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VALIDATION OF SOME EXTRAPOLATION METHODS USED FOR EFFECT ASSESSMENT

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Abstract—For effect assessment several extrapolation methods can be used to derive the concentration of toxic chemicals above which adverse effects on aquatic ecosystems may occur. These methods are based on single-species toxicity data. At present, however, it is uncertain whether the values calculated with these extrapolation methods really represent accurate estimations of concentrations harmless to ecosystems. Therefore a validation of extrapolation methods was carried out by comparing NOECs derived from multiple-species (semi-) field experiments with extrapolated values. In this study validation was restricted to the methods of Aldenberg and Slob and Wagner and Løkke and a modification of the method of the U.S. Environmental Protection Agency. Multiple-species experiments for organic compounds and metals in aquatic ecosystems were studied. For only 29 compounds, 19 organic compounds, and 10 metals, one or more multiple-species NOECs could be derived. For 11 of these compounds an insufficient amount of single-species toxicity data was available to apply the methods of Aldenberg and Slob and Wagner and Løkke. With reservations, due to this paucity of data, it is concluded that single-species toxicity data can be used to derive "safe" values for the aquatic ecosystem. Furthermore, extrapolation methods seem to be a good basis for determining these values. Based on the results of this study, the best correlation between multiple-species NOECs and extrapolated values can be obtained with the methods of Aldenberg and Slob and Wagner and Løkke, both with a 95% protection level and a 50% confidence level.

Keywords—Extrapolation methods Validation Multiple-species experiments Single-species data

INTRODUCTION

For effect assessment several extrapolation methods can be used to derive concentrations of toxic chemicals above which adverse effects on aquatic ecosystems may occur. These "safe" concentrations are calculated on the basis of results of single-species toxicity experiments. In the past, several extrapolation methods were developed, most of them with a different theoretical background. The Health Council of The Netherlands [1] has reported on the usefulness and limitations of the various methods. On the basis of available information, a procedure was proposed comprising a combination of the methods of Kooijman [2], Slooff et al. [3], and Van Straalen and Denneman [4]. In the Dutch policy memorandum "Premises for Risk Management" [5] this approach was not followed, but the method of Van Straalen and Denneman was adapted to derive quality objectives. This method was subsequently subject of several modifications to improve the statistical basis. Modifications were developed by Aldenberg and Slob [6] and Wagner and Løkke [7]. These two methods, as well as the method of Stephan et al. [8], were recommended by the Organization for Economic Cooperation and Development (OECD) [9] for refined effect assessment. For preliminary effect assessment, a modification of the U.S. Environmental Protection Agency (EPA) method [9] was recommended.

However, it still is uncertain whether the values calculated with extrapolation methods really represent accurate estimations of concentrations harmless to ecosystems. Doubts are raised mainly because of the differences in sensitivity of similar species under (semi-) field (multiple-species [MS]) and laboratory (single-species [SS]) conditions. Therefore, as recommended by the OECD [10] and the Health Council of The Netherlands [1], a validation was carried out by comparing NOECs de-

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rived from MS experiments with extrapolated values. In the first instance only MS experiments for organic compounds in aquatic ecosystems were considered [11]. In the underlying study MS experiments for both organic compounds and metals in aquatic ecosystems were used. In addition, to obtain as many MS data as possible, a new procedure was introduced to derive NOECs from LOECs. Validation was restricted to the methods of Aldenberg and Slob [6], Wagner and Løkke [7], and the modification of the EPA method [9]. The method of Stephan et al. [8] was not used here because this method needs at least eight chronic SS NOECs from specified families as input data, and these data were not available in most of the cases. Aims of the research were (a) to determine whether there are relevant differences in NOECs derived from MS and SS experiments for similar or related species and corresponding effect parameters, (b) to determine whether ecosystems are protected by calculating a "safe" value from SS NOECs by extrapolation, and (c) to determine whether there are relevant differences between the values calculated with the three extrapolation methods used.

MATERIALS AND METHODS

MS data

An extensive on-line literature search was performed for toxicity data on organic compounds and metals tested in freshwater systems. From DIMDI more than 3,000 references on MS experiments were obtained. Furthermore, several research centers and chemical industries were asked for data on MS experiments. From these searches about 300 references were selected as potentially useful. These experiments were evaluated using the following criteria, derived from Touart [12], Dortland [13], and Persoone [14]:

1. A distinct concentration–effect relationship should be obtained.
2. A reliable MS NOEC should be derived.
3. Several taxonomic groups, in more or less natural ecosystems, should be exposed to one test concentration for a longer period.
4. In each experiment several concentrations should be tested, consisting of one control and at least two test concentrations.
5. Each test concentration should have at least one replica.
6. The concentration of the test compound should be measured several times during the experiment.
7. Physicochemical parameters like pH, temperature, and hardness should be measured.
8. Apart from effect parameters like population density and biomass, effect parameters on higher integration levels such as species diversity and species richness should be measured.

The original criteria of these authors were modified because some were found to be too stringent to be useful, as none of the experiments were performed in accordance with all criteria. With respect to the modified criteria, three classifications could be distinguished: reliable, less reliable, and unreliable MS NOECs. Criteria 1, 2, 3, and 6, and for metals criterion 7, were considered to be the most important. An experiment was classified as reliable if it was carried out according to all criteria or was missing one or two less important criteria. If one important or several less important criteria were missing, the experiment was classified as less reliable, whereas an experiment was classified as unreliable if several important criteria were missing.

In the selected MS experiments, species from different taxonomic groups were tested. These species were classified in several taxonomic groups based on Mol [15] and Streble and Krauter [16] (Table 1). Differences in ecological function (trophic level) and morphological structure were also considered.

MS NOECs were calculated only for direct effects that influenced the population level, for example, effects on growth, reproduction, and survival. Indirect effects were left out of consideration, because for these effects often no concentration–effect relationship could be distinguished. Within several experiments more than one MS NOEC could be derived, that is, MS NOECs for different species that differ in sensitivity. Only the lowest MS NOEC, the one for the most sensitive tested species, was used for the comparison with extrapolated values.

The MS NOEC values were determined by means of three procedures:

1. Comparison of effect parameters in treated and control systems. The highest concentration in the treated system showing no significant differences compared to the control system was considered as the MS NOEC.

2. Determination of the concentration at the start of recovery of the most sensitive species. For that purpose the concentration of the compound immediately after the last application was determined. In addition, the time needed for recovery of the population density of the most sensitive species

Table 1. Classification of test organisms in taxonomic groups

Taxonomic group	Abbreviation
Bacteriophyta	Bac
Algae	Alg
Cyanophyta	Cya
Chrysophyta	Chr
Euglenophyta	Eug
Dinophyta	Din
Cryptophyta	Cry
Chlorophyta	Chl
Mycophyta	Myc
Macrophyta	Mac
Protozoa	Pro
Metazoa	Met
Rotatoria	Rot
Nematoda	Nem
Gastropoda	Gas
Oligochaeta	Oli
Hirundinae	Hir
Branchiopoda	Bra
Ostracoda	Ost
Copepoda	Cop
Malacostraca	Mal
Ephemeroptera	Eph
Odonata	Odo
Plecoptera	Ple
Heteroptera	Het
Coleoptera	Col
Diptera	Dip
Trichoptera	Tri
Hemiptera	Hem
Pisces	Pis

after the last application was calculated. This mean recovery time was derived as the average of the last observation day of no recovery and the first observation day of recovery. By means of the estimated half-life of the test compound (in this study defined as the time needed for 50% of the test substance to disappear assuming first-order kinetics), the concentration at the mean recovery time of the population density was determined. This concentration was regarded as the MS NOEC. An example of this method is presented in Figure 1.

3. Derivation of an MS NOEC from an MS LOEC. This procedure was used in experiments in which the lowest tested concentration showed a significant effect in comparison to the control system. The NOEC was derived as follows:

- If the LOEC showed 10 to 20% effect, NOEC = LOEC/2.
- If the LOEC showed ≥20% effect and there was a clear concentration-effect relationship, the EC10 was calculated by extrapolation of this relation, and regarded as the NOEC.
- If the LOEC showed ≥20% effect and there was no concentration-effect relationship, NOEC = LOEC/3 in case of 20 to 50% effect and NOEC = LOEC/10 in case of ≥50% effect.

The first procedure is considered as the most reliable one, although control and treated systems

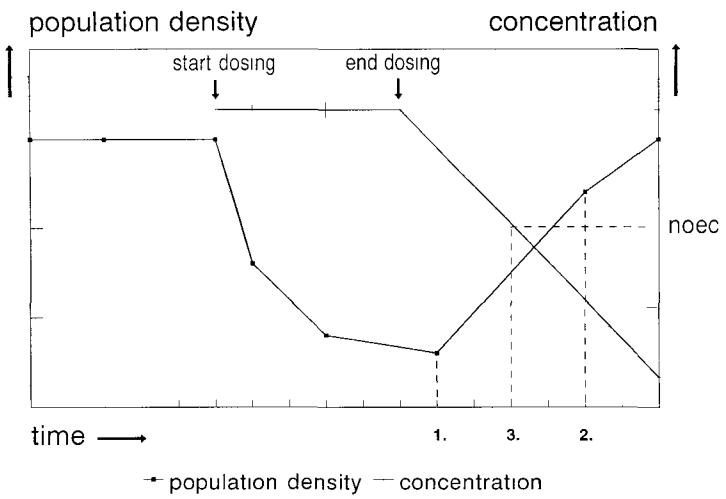

Fig. 1. Calculation of MS NOEC by determining the concentration of the test compound at the mean recovery time of population density (3). Mean recovery time (3) is derived as the average of the last observation day of no recovery (1) and the first observation day of recovery of population density (2).

were not always exactly alike due to natural deviation with respect to physicochemical parameters and species composition. Some comments can be made on the second procedure. In the first place, the half-life calculation was based on first-order kinetics. It is known, however, that in the course of time deviations will occur from first-order kinetics [17]. In the second place, the mean recovery time could often only be estimated inaccurately, because in most cases no raw data were available. In the third place, the mean recovery time was an estimation of a point in time at which recovery starts; therefore, it is not a very accurate estimation of a no-effect situation. Finally, it is known that population recovery does not necessarily occur when the concentration of the test compound decreases below the no-effect concentration but can be restricted to specific periods in the season (P. Leeuwangh, personal communication). For these reasons an MS NOEC derived with this procedure was considered either as less reliable or as unreliable. Comments can also be made on the third procedure. This procedure is based on expert judgment, and the mentioned factors are not experimentally determined. Especially when there is no concentration–effect relationship or when the effect percentage of the LOEC exceeds 50%, the usefulness of the procedure is questionable. To be classified as reliable, MS NOECs obtained with this third procedure had to be carried out in accordance with criteria slightly different from those presented before: a distinct concentration–effect relationship and the use of more than one test concentration are not strictly necessary. On the other hand, the effect percentage of the lowest concentration should be lower than 50%.

SS data

For all compounds for which an MS NOEC was derived, chronic SS NOECs were gathered. If no chronic data were available for algae, crustaceans, and fish, then acute toxicity data were sought. The reliability of these data was evaluated, based on criteria of the OECD *Guidelines for Testing of Chemicals* [18]. For one compound, 1,2,4-trichlorobenzene, estimates of chronic toxicity data also were made using QSARs. SS toxicity data were used as a basis for the application of extrapolation methods.

SS data were also used to obtain an indication about differences in sensitivity of species in MS experiments compared to similar or related species in SS experiments. For that purpose chronic data of similar or related species on the genus level, tested on corresponding effect parameters in MS and SS experiments, were compared. For this comparison MS NOECs of less sensitive species also were used.

Extrapolation methods

The extrapolation methods developed by Aldenberg and Slob [6], Wagner and Løkke [7], and the modified EPA method [9] were used for validation. The method of Aldenberg and Slob calculates a hazardous concentration (HCp), defined as the concentration at which p% of the species in the community may be adversely affected. The decision on what is an acceptable value for p% is not a matter of science, but a political compromise. As stated in "Premises for Risk Management" [5], the aquatic ecosystem is supposed to be protected if 95% of the species is protected, which means that in the ecosystem the NOEC is exceeded for at most 5% of the species. This implies that a HC5 is calculated (see Fig. 2). The 95% protection level can be calculated with 50 and 95% confidence levels. The 95% protection level with 50% confidence level means, in theory, that there is a 50% chance of this value being too high or too low. In The Netherlands this value is set equal to the maximum permissible concentration (MPC), which is used for setting environmental quality objectives [19]. The 95% protection level with 95% confidence level means that there is only a 5% chance of this value being too high. It is therefore a safer value. The difference between the 95% protection level with a 50% and with a 95% confidence level can be seen as a degree of uncertainty.

The method of Aldenberg and Slob uses the lowest SS NOECs per species as input data and is applied when at least four SS NOECs for different taxonomic groups, with preference for sensitive or target species, are available. This approach corresponds to the proposal of Okkerman et al. [20] for the former method of Van Straalen and Denneman [4]. SS NOECs used as input data for the extrapolation methods were evaluated according to Van de Meent et al. [21]: (a) if for a single species several NOECs were derived for *different* effect parameters, the lowest one was selected for the use in extrapolation methods; (b) if for a single species several NOECs were derived for the *same* effect parameter, a geometric mean was calculated for the use in extrapolation methods. Like Van Straalen and Denneman, Aldenberg and Slob assume that the SS NOECs used for calculation fit a log-logistic distribution. For checking this assumption the available SS data were tested statistically with the so-called empirical distribution function (EDF). The test performed here was the Kolmogorov–Smirnov

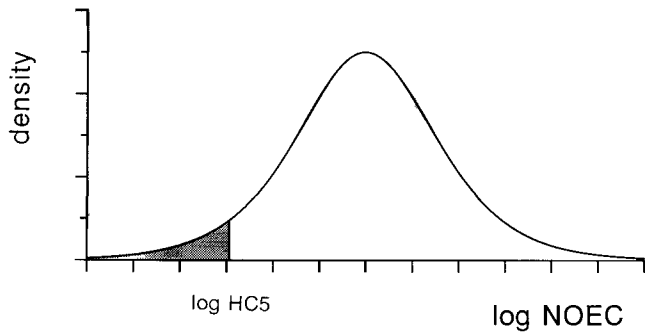

Fig. 2. The standard logistic distribution of log (NOEC) values, according to the extrapolation method of Aldenberg and Slob [6]. Log HC5 is the hazardous concentration to be calculated. The fraction of the species possibly adversely affected is shaded.

test [22]. With respect to the outcome of this test, it should be stated that there will be no absolute certainty, whether the data fit the log-logistic distribution or not. The test only calculates how (un)likely it is that the data are derived from the supposed distribution. In this study the (un)likeliness is calculated with a 1% significance level. Okkerman et al. [11] used a significance level of 5%.

The extrapolation method of Wagner and Løkke is a variant of the previous approach. The main difference is that is assumes a log-normal distribution instead of a log-logistic one. Here also the 95% protection level (HC5) is calculated with 50 and 95% confidence levels.

The modified EPA method estimates an environmental concern level, as does the former EPA method [23]. The method assumes constant differences between acute and chronic toxicity and between laboratory SS toxicity and field effects. Assessment factors in relation to the availability of SS data are presented in Table 2. Compared to the other methods, the modified EPA is straightforward and can be applied if only one acute or chronic test result or even one QSAR estimate of toxicity is available. The modified EPA method is also used in The Netherlands for setting environmental quality objectives, if fewer than four NOEC values are available. In that case, the extrapolated value is called an indicative MPC [19].

Statistical procedures

To quantify the differences between MS NOECs and values calculated with the extrapolation methods, standard regression techniques are not valid, as they assume that only one of the measured variables is subject to error, the so-called dependent variable. If, as in this study, both X and Y values contain errors, the standard regression model will underestimate the slope of the regression line. Therefore, a model II regression procedure was performed, which avoids this bias. This procedure will yield two different lines, depending on whether Y or X is used as the dependent variable. The line indicating the real relationship between X and Y will lie somewhere in between and can be calculated [24]. This method, however, does not allow signif-

Table 2. Assessment factors applied to derive environmental concern levels, according to the modified EPA method [9]

Available information	Assessment factor
Lowest acute L(E)C50 value or QSAR estimate for acute toxicity	1,000
Lowest acute L(E)C50 value or QSAR estimate for minimal algae/crustaceans/fish	100
Lowest NOEC value or QSAR estimate for chronic toxicity	10[a]
Lowest NOEC value or QSAR estimate for chronic toxicity for minimal algae/crustaceans/fish	10

[a]This value is subsequently compared to the extrapolated value based on acute L(E)C50 toxicity values. The lowest is selected.

icance tests on the obtained values. For this reason, separate tests were performed for significance of correlation coefficients and for differences between means (t test for paired comparisons) [25]. A condition for employing the latter test is that variances of the data sets to be compared are equal. If the variances are equal, the slope of the regression line can be considered to be equal to one. If the calculated t_s value is not significantly different from zero, the two data sets can be considered to be equal. If so, the intercept of the regression line will be equal to zero, so the regression line fits the line $Y = X$.

In addition, for the comparison of the sensitivity of species in MS experiments and similar or related species in SS experiments, a model II regression analysis and a t test for paired comparisons were performed.

RESULTS

MS and SS experiments

The results of the MS experiments are presented in Table 3. The NOECs presented are the lowest NOECs found in these experiments.

For 19 organic compounds and 10 metals, one or more MS NOECs could be estimated. According to the criteria presented before, the MS NOEC could be considered as reliable in seven cases (atrazine, copper (twice), 2,4-dinitrotoluene, fluorene, parathion, and 2,4,6-trinitrotoluene). The other MS NOECs were considered as less reliable (24 cases) or unreliable (27 cases). Population density and density recovery were the most sensitive parameters.

For seven compounds (aluminum, atrazine, cadmium, copper, mercury, selenium, and zinc) more than one MS NOEC could be obtained. It appeared that the factor between the highest and lowest MS NOECs for one compound was considerably high, particularly for atrazine, cadmium, and selenium, being 22, 33, and 1,200 respectively. There were several explanations for this: The experiments differed in reliability and different taxonomic groups or effect parameters were used. An exception was atrazine: Two NOECs of less reliable MS experiments both with algae and chlorophyll-*a* content as criterion differed by a factor 14.

For aziphos methyl, dichlobenil, 3,4-dichloroaniline, methyl parathion, permethrin, 1,2,4-trichlorobenzene, and trichloroethylene, an MS NOEC could be obtained by using the second procedure as well as the third. MS NOECs derived from both procedures are presented in Table 3. It appeared that for dichlobenil, methyl parathion, and trichloroethylene the two MS NOECs were considerably different, the factor between the values calculated with the third and the second procedures being 20, 90, and 890, respectively. It has to be noted, however, that the procedures had different effect parameters, namely density and density recovery.

The results of the (semi-) chronic SS experiments are presented by Emans et al. [26]. For most pesticides chronic SS data were found for target and therefore sensitive species. Exceptions were dieldrin and methyl parathion, both insecticides, and toxaphene, a herbicide. For dieldrin, however, other organisms like fish and, for toxaphene, other organisms like crustaceans and fish were also sensitive. For 1,2,4,-trichlorobenzene additional information was obtained by using QSARs [27]. For some compounds no chronic SS data were available for algae, crustaceans, and fish. For these compounds acute SS data were sought in order to apply the EPA method correctly. No reliable chronic data at all were found for dichlobenil and methyl parathion. For these compounds acute data were used for extrapolation. For methyl parathion acute SS data were available for target and therefore sensitive species, in contrast to dichlobenil.

Comparison of NOECs from MS and SS experiments for similar or related species

Results of the comparison of NOECs from MS and SS experiments for similar species were presented in Figure 3 and by Emans et al. [26]. These data can be compared only for similar or related species on the genus level and for corresponding effect parameters, which was not possible for all compounds. For atrazine, cadmium, copper, parathion, pentachlorophenol, and 1,2,4-trichlorobenzene a comparison could be made for two species.

For most comparisons the MS NOEC-to-SS NOEC ratio varied between 0.2 and 5.0. This result is consistent with findings stated by Slooff et al. [3]. It should be noted, however, that the ratios calculated for mercury, pentachlorophenol, trichloroethylene, toxaphene, and one for parathion are "greater than or equal to" values. In five cases (cadmium [twice], copper, pentachlorophenol, and toxaphene) the MS NOEC-to-SS NOEC ratio was <0.2 or >5.0, which had several reasons, like differences in test conditions between MS and SS experiments (e.g., copper) or unreliability of the tests (e.g., cadmium) [26]. Model II regression analysis of NOECs derived from MS experiments on NOECs derived from SS experiments for similar or related species

and corresponding effect parameters gave the following relationship:

$$\log \mathrm{NOEC}_{\mathrm{MS\ experiment}} = 0.750 \cdot \log \mathrm{NOEC}_{\mathrm{SS\text{-}experiment}} + 0.263 \quad (r = 0.935)$$

Unreliable cadmium data for algae and "greater than or equal to" values were left out of consideration. The corresponding correlation coefficient of 0.935 was significant, with $P < 0.001$, so both data sets were similar or related to each other. The regression line is presented in Figure 3.

Variances of both data sets were not significantly different ($P > 0.05$), indicating that the slope of the regression line could be considered to be equal to one. The t test for paired comparisons gave a t_s value of 0.407, which was not significant, at $P > 0.05$. It can therefore be stated that the regression line fits the line $Y = X$. In other words, species tested in MS experiments appear to be as sensitive as similar or related species in SS experiments.

Comparison of MS NOECs with extrapolated values

MS NOECs and extrapolated values are presented in Table 4. For all compounds the SS data did fit the log-logistic distribution according to the Kolmogorov-Smirnov test, with a significance level of 0.01.

With the method of Aldenberg and Slob [6], 95% confidence level, the lowest extrapolated values were calculated. Table 5 shows that the geometric means of values calculated with Wagner and Løkke [7], 95% confidence, and the EPA method [9] were in the same order of magnitude as those of Aldenberg and Slob, 95% confidence, being on average a factor 1.3 and 1.5 higher, respectively. The geometric means of values calculated with the methods of Aldenberg and Slob and Wagner and Løkke, both with 50% confidence, were much higher than those of Aldenberg and Slob, 95% confidence, namely a factor 14 and 11, respectively. In relation to the MS NOECs, extrapolated values calculated with Aldenberg and Slob, and Wagner and Løkke, both with 95% confidence, and the modified EPA method were much lower, on average differing by a factor 48, 36, and 31, respectively. Values calculated with Aldenberg and Slob, and Wagner and Løkke, both with 50% confidence levels, were about as high as the reliable and less reliable MS NOECs, differing by a factor 3.4 and 4.3, respectively.

In Table 6 the results of the model II regression analysis are presented. A distinction was made in reliable and less reliable MS NOEC data on one side and unreliable MS NOEC data on the other. This arrangement allowed determination of whether the criteria on which the classification of MS NOECs in reliable, less reliable, and unreliable was based were useful. Reliable and less reliable MS data were

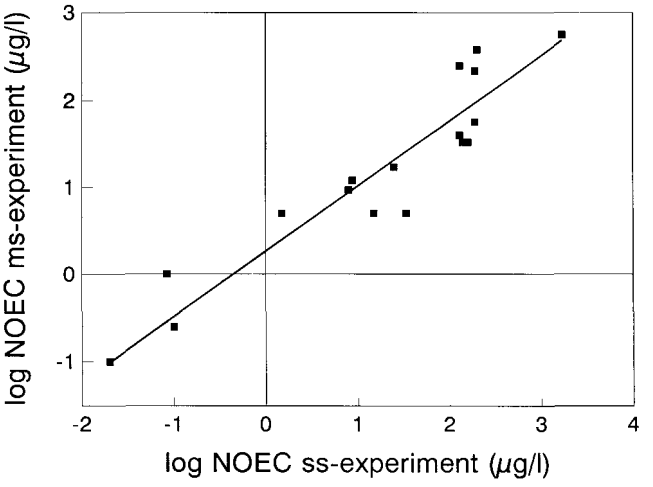

Fig. 3. Regression of $\mathrm{NOEC}_{\mathrm{MS\text{-}experiment}}$ on $\mathrm{NOEC}_{\mathrm{SS\ experiment}}$ for similar or related species and corresponding effect parameters, based on 17 data pairs: $\log \mathrm{NOEC}_{\mathrm{MS\text{-}experiment}} = 0.750 \cdot \log \mathrm{NOEC}_{\mathrm{SS\ experiment}} + 0.263$, $r = 0.935$.

Table 3. Test results of MS experiments

Compound	Quality[a]	Taxonomic groups tested[b]	Criterion[c]	MS NOEC[d] (μg/L)	Ref.
Aluminum	U	Bac, Cop (3)	D	33[e]	[29]
Aluminum	U	Alg	P	50[e]	[30]
Aluminum	U	Gas, Mal, Ple, Tri (5)	D	250	[31]
Atrazine	R	Cya[f], Chl[f], Rot, Nem, Ost, Cop (>11)	D	5.0	[32]
Atrazine	Lr	Bac, Alg, Myc, Pro, Met	C	8.0	[33]
Atrazine	Lr	Cya, Chr, Eug, Din, Cry, Chl, Rot, Bra, Cop, Mal (>59)	Npp	20	[34]
Atrazine	Lr	Bac, Alg, Myc, Pro[f], Met (150–200)	B,C	110	[35]
Azinphos methyl	Lr	Chl, Gas, Bra[f], Ost, Cop, Dip (11)	Dr/D	0.25/0.081[c]	[13]
Cadmium	U	Alg, Rot, Bra[f], Cop (23)	D,Do	0.07	[36]
Cadmium	U	Cya, Chr[f], Din, Cry, Chl (21)	D	0.1	[37]
Cadmium	Lr	Pro (170)	Co	0.7	[38]
Cadmium	U	Chl, Bra[f] (4)	D	1.0	[39]
Cadmium	Lr	Bac, Cya, Chl, Myc, Pro, Rot, Nem, Bra, Ost, Cop (17)	D,C	1.0	[40]
Cadmium	U	Alg	P	2.3	[41]
Chromium	U	Cya, Chr[f], Chl	B	50.0	[42]
Copper	Lr	Ep[f], Dip, Tri (6)	D	0.3[e]	[43]
Copper	U	Ep[f], Dip, Tri (9)	D	0.55[e]	[44]
Copper	R	Alg, Pro	D,C	0.66[e]	[45]
Copper	U	Alg	P	0.76	[41]
Copper	Lr	Cy[f], Chr, Chl, Eph, Ple, Cop, Dip, Tri (67)	D	2.5	[46–49]
Copper	R	Chr[f], Mac, Gas, Oli, Hir, Bra, Ost, Dip (13)	D	4.0	[50]
Dichlobenil	U	Cya, Chr, Chl, Mac[f], Rot, Ost, Mal, Odo, Pis (18)	Dr/D	5.0/100[e]	[51]
3,4-Dichloroaniline	Lr	Gas, Bra[f], Ost, Cop, Dip (12)	Dr/D	12/3.3[e]	[52,53]
Dieldrin	Lr	Alg, Gas, Pis[f] (>3)	B	0.25[e]	[54]
2,4-Dinitrotoluene	R	Chl, Oli, Bra[f] (3)	R	250[e]	[55]
Fluorene	R	Alg, Mac, Rot, Bra, Cop, Eph, Odo, Het, Col, Dip, Tri, Pis[f] (>78)	Y	12[e]	[56]
Lead	U	Alg	P	42	[41]

Lindane	U	Alg, Rot[f], Cop (>7)	D	2.6[e]	[57]
Manganese	U	Alg, Pis (2)	Rs,D	≥3,200	[58]
Mercury	U	Cya, Cry, Chl, Rot[f], Bra, Cop	D	0.1[e]	[41]
Mercury	U	Alg	P	0.2	[59]
Mercury	U	Bac, Chl, Pro, Rot, Bra, Ost (8)	D,C	≥0.5	[60]
Methyl parathion	Lr	Mac, Chl, Chr, Gas, Bra, Cop, Epi[f], Dip[f] (13)	Dr/D	0.1/9.0[e]	[61,62]
Parathion	R	Cya, Pro, Bra[f], Gas, Oli, Hir, Ost, Cop, Epi, Odo, **Het, Col (14)**	D	0.1	[13]
Pentachlorophenol	Lr	Chl[f], Mac[f], Gas, Oli, Bra, Cop, Eph, Odo, Cop, **Dip, Hem (15)**	B	20	[63]
Permethrin	U	Rot, Bra[f], Cop[f] (16)	Dr/D	0.023/0.05[e]	[64]
Selenium	Lr	Alg[f], Pro	P	8.3	[65]
Selenium	Lr	Alg, Pro[f] (±100)	S	10.0	[65]
Selenium	U	Cop, Gas, Pis[f] (4)	D	100	[66]
Selenium	U	Cya, Chr[f], Chl	Di	10,000	[43]
Simazine	Lr	Bac, Cya, Chl (>72)	C	33[e]	[67,68]
Terbutryne	Lr	Bac, Cya, Chl (>72)	Bv	1.0[e]	[67,68]
Toxaphene	U	Bac, Chl, Pro, Rot, Bra (7)	D	≥1.5	[61]
1,2,4-Trichlorobenzene	Lr	Cya, Chr, Eug, Cry, Chl, Bra[f] (32)	Dr/D	57/22[e]	[69]
Trichloroethylene	Lr	Cya, Cry, Chl, Pro, Bra[f] (12)	Dr/D	2.8/2,500[e]	[70]
Trifluralin	Lr	Chl, Gas, Bra, Pis[f] (4)	Be	0.5	[71]
2,4,6-Trinitrotoluene	R	Chl, Oli, Bra[f] (3)	R	167[e]	[55]
Vanadium	U	Cya, Chr[f], Chl	Di	9.3	[42]
Zinc	U	Alg, Rot, Bra, Cop[f] (33)	D	1.7[e]	[71]
Zinc	U	Alg	P	4.3	[41]

[a]R = well-performed experiment, reliable MS NOEC; Lr = experiment with shortcomings, less reliable MS NOEC; U = experiment with severe shortcomings, unreliable MS NOEC.
[b]See Table 1 for abbreviations (if possible, total number of tested species is given in parentheses).
[c]B = biomass; Be = (relevant) behavior; Bv = biovolume; C = chlorophyl-*a*; Co = colonization; D = density; Dr = density recovery; Di = diversity; Do = dissolved oxygen; Npp = net primary productivity; P = photosynthesis; R = reproduction; Rs = respiration; S = species richness; Y = yield.
[d]Of the most sensitive tested species.
[e]MS NOEC derived from MS LOEC. Slash indicates that both second and third procedures were performed.
[f]Most sensitive tested species giving the lowest NOEC; when no superscript is given, all tested species had the same NOEC.

Table 4. MS NOECs and extrapolated values calculated with the methods of Aldenberg and Slob (A & S) [6], 50 and 95% confidence, Wagner and Løkke (W & L) [7], 50 and 95% confidence, and the modified EPA method [9]

Compound	MS NOEC[a] (μg/L)	A & S 50% (μg/L)	W & L 50% (μg/L)	A & S 95% (μg/L)	W & L 95% (μg/L)	EPA (μg/L)
Aluminum	33–250	–[b]	–	–	–	0.75
Atrazine	5.0–110	2.2	1.7	0.12	0.17	0.15
Azinphos methyl	0.25/0.081	0.085	0.074	0.015	0.018	0.01
Cadmium	0.07–2.3	0.16	0.15	0.029	0.035	0.0085
Chromium	50.0	4.9	4.6	1.5	1.6	0.035
Copper	0.3–4.0	3.9	3.7	2.2	2.3	0.3
Dichlobenil	5.0/100	–	–	–	–	7.8
3,4-Dichloroaniline	12/3.3	–	–	–	–	0.067
Dieldrin	0.25	0.083	0.054	0.0011	0.0017	0.012
2,4-Dinitrotoluene	250	78	72	20	23	6.5
Fluorene	12	9.3	6.0	0.11	0.17	6.3
Lead	42	5.5	5.1	1.4	1.6	0.72
Lindane	2.6	0.86	0.72	0.061	0.083	0.22
Manganese	≥3,200	–	–	–	–	15
Mercury	0.1–≥0.5	0.02	0.016	0.00093	0.0013	0.002
Methyl parathion	0.1/9.0	–	–	–	–	0.024
Parathion	0.1	0.013	0.009	0.00013	0.00023	0.002
Pentachlorophenol	20	3.2	2.9	0.53	0.66	0.32
Permethrin	0.023/0.05	–	–	–	–	0.066
Selenium	8.3–10,000	2.5	2.2	0.31	0.39	0.09
Simazine	33	–	–	–	–	0.1
Terbutryne	1.0	–	–	–	–	0.024
Toxaphene	≥1.5	0.003	0.0018	<0.0001	<0.0001	0.0025
1,2,4-Trichlorobenzene	57/22	44	32	1.1	1.7	19
Trichloroethylene	2.8/2,500	2,100	1,300	20	32	130
Trifluralin	0.5	–	–	–	–	0.19
2,4,6-Trinitrotoluene	167	–	–	–	–	4.6
Vanadium	9.3	–	–	–	–	3.5
Zinc	1.7–4.3	4.0	3.5	0.46	0.58	0.33
Geometric mean	4.7[c]	1.4	1.1	0.098	0.13	0.15[d]

[a]When more than one MS NOEC was available, the lowest and highest values are presented (with a dash). When the second as well as the third procedure could be performed, both values are presented (with a slash).
[b]Lack of data for application of extrapolation method.
[c]Geometric mean of reliable and less reliable MS NOECs for those compounds for which refined effect assessment could be performed.
[d]Geometric mean of extrapolated values of those compounds for which refined effect assessment could be performed.

Table 5. Ratios[a] between geometric means of reliable and less reliable MS NOECs and extrapolated values calculated with the methods of Aldenberg and Slob (A & S) [6], 50 and 95% confidence, Wagner and Løkke (W & L) [7], 50 and 95% confidence, and the modified EPA method [9]

Method	MS NOEC	A & S 50%	W & L 50%	A & S 95%	W & L 95%	EPA
MS NOEC	–	0.30	0.23	0.021	0.028	0.032
A & S 50%	3.4	–	0.79	0.070	0.093	0.11
W & L 50%	4.3	1.3	–	0.089	0.12	0.14
A & S 95%	48	14	11	–	1.3	1.5
W & L 95%	36	11	8.5	0.75	–	1.2
EPA	31	9.3	7.3	0.64	0.87	–

[a]Horizontal axis is divided by vertical axis.

Table 6. Model II regression analysis of MS NOECs and extrapolated values calculated with the methods of Aldenberg and Slob (A & S) [6], 50 and 95% confidence, Wagner and Løkke (W & L) [7], 50 and 95% confidence, and the modified EPA method [9]

Method	Reliability[a]	n[b]	Relation	r[c]	Significance[d]
A & S 50%	R + Lr	23	log MS NOEC = 0.817·log (A & S 50%) + 0.276	0.739	***
	U	17	log MS NOEC = 1.490·log (A & S 50%) + 0.937	0.530	*
W & L 50%	R + Lr	23	log MS NOEC = 0.835·log (W & L 50%) + 0.344	0.738	***
	U	17	log MS NOEC = 1.417·log (W & L 50%) + 0.991	0.517	*
A & S 95%	R + Lr	23	log MS NOEC = 0.793·log (A & S 95%) + 1.159	0.623	**
	U	17	log MS NOEC = 0.943·log (A & S 95%) + 1.561	0.453	*
W & L 95%	R + Lr	23	log MS NOEC = 0.817·log (W & L 95%) + 1.075	0.645	***
	U	17	log MS NOEC = 0.968·log (W & L 95%) + 1.512	0.454	*
EPA	R + Lr	23	log MS NOEC = 0.764·log EPA + 1.081	0.711	***
	U	17	log MS NOEC = 2.036·log EPA + 3.423	0.351	*

[a]R + Lr: only reliable and less reliable MS NOECs used; U: only unreliable MS NOECs used.
[b]Number of data on which regression is based.
[c]Regression correlation coefficient.
[d]Significance level of hypothesis $r \geq 0$: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

taken together to prevent the small number of reliable MS NOECs playing a major role in the statistical calculations. For those compounds for which more than one MS NOEC was available, all values were used, that is, no geometric mean of the MS NOECs was calculated.

When only reliable and less reliable MS NOECs were used, the highest correlation coefficients were obtained with the methods of Aldenberg and Slob [6], and Wagner and Løkke [7], both with 50% confidence, and the EPA method [9]. The lowest values were obtained with the methods of Aldenberg and Slob, and Wagner and Løkke, both with 95% confidence. All correlation coefficients were significant, with $P < 0.01$. When unreliable data were used, all correlation coefficients were significant, with $P < 0.05$. As an example, the regression lines for the values calculated with the modified EPA method are presented in Figure 4. The figures illustrate that the regression line fits less well when unreliable MS NOECs are used. From these results it can be concluded that the criteria presented before, on which the distinction in reliable, less reliable, and unreliable MS data is based, appear to be useful.

Table 7. t Test for paired comparisons between MS NOECs and extrapolated values calculated with the methods of Aldenberg and Slob (A & S) [6], 50 and 95% confidence, Wagner & Løkke (W & L) [7], 50 and 95% confidence, and the modified EPA method [9]

Method	Reliability[a]	t_s[b]	Significance[c]
A & S 50%	R + Lr	1.01	NS
	U	2.66	*
W & L 50%	R + Lr	1.52	NS
	U	2.81	*
A & S 95%	R + Lr	5.81	***
	U	4.82	***
W & L 95%	R + Lr	5.47	***
	U	4.61	***
EPA	R + Lr	5.98	***
	U	7.05	***

[a]R + Lr: only reliable and less reliable MS NOECs used; U: only unreliable MS NOECs used.
[b]For paired comparisons.
[c]Significance level of hypothesis $t_s = 0$: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant.

In all cases the variances of the MS NOECs and the extrapolated values could be considered as equal, so the t test for paired comparisons could be employed. The results of these t tests between MS NOECs on one side and the extrapolated values for the various methods on the other are presented in Table 7.

It appeared that the t_s values of Aldenberg and Slob [6], and Wagner and Løkke [7], both with 50% confidence, were not significant ($P > 0.05$) if reliable and less reliable MS NOECs were used. This means that the mean of the values calculated with these extrapolation methods can be considered equal to the mean of the MS NOECs. In all other cases the t_s values were significant ($P < 0.05$), which indicates that the means of these extrapolated

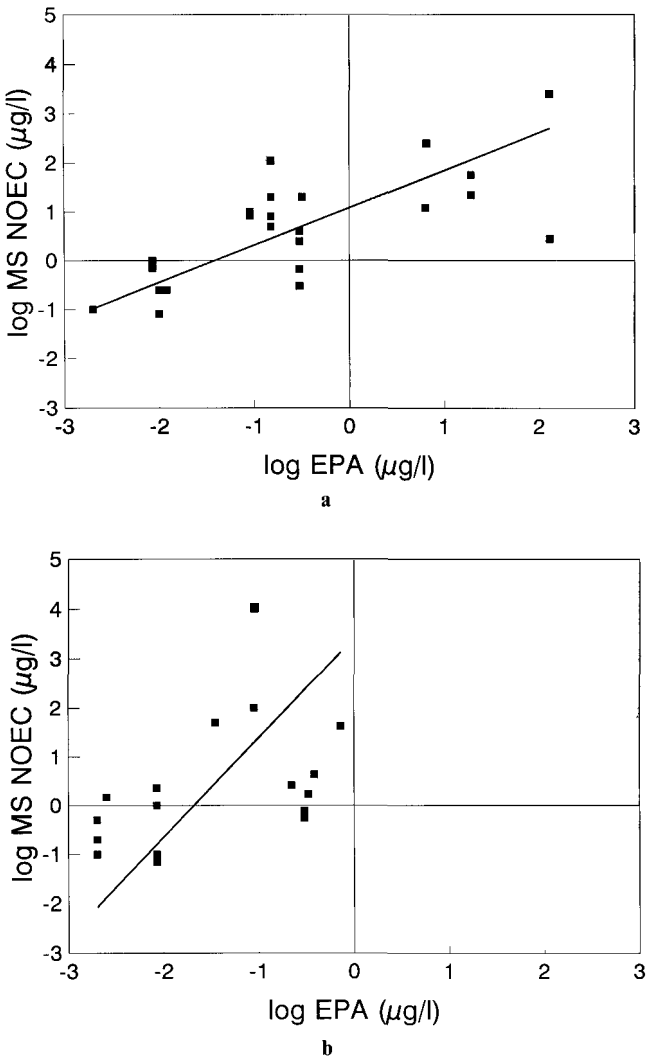

Fig. 4. Model II regression analysis of MS NOECs on extrapolated values calculated with the modified EPA method: (**a**) reliable and less reliable MS data, regression based on 23 data pairs: log MS NOEC = 0.764·log EPA + 1.081, $r = 0.711$; (**b**) unreliable MS data, regression based on 17 data pairs: log MS NOEC = 2.036·log EPA + 3.423, $r = 0.351$.

values are significantly lower than the mean of the MS NOECs.

DISCUSSION AND CONCLUSIONS

The first objective of this research was to study whether there are relevant differences in NOECs derived from MS and SS experiments for similar or related species and corresponding effect parameters. Based on the results of this study, it can be noted that there seems to be no reason to believe that organisms differ in sensitivity under field and laboratory conditions. When species tested in MS experiments were compared with similar or related species in SS experiments, for corresponding effect parameters and exposed to equal concentrations, they appeared to be equally sensitive. This result was supported by statistical analysis of the available data by means of model II regression and a *t* test for paired comparisons.

The second objective was to study whether ecosystems can be protected by setting a "safe" value that is derived from SS NOECs by extrapolation. It is assumed that an extrapolated value is a "safe"

value if it is equal to or lower than an experimentally derived MS NOEC. In the first place, it can be stated that validation of extrapolation methods is seriously hampered by the lack of useful and reliable data. It is therefore not possible to draw definite conclusions concerning this validation. The lack concerns both experimental MS and SS data:

1. In this study MS NOECs for organic compounds and metals were sought, and three procedures to obtain MS NOECs were used. A distinction was made in reliable, less reliable, and unreliable MS NOECs. Only for six different compounds could a reliable MS NOEC be obtained. For 14 different compounds a less reliable MS NOEC and for another 14 different compounds an unreliable MS NOEC could be obtained. To prevent the small number of reliable MS data playing a major role in the statistical procedures, reliable and less reliable MS data were taken together. It appeared that the criteria on which the distinction in reliable and less reliable MS data on one side and unreliable MS data on the other were useful; validation should therefore be restricted to comparing extrapolated values with reliable and less reliable MS NOECs. Unfortunately, due to paucity of data, it was not possible to test whether the distinction in reliable data on one side and less reliable data on the other was useful.

2. The reliability of both the second and the third procedures to obtain MS NOECs is questionable. For six compounds both procedures could be applied. Especially for trichloroethylene the third procedure gave an MS NOEC that seemed much more realistic compared to SS data than the MS NOEC obtained with the second procedure. Nevertheless, the number of comparable data is too low to give preference to either the second or the third procedure. It has to be mentioned, however, that the first procedure is always preferable.

3. It is noted that the MS NOECs are presented as fixed values, but these data are accompanied by an experimental uncertainty—the spread in MS NOECs for one compound derived from comparable experiments can be considerably high, as illustrated for atrazine.

4. Useful SS data were often missing. Data on sensitive species were not always available. For 11 compounds fewer than four chronic SS NOECs were available, so the methods of Aldenberg and Slob [6], and Wagner and Løkke [7] could not be applied. Validation of these methods was therefore restricted to 18 compounds.

In the second place, it can be noted that employing extrapolation methods leads to equal or lower, but not to higher, values than the reliable and less reliable NOECs from the MS experiments. Employing model II regression analysis showed that the extrapolated values calculated with methods of Aldenberg and Slob [6], and Wagner and Løkke [7], both with 50% confidence, were in good agreement with reliable and less reliable MS NOECs, contrary to the values calculated with the methods of Aldenberg and Slob, and Wagner and Løkke, both with 95% confidence level and the modified EPA method [9]. These values are much lower than the reliable and less reliable MS NOECs.

With restrictions, due to the paucity of data, it seems therefore acceptable to derive "safe" values from SS data. The studied extrapolation methods seem to be a good basis for determining these values. A condition is that sufficient toxicity data are available for sensitive taxonomic groups.

The third objective was to study whether there are relevant differences between the values calculated with the various extrapolation methods. The lowest extrapolated values were calculated with the method of Aldenberg and Slob [6], 95% confidence, although values calculated with Wagner and Løkke [7], 95% confidence, and the modified EPA method [9] were in the same order of magnitude. Aldenberg and Slob, and Wagner and Løkke, both with 50% confidence, gave the highest extrapolated values. Based on the results of this study, there is no reason to choose either the method of Aldenberg and Slob or the method of Wagner and Løkke. The modified EPA method has some disadvantages. An important one is that, when using only one SS test result or QSAR estimate, the chance increases of underestimating the safe value for ecosystems. In addition, the modified EPA method uses only the lowest L(E)C50 or NOEC value, whereas more toxicity data may be available. Besides, it is an empirical method that has almost no possibilities for further improvement. The results support the recommendations of the OECD [9] that the modified EPA method should be used only for preliminary effect assessment. Whether the methods of Aldenberg and Slob or Wagner and Løkke should be used with 50 or 95% confidence is a political issue, which was also mentioned by Van den Berg and Bodar [28]: the choice is between the most likely estimate, values calculated with a 50% confidence level, and the safer estimate, values calculated with a 95% confidence level.

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