narc	LC50	µg/L	various lengths	survival	6	4.88	0.37	0.82	1.75	-0.36	0.70
4	Staples et al. 1997	DEP	pol narc	LC50	µg/L	4 d	survival	5	4.53	0.56	0.89	-0.35	-0.65	0.59
5	Staples et al. 1997	DBP	pol narc	LC50	µg/L	4 d	survival	14	3.60	0.44	0.94	-0.80	-0.90	0.63
6	Staples et al. 1997	BBP	pol narc	LC50	µg/L	4 d	survival	9	3.31	0.35	0.90	0.87	-0.35	0.68
7	Vaal et al. 1997	Aniline	polar arc	mean LC50	µmol/L	variable	survival	13	3.25	0.95	0.82	-0.52	-2.38	2.05
8	Vaal et al. 1997	Dieldrin	OP-anti-ChE	mean LC50	µmol/L	variable	survival	2	-1.29	1.57	1.00	.	-1.11	1.11
9	Vaal et al. 1997	Lindane	neurotoxic	mean LC50	µmol/L	variable	survival	5	0.38	0.93	0.91	-1.02	-1.45	0.87
10	Vaal et al. 1997	Malathion	OP-anti-ChE	mean LC50	µmol/L	variable	survival	3	-1.49	0.86	0.97	0.84	-0.77	0.93
11	Vaal et al. 1997	Parathion	OP-anti-ChE	mean LC50	µmol/L	variable	survival	5	-2.05	0.59	0.93	0.72	-0.57	0.86
12	Vaal et al. 1997	Pentachlorophenol	uncoupler	mean LC50	µmol/L	variable	survival	14	0.75	0.87	0.92	0.74	-1.13	1.68
13	Morton et al. 1997	Pzinphos-methyl	OP-anti-ChE	LC50	µg/L	most 4d	survival	9	0.12	1.17	0.84	1.60	-1.12	2.67
14	van Wijngaarden et al. 1996	Chlorpyrifos	OP-anti-ChE	LC50	µg/L	4 d	survival	5	-0.22	0.42	0.94	0.97	-0.47	0.66
15	van Wijngaarden et al. 1993	Chlorpyrifos	OP-anti-ChE	LC50	µg/L	4 d	survival	6	-0.23	0.70	0.96	0.41	-0.92	1.04
	Average(s) per reactive group						**narcotics**	**28**		**0.37**	**0.96**	**-0.71**	**-0.74**	**0.59**
							polar narcotics	**47**		**0.59**	**0.89**	**-0.60**	**-0.72**	**0.68**
							reactives	**59**		**0.76**	**0.97**	**0.05**	**-1.20**	**1.44**
	Terrestrial invertebrates													
16	Løkke and van Gestel 1998	Dimethoate	OP-anti-ChE	LC50	mg ai /kg	variable	survival	10	0.88	1.03	0.87	0.02	-1.20	1.44
17	Croft and Whalon 1982	Cypermethrin	Neurotoxic	LC50	µg/g	variable	survival	9	-0.29	0.87	0.97	0.05	-1.36	1.47
18	Croft and Whalon 1982	Fenvalerate	Neurotoxic	LC50	g ai/ha	variable	survival	14	1.59	0.47	0.93	0.25	-0.75	0.75
	Average(s) per reactive group						**reactives**	**35**	**0.73**	**0.79**	**0.92**			

Aquatic plants

		Compound		Measure	Units	Time	Param	Spec's /ob's	Avg(log (LC50))	STD(log (LC50))	P NORM	SK	CL5C	CL95C
1	Staples et al. 1997	DMP	pol-narc	EC50 or LC50	µg/L	4 d	various growth related	8	4.85	0.39	0.92	0.53	-0.44	0.64
2	Staples et al. 1997	DEP	pol-narc	EC50 or LC50	µg/L	4 d	various growth related	12	4.56	0.39	0.90	-1.05	-0.91	0.44
3	Staples et al. 1997	DAP	pol-narc	EC50 or LC50	µg/L	4 d	various growth related	3	3.66	0.08	1.00	0.26	-0.08	0.08
4	Staples et al. 1997	DBP	pol-narc	EC50 or LC50	µg/L	4 d	various growth related	8	3.01	1.00	0.92	-1.14	-2.01	1.10
5	Staples et al. 1997	BBP	pol-narc	EC50 or LC50	µg/L	4 d	various growth related	9	2.46	0.34	0.93	0.37	-0.42	0.54
6	Kasai and Hatakeyama 1993	Simetryn	reactive	EC50	µg/L		biomass	4	1.79	1.02	0.78	-0.03	-0.98	0.90
7	Kasai and Hatakeyama 1993	Pretilachlor	reactive	EC50	µg/L		biomass	4	1.72	1.72	0.85	0.10	-1.61	1.79
8	Kasai and Hatakeyama 1993	Thiobencarb	reactive	EC50	µg/L		biomass	4	2.50	1.22	0.80	-0.04	-1.19	1.08
9	Fairchild et al. 1998	Metribuzin	reactive	EC50	µg/L	4d alg 14d macro	wet weight biomass	11	1.70	0.67	0.75	2.15	-0.55	1.78
10	Fairchild et al. 1998	Alachlor	reactive	EC50	µg/L	4d alg 14d macro	wet weight biomass	11	2.68	0.88	0.86	-0.92	-1.68	0.80
11	Fairchild et al. 1998	Metolachlor	reactive	EC50	µg/L	4d alg 14d macro	wet weight biomass	11	2.85	0.66	0.84	-0.41	-1.01	0.63
12	Fairchild et al. 1998	Atrazine	reactive	EC50	µg/L	4d alg 14d macro	wet weight biomass	11	2.01	0.60	0.83	1.33	-0.68	1.47
13	Tang 1997	Atrazine	reactive	EC50	µg/L	28 d	Chl a	8	2.00	0.34	0.95	-0.32	-0.53	0.41
14	Bednarz 1981	Atrazine	reactive	EC50	µg/L	14 d	biomass	12	2.15	0.76	0.93	-0.48	-1.28	1.02
15	Bednarz 1981	2,4-D acid	reactive	EC50	µg/L	14 d	biomass	12	2.49	2.09	0.92	-0.34	-3.32	2.51
16	Bednarz 1981	Diuron	reactive	EC50	µg/L	14 d	biomass	12	1.49	0.79	0.86	1.05	-0.79	1.68
17	Bednarz 1981	Monuron	reactive	EC50	µg/L	14 d	biomass	12	2.58	0.79	0.87	-1.22	-1.76	1.04
18	Bednarz 1981	Simazine	reactive	EC50	µg/L	14 d	biomass	12	2.25	1.18	0.92	0.57	-1.48	2.19
19	Bednarz 1981	1,4 p-naphtoquinone	reactive	EC50	µg/L	14 d	biomass	12	4.35	1.28	0.56	-1.95	-2.75	0.65
20	Bednarz 1981	TCA	reactive	EC50	µg/L	14 d	biomass	12	4.05	0.96	0.86	-0.41	-1.57	0.95
21	Sáenz et al. 1997	paraquat	reactive	EC50	µg/L	4 d	cells	5	2.42	0.57	0.98	-0.09	-0.75	0.69
22	Blanck et al. 1988	paraquat	reactive	EC100	µg/L	?	?	12	1.65	0.60	0.91	0.52	-0.85	0.95
23	Blanck et al. 1988	diuron	reactive	EC100	µg/L	?	?	12	2.40	0.61	0.73	1.76	-0.60	1.50
24	Blanck et al. 1988	glyphosate	reactive	EC100	µg/L	?	?	12	4.13	0.20	0.77	-0.74	-0.43	0.18
25	Blanck et al. 1988	norflurazon	reactive	EC100	µg/L	?	?	12	2.00	0.64	0.84	1.42	-0.60	1.50
26	Blanck et al. 1988	tributyltin oxide	reactive	EC100	µg/L	?	?	12	2.35	0.42	0.85	1.06	-0.55	0.95
27	Bednarz 1981	DDT	reactive	EC50	µg/L	14 d	biomass	12	4.40	0.63	0.88	-1.11	-1.44	0.60
28	Bednarz 1981	Methoxychlor	reactive	EC50	µg/L	14 d	biomass	12	3.64	0.55	0.90	-0.72	-0.96	0.83
29	Kent and Currie 1995	fenitrothion	reactive	EC50	µg/L	4 d	max biomass	12	3.76	0.25	0.85	-0.55	-0.42	0.24
	Average(s) per reactive group						**polar narcotics**	**40**		**0.52**	**0.92**	**-1.18**	**-0.72**	**0.79**
							reactives	**249**		**0.83**	**0.97**	**-0.39**	**-1.35**	**1.35**

Terrestrial plants

		Compound		Measure	Units	Time	Param	Spec's /ob's	Avg(log (LC50))	STD(log (LC50))	P NORM	SK	CL5C	CL95C
1	Fletcher et al. 1985	dalapon (uM/ha)	herbicide	35-70% 'effect'	µM/ha	variable	'most sens. out of 11'	4	4.22	1.44	0.97	-0.74	-1.89	1.48
2	Fletcher et al. 1985	2,4-D (uM)	herbicide	35-70% 'effect'	µM/ha	variable	'most sens. out of 11'	11	2.05	1.15	0.82	-0.08	-1.35	1.95
3	Fletcher et al. 1985	dicamba (uM)	herbicide	35-70% 'effect'	µM/ha	variable	'most sens. out of 11'	4	1.02	0.65	0.63	2.00	-0.33	0.98
4	Fletcher et al. 1985	diphenamid (uM)	herbicide	35-70% 'effect'	µM/ha	variable	'most sens. out of 11'	15	0.81	0.40	0.88	0.11	-0.51	0.59
5	Fletcher et al. 1985	trifluralin (uM)	herbicide	35-70% 'effect'	µM/ha	variable	'most sens. out of 11'	7	0.25	0.91	0.89	0.77	-0.95	1.49
6	Fletcher et al. 1985	picloram (uM)	herbicide	35-70% 'effect'	µM/ha	variable	'most sens. out of 11'	5	0.97	0.82	0.92	0.30	-0.97	1.03
7	Fletcher et al. 1985	2,4-D (kg)	herbicide	35-70% 'effect'	kg/ha	variable	'most sens. out of 11'	9	-0.15	0.50	0.86	0.25	-0.85	0.80
8	Fletcher et al. 1985	diphenamid (kg)	herbicide	35-70% 'effect'	kg/ha	variable	'most sens. out of 11'	9	0.30	0.52	0.85	0.19	-0.60	0.65
9	Fletcher et al. 1985	dinoseb (kg)	herbicide	35-70% 'effect'	kg/ha	variable	'most sens. out of 11'	5	0.27	1.46	0.79	-0.67	-1.88	1.16
10	Fletcher et al. 1985	linuron (kg)	herbicide	35-70% 'effect'	kg/ha	variable	'most sens. out of 11'	4	-0.23	1.00	0.76	-1.80	-1.47	0.62
11	Fletcher et al. 1985	terbacil (kg)	herbicide	35-70% 'effect'	kg/ha	variable	'most sens. out of 11'	4	-1.05	0.96	0.86	-0.85	-1.25	0.75
12	Løkke et al. 1995	glyphosate	herbicide	various	kg/ha	5-730 d	variable	9	-0.87	1.46	0.84	1.71	-1.53	3.36
13	Fletcher et al. 1990	promethryn	herbicide	EC50	kg/ha?	not stated	not stated	31	0.04	0.41	0.93	0.22	-0.59	0.70
14	Boutin	Sulfonylyrea	herb (post)	Ec25 (g)	kg/ha	variable	variable	46	-2.12	0.90	0.85	0.20	-1.17	1.21
15	Boutin	Dinitroanaline	herb (pre)	Ec25 (g)	kg/ha	variable	variable	47	-0.40	0.78	0.97	-0.17	-1.40	1.06
16	Boutin	Imidazolinone	herb (Postemergence)	Ec25 (g)	kg/ha	variable	variable	68	-1.65	0.55	0.94	0.72	-0.75	1.18
	Average(s) per reactive group						**reactives**	**278**		**0.75**	**0.99**	**0.30**	**-1.18**	**1.21**

Appendix 2

Using lumped sensitivity data to calculate and compare distribution based and fixed (OECD) safety factors

In this section we focus on the use of lumped data. The aim of lumping is to solve problems with small data sets. Particularly when only a single sensitivity test is available, neither the position of this sensitivity value compared to other species nor the variability in the sensitivity values of other species are known. This makes it impossible to calculate a distribution-based safety factor.

A solution to the above situation is offered by assuming that the width of the sensitivity distribution can be estimated from a reference data set containing results from similar compounds and comparable species.

If a large reference database can be used to estimate the width of the sensitivity distribution, this makes it relatively easy to calculate safety factors for all sample sizes.

An example of data-lumping for data on birds and mammals were presented by Luttik and Aldenberg 1997.

Subsequently, we will apply this method to other taxa.

Finally, we will use the results to compare safety limits based fixed safety factors with safety factors calculated from sensitivity distributions.

An example of lumping for bird and mammal data

Luttik and Aldenberg's (1997) data on birds and mammals encompassed a minimum of four sensitivity data per compound. Ranges, e.g. 10-40 mg/kg, were treated as separate values. Luttik & Aldenberg assumes a log-logistic sensitivity distribution.

If data are available which have been centred around the mean sensitivity per compound, this can be used to directly calculate an average standard deviation.

If the data only give standard deviations per compound the following equation can be used:

$$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 + \ldots + (n_m - 1)s_m^2}{n_1 + n_2 + \ldots + n_m - m} \qquad (1)$$

Here $S_1^2, S_2^2, \ldots, S_m^2$ are the sample variances of the m datasets with the ln(LD50) values for the respective toxicants and n_i are the number of species/observations per test. S_p can now be considered the estimate of the average standard deviation across all toxicants, and all species.

Application of this method implies that the standard deviations are independent of the mean, for example, do not increase with increasing toxicity. Furthermore it is required that it is reasonable to assume normal distributions for the separate data sets.

The average standard deviation (S_p) can be used in combination with the mean ($\bar{x}$) of any sample to estimate the dose which represents a hazard for only 5% of the species (the HD5) as follows:

$$\ln(HD5) = \bar{x} - 1.62 s_p \qquad (2)$$

The left 95% confidence limit of this HD5 value is now given by:

$$\ln(HD5)_{95} = \bar{x} - (1.62 s_p + 1.64 / \sqrt{n}) s_p \qquad (3)$$

As this equation is based on logarithmic values, the subtraction in the equation actually implies a factor by which the geometric mean of the untransformed data has to be divided to obtain the toxicological value of concern. When applied to the geometric mean of the original data, this factor yields the left 95% confidence limit. This factor has been named the 'safety factor' (SF) by Kooijman (1987).

Birds

The calculations of Luttik and Aldenberg (1997) for birds included 55 compounds, mainly choline-esterase inhibiters resulting in an overall standard deviation of 1.07 (= 0.46 on a 10log scale). From this value, safety factors can be calculated for any other data set with sensitivity data for one or more birds (Table A.1).

Mammals

The calculations for mammals included 69 compounds. A goodness of fit test did not reject a logistic distribution of the input data. From the overall data, a standard deviation was calculated of 0.83 (= 0.36 on a 10log scale). Corresponding safety values for samples of different size are given in Table A.1.

Table A.1. Safety factors based on LD50 values for birds and mammals. LD50 values were based on many compounds and many species.

	Safety factors:		**Safety factors: mammals birds**	
Number of LC50s	**SF50***	**SF95****	**SF50**	**SF95**
1	5.7	32.9	3.8	14.9
2	5.7	19.6	3.8	10.0
3	5.7	15.6	3.8	8.4
4	5.7	13.7	3.8	7.6
5	5.7	12.4	3.8	7.0
6	5.7	11.6	3.8	6.7
7	5.7	11.0	3.8	6.4
8	5.7	10.6	3.8	6.2
9	5.7	10.2	3.8	6.0
10	5.7	9.9	3.8	5.9

From these results, it can be concluded that the data show modest variation, as is indicated by standard deviations being roughly equal to 1, which implies a factor $e^{1.07} = 2.9$ and $e^{0.83} = 2.3$ for the untransformed data of birds and mammals.

Using the overall variance to calculate safety limits for the present data

In chapter 2 we presented estimates of the overall standard deviation for the aquatic invertebrates, the fish, the birds, the aquatic plants and the terrestrial plants. The applicability of these estimates in the calculation of safety factors using equation (3), depends on the independence of the standard deviations and means of the compounds, and on the validity of the assumption of normality of the log-transformed data.

Probabilities for the normality of the data are included in the tables in chapter 2. All compounds had probabilities equal to or higher than 94%, indicating no major problems with the assumption of normality.

Then, the independence of the variance and the mean needs to be investigated. For this purpose, linear regressions were performed for all datasets on reactive compounds for groups of data measured with equal units. The regression curves for birds and aquatic plants are shown in Figure 3.1A and B; regression coefficients and significances are given in Table A.2.

With the exception of the terrestrial plants (uM/ha) none of the regressions indicated a significant relationship between the standard deviation and the mean. In fact, the significance of the terrestrial plants was caused by a single outlayer, and may, therefore, be considered irrelevant.

Table A.2. Regressions of the standard deviation against the mean for log-transformed LC50 data per compound. Regression based on pesticides and on data measured in the same units.

Spec. Group	Units	No. obs.	Prob. > |T|	Sign.
Aquatic invertebrates	umol	4	0.95	no
Fish	ug ai/L	9	0.64	no
Fish	umol/L	8	0.92	no
Birds	mg/kg	15	0.12	no
Aquatic plants	ug/L	23	0.56	no
Terrestrial plants	uM/ha	6	0.05	yes*
Terrestrial plants	kg/ha	9	0.88	no

* indicates that the significance depended strongly on a single outlayer.

It is concluded that the requirements for the calculations on lumped data seem to be met and calculations using equation (3) can be performed without major problems. Accordingly, similar data as given in Table A.1 were calculated for the different taxa of the present study (Table A.3.).

The calculated safety factors in Table A.3 reflect the substantial differences found in the variability within the studied groups. The size of the required safety factors diminishes rapidly as more data becomes available for a better estimate of the mean sensitivity. The safety factors for birds reported in Luttik and Aldenberg (1997) was twice the size of the safety factor estimated in our study. This might be caused by differences in variability of the data of different test methods. The present study is based on forced feeding experiments (Tucker and Haegele 1971, Schafer 1983).

Table A.3. Calculations of safety factors (SF5,95) for reactive chemicals. Data for taxon/environment groups are based on the inventory of Chapter 2. All results are based on LC50 values. SF's are calculated for samples of different size, up to 10 sensitivity values. Calculations are based on equation (3). Standard deviations are based on 10log values. Note the similarity between the SF's for a single sample, and the SR's calculated in Chapter 2.

No. of obs.	**Taxon/biotope-group (with std(log(LC50))**				
	Aquatic invertebrates (0.82)	Fish (0.58)	Birds (0.35)	Aquatic plants (0.85)	Terrestrial plants (0.75)
	Safety factors required for an SF5:95				
1	473	78	14	593	280
2	191	41	9	231	122
3	128	31	8	153	84
4	100	26	7	119	68
5	85	23	7	100	58
6	76	21	6	89	52
7	69	20	6	80	48
8	64	19	6	74	45
9	60	18	6	70	42
10	57	17	6	66	40

Comparison of fixed (OECD) and distribution based safety factors

When comparing distribution based safety factors with a tiered approach with fixed safety factors, some assumptions have to be made to enable quantification of differences.

One aspect is that the OECD method in principle aims at protection at no observable effect level (NOEC) whilst only LC50 values were used as the basis for the sensitivity distributions in the above text. As can be derived from Table 1.1 in the introduction, the OECD presumes that a factor 10 can account for the acute LC50- chronic NOEC conversion for fish and daphnia (point 2.5.2.2 of directive 97/57/EC, 1997), and a factor 2 for birds (point 2.5.2.1 of the same directive). The validity of this factor 10 forms the topic of the next chapter.

Another point is that when several data within a particular organism group, such as crustaceans, fish or algae, are available, the OECD text implies that the lowest value has to be used. This rule is applied in the present practice in registration procedures. With respect to 'rich' samples, for example with six sensitivity values, we will use the most probable position for a most sensitive species out of six as the basis for the OECD method (see Table A.4), whilst the size of the safety factors for the distribution based approach is determined at sample size six in Table A.3.

Table A.4. Most probable positions in a normal sensitivity distribution of the lowest sample for sample sizes ranging from 1 to 10. The most probable position of the lowest value is indicated as a fraction of the std below the mean.

Sample size	1	2	3	4	5	6	7	8	9	10
Fraction of std	0	0.56	0.85	1.03	1.16	1.27	1.35	1.42	1.49	1.54

Before presenting the results in a table, we will give an example of the comparison method used, based on fish data.

Example: A single sample for fish

Assuming that only a single sample is available, the data of Table A.3 requires an SF(95) of 78 for fish. This factor is based on LC50 values. Assuming that a factor 10 can be used for the conversion of LD50 data to NOEC levels (US EPA 1991, OECD 1992), this yields a safety factor at NOEC level of 780.

Comparison with the standard OECD (1992) method can be performed by assuming that a fish species was the most sensitive. The OECD method indicates that a factor of 100 should be applied.

Sensitivity distribution based safety factor is thus 7.8 times larger than that of the OECD.

Example: Six sensitivity data for fish

Assuming that six samples are available, the lowest value hereof has to be used for the OECD method. The most probable position for the lowest sample can be calculated as a fraction of the standard deviation (Kotz *et al.* 1983). For six samples, this fraction is 1.27. The fractions for other sample sizes are given in Table A.4. Accordingly, the most probable position for a low value out of six, given the std of 0.58 for fish, becomes 0.74. For the actual data this implies a factor $10^{0.74}$ = 5.5. Additionally, a factor 100 has to be applied (see introductory chapter). This yields a safety factor of 550 below the mean. Note that the availability of more data will lead to increasingly large safety factors.

Table A.5 for samples/compounds with six observations shows that the safety factor for six fish samples is 21. Applying a factor 10 to transform from LD50 to NOEC, we obtain safety factors of 210, relative to the estimated mean.

For a sample of six sensitivity values, the sensitivity distribution based extrapolations lead to safety factors for fish which are 0.38 times the size of the OECD values.

Comparison of methods for all investigated groups

The above calculations can also be performed using the data of the other taxa/environment combinations (Table A.5.).

Table A.5. Safety factors for the different methods based on the assumptions and calculations as explained in the example in the text.

	Single sample		**Six samples**	
	OECD	Distribution based	OECD (lowest[1])	Distribution based
Aquatic invertebrates	100	4730	1100	760
Fish	100	780	550	210
Birds	100	140	280	60
Aquatic plants	100	5930	1200	890
Terrestrial plants	100	2800	890	520

1: The most probable lowest value is chosen as the starting point for the risk assessment.

Summary of test method comparison

If only a single sensitivity measurement is available, the protection level offered by the OECD method is always smaller than the HC5(95) based on a large data set.

If several measurements are available the OECD method yields the largest factors.

The protection level of the OECD method varies between the different groups depending

Table A.5. Safety factors for the different methods based on the assumptions and calculations as explained in the example in the text.

	Single sample		**6 samples**	
	OECD	Distribution based	OECD (lowest[1])	Distribution based
Aquatic invertebrates	100	4730	1100	760
Fish	100	780	550	210
Birds	100	140	280	60
Aquatic plants	100	5930	1200	890
Terrestrial plants	100	2800	890	520

1: The most probable lowest value is chosen as the starting point for the risk assessment.

Appendix 3

The prediction interval for any LC50-derived NOEC value was calculated in the present study via the following equation:

$$\hat{\gamma}_0 \pm ts\left(1+\frac{1}{n}+\frac{(x_0-\bar{x})^2}{\sum_1^n (x_i-\bar{x})^2}\right)^{\frac{1}{2}} \qquad (4)$$

Were $\hat{\gamma}_0$ represents the regression prediction of the log(NOEC), t the 5% probability value (for 95% confidence limits) of a two-tailed t distribution, s the root of the mean square of the error terms of the regression ('MSE' in statistical programmes), n the number of observations, x_0 any actual log(LC50) value, $\bar{x}$ the mean of all log(LC50) values. The summary term in the equation can be obtained in statistical programmes as the CSS, the sum of squares corrected for the mean. In order to enable the calculation of confidence limits for any NOEC value, publications on regressions have to provide values for n, $\bar{x}$, the MSE and the CSS.

A simpler approach, which underestimates the confidence interval at low and high values, is to assume a constant prediction interval for all values. In that case the prediction limits can be calculated as:

$$\hat{\gamma}_0 \pm ts\left(1+\frac{1}{n}\right)^{\frac{1}{2}} \qquad (5)$$

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