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Variation in the Sensitivity of Aquatic Species to Toxicants

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Ecotoxicological hazard assessment for ecosystems is primarily based on single-species toxicity data. Economic, practical and ethical considerations further urge to apply laboratory-to-ecosystem extrapolation methods on toxicity data of only a few species, although large differences in sensitivity between species exist. Therefore it is important to study the existing body of data to trace down the factors that determine the sensitivity of species to chemicals. This may open the way to prediction for untested combinations of species and chemicals. This paper reports the first results of a research program concerned with the analysis and explanation of differences in sensitivity of species to toxic substances, using biological properties of the species. The project aims at the development of predictive models, which, in analogy to QSARs, are called Quantitative Species Sensitivity Relationships. The distributions of acute toxicity data of different species are studied for 26 chemicals. A measure for quantification of among-species variation in sensitivity to a chemical is introduced, and differences between species are linked with their taxonomic positions. Finally, problems associated with the analysis of toxicity data from different sources will be discussed.

MATERIALS AND METHODS

In this first analysis the acute LC_{50} was used as a measure of toxicity, because of its abundance in literature and its clear definition in statistical and biological sense. The LC_{50}-data were derived from preferably 96 hours or otherwise 48 hours toxicity experiments. The data were retrieved from integrated criteria documents developed at our institute and some reviews in open literature. More than 80% of the data origins from one of the following sources: Mayer and Ellersieck (1986), Phipps and Holcombe (1985), Ros and Slooff (1988), and Slooff *et al.* (1983a,b). Information on other (minor) sources can be obtained from the authors. Both data on freshwater and marine species were included. The tests were scrutinized on the basis of reported information on pH, hardness, temperature, test medium, duration

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of test, purity and specification of the chemical tested. Tests with exceptional values for these experimental conditions were excluded. No test was excluded on the basis of its outlying LC_{50}-value. Data of different life stages of one species were treated as separate organisms in the analysis. If more toxicity values per life stage of a species per chemical were available, the geometric mean was used. If a range was given between which the LC_{50} would fall, the range was replaced by the geometrical mean of the lowest and highest point.
The distribution of LC_{50}s per chemical is described by a number of summary statistics. The median (50% percentile) is used as measure of the center of the distribution, because of the observed skewness of the distributions. Measures of skewness (S) and kurtosis (K) of logtransformed LC_{50}s are presented, to facilitate comparison of the observed distributions with the lognormal and loglogistic distribution. The measures are defined as follows:

$$S = \frac{\sum_{i=1}^{N} (x_i - \bar{x})^3 / N}{\{\sum_{i=1}^{N} (x_i - \bar{x})^2 / N\}^{1.5}} \qquad K = \frac{\sum_{i=1}^{N} (x_i - \bar{x})^4 / N}{\{\sum_{i=1}^{N} (x_i - \bar{x})^2 / N\}^2} - 3$$

where N=number of species (life stages taken apart) with LC_{50} for chemical concerned, x_i=log(LC_{50}) of ith species, and $\bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$. If x follows the normal distribution, S=0 and K=0; for the logistic distribution, S=0 and K=1.2 (Johnson and Kotz 1970). It was tested whether calculated values of S and K complied with the normal distribution, using Tables 9.2, 9.3 and 9.5 from D'Agostino and Stephens (1986) (two-sided, α=0.05). The variation in the distributions is expressed by the ratio between the 95% percentile to the 5% percentile. This statistic is named "95% : 5% Sensitivity Ratio" ($SR_{95:5}$). It measures the spread in the distribution in a way that is easily interpretable without knowing the underlying distribution of LC_{50}s. The $SR_{50:5}$, defined in similar way, is also calculated. For distributions symmetric on log scale, $SR_{95:5} = SR_{50:5}^2$. The ratios are illustrated in Figure 1. Sensitivity Ratios were also presented by Fletcher *et al.* (1990), but their quantification leads to an increase of the ratio with increasing sample size. The ratios presented here are independent of sample size, just as the standard deviation.

Two different levels of taxonomic classification were incorporated in the analysis: phylum and class. Taxonomic classifications below class-level were not considered because of sparsity of data per taxonomic category. Analyses of variance were carried out on the log(LC_{50})-values for each chemical separately. The analyses were based on a hierarchical statistical model involving a phylum, class-within-phylum and random component. The random component was assumed to follow a normal distribution. Data from different life stages of one species were treated as independent observations. A preliminary analysis including a life stage-within-species component showed that the variation between life stages within the species

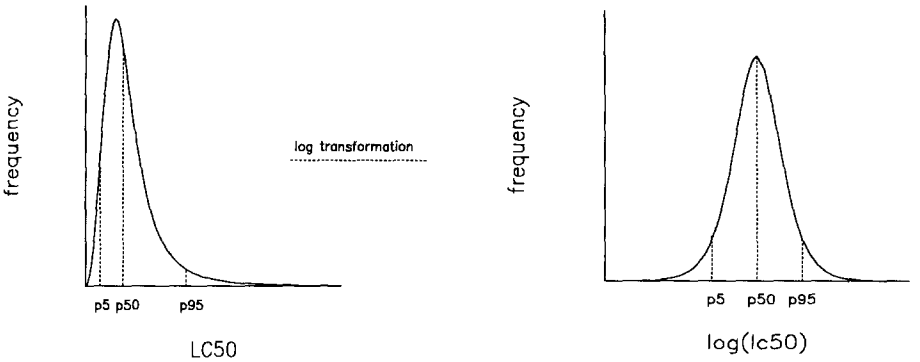

Figure 1. Sensitivity Ratios illustrated for distribution of LC_{50}s of different species, symmetric on log scale. $SR_{95:5}$ = p95/p5 ; $SR_{50:5}$ = p50/p5.

was about as large as the variation between different species.
Right censored data (i.e., cases where a lower limit to the LC_{50} was given; about 3% of the data) were replaced by estimated values based on the lognormal distribution of all the data of the chemical concerned, using maximum likelihood. There were only a few left censored data (4); these were disregarded.

RESULTS AND DISCUSSION

Toxicity data of 26 substances and, in total, 196 species were collected. The distribution of the test species over classes and phyla is given in Table 1. The fraction of species represented by more than one life stage in our data set is on average 8% per chemical. A summary of the LC_{50}data per chemical is given in Table 2. Skewness and kurtosis of $\log(LC_{50})$ are displayed in Table 3. The values of S and K are often close to 0, in which case the normal distribution is a reasonable approximation. There are no cases in which the logistic distribution seems particularly adequate: in all cases where kurtosis was positive as in the logistic distribution, the data also showed considerable asymmetry which is a contra-indication for the logistic distribution. Sensitivity Ratios are displayed in Table 3 and Figure 2. Figure 2 is a powerful presentation tool in the interpretation of sensitivity distributions. The $SR_{95:5}$ measures variation in the direction of sensitivities both larger and smaller than the median. The $SR_{50:5}$ quantifies the distance from the median species to the 5% most sensitive species. The theoretical relation for symmetric distributions, is shown as a solid line. The skewness measure S corresponds well to the closeness of the toxicants to the line.
Extreme differences exist between the chemicals with respect to the variability among species in LC_{50}s. Chemicals with a specific mode of action have large Sensitivity Ratios, whereas the inert chemicals with baseline toxicity (acetone, benzene, n-heptanol, n-propanol and trichloroethylene; Verhaar *et al.* 1992) have the smallest. For chromium-VI, aniline, carbaryl, mexacarbate and parathion distributions have long tails to the left, with strongly sensitive species: when

Table 1. Number of test species per phylum and class.

Phylum	Class	Phylum	Class
Coelenterata(1)	Hydrozoa(1)	Annelida (13)	Hirudinea (2)
			Oligochaeta (5)
Echinodermata (2)	Stelleroidea(2)		Polychaeta (6)
Mollusca (22)	Bivalvia (8)	Arthropoda(99)	Malacostraca (34)
	Gastropoda(14)		Maxillopoda (12)
			Phyllopoda (12)
Platyhelminthes (3)	Turbellaria(3)		Insecta (41)
Rotatoria (4)	Digononta (2)	Chordata (52)	Amphibia (5)
	Monogononta(2)		Osteichtyes (47)

Table 2. Summary of LC_{50} (48-96 hours) values used in this study (in mg/l unless stated otherwise) N=no. of observations, life-stages of species taken apart.

Chemical	Min.	Median	Max.	N
Inorganic Arsenic	0.23	11	73	25
Cadmium	0.0020	2.2	4600	143
Chromium-VI	0.050	9.1	192	40
Mercury-II	0.0022	0.10	10	56
Zinc	0.040	2.4	114	32
Acetone	5000	9600	24000	23
Allylamine	1.8	30	171	23
Aniline	0.10	131	800	30
Benzene	10	130	420	23
Carbaryl*	5.0	2200	20000	34
o-Cresol	8.4	38	160	23
DDT*	0.52	6.6	1000	39
Ethylacetate	125	480	3950	23
Ethylpropionate	20	125	1000	23
n-Heptanol	32	65	200	23
Lindane*	1.0	66	3200	30
Malathion*	1.0	168	45500	36
Methylparathion*	4.0	4960	9415	18
Methoxychlor*	0.5	14	333	28
Mexacarbate	0.010	13	22	27
Parathion*	0.04	94	2216	22
Pentachlorophenol	0.036	0.42	131	54
n-Propanol	1000	4200	6800	23
Pyridine	30	410	2470	23
Salicylaldehyde	1.3	6.5	54	23
Trichloroethylene	24	55	270	23

* µg/l

Table 3. Sample Skewness (S) and Kurtosis (K) of log(LC_{50})-values, 95%:5% Sensitivity Ratio ($SR_{95:5}$), $SR_{50:5}$ and P-value corresponding to F-test on differences between Class means (P-Class).

Chemical	S	K	$SR_{95:5}$	$SR_{50:5}$	P-Class
Inorganic Arsenic	-0.59	0.16	49	10	0.10
Cadmium	0.03	-0.03	16667	92	<.001
Chromium-VI	-0.79*	-0.25	2737	181	<.001
Mercury-II	0.15	-0.82*	1781	31	0.02
Zinc	-0.08	-0.11	1419	36	0.08
Acetone	0.32	-0.96	3	2	0.13
Allylamine	-0.35	-0.56	81	14	0.17
Aniline	-1.80*	3.10*	1392	205	<.001
Benzene	-0.50	-0.29	12	4	0.40
Carbaryl	-1.06*	-0.26	3089	393	0.05
o-Cresol	0.02	-0.73	14	4	0.03
DDT	1.22*	1.46*	800	7	0.003
Ethylacetate	0.47	-0.69	23	4	0.30
Ethylpropionate	0.50	-0.24	22	3	0.83
n-Heptanol	0.23	-1.28*	5	2	0.26
Lindane	0.08	-0.19	828	21	0.002
Malathion	0.09	-0.82	65000	240	0.29
Methoxychlor	-0.27	-0.41	125	18	0.01
Methylparathion	-1.80*	1.58*	-**	-**	0.05
Mexacarbate	-1.95*	2.58*	684	409	<.001
Parathion	-0.37	-1.13	3322	157	0.90
Pentachlorophenol	1.07*	0.63	2043	9	0.13
n-Propanol	-0.86	-0.16	5	3	0.05
Pyridine	-0.29	-0.78	36	6	0.02
Salicylaldehyde	0.24	1.01	12	5	0.12
Trichloroethylene	1.00*	0.50	7	2	0.09

*: value significantly different from that of normal distribution (2-sided; α=0.05)
** : too few data to calculate ratio

members of the family Daphnia were included in these data (chromium-VI, aniline, and carbaryl), these belonged to the strongly sensitive group, with an LC_{50} more than a factor 100 below the median LC_{50}. The remaining species satisfying this criterion belonged to other families of the Arthropoda, with the exception of *Physa integra* (a gastropod highly sensitive to chromium-VI), *Tubifex tubifex* (an oligochaet highly sensitive to chromium-VI), and *Ictalurus punctatus* (a fish highly sensitive to mexacarbate). For PCP and DDT there seem to be some extra resilient species, belonging to Chordata and Insect classes in both cases.

The large differences between chemicals in among-species variation should have implications for the data-collection and estimation of safe levels for ecosystems. Methods that derive ecotoxicological protection levels from observed variation in

sensitivity seem sensible (Kooijman, 1987; Wagner and Løkke, 1991; Aldenberg and Slob, 1993). If approximately equally precise estimates are required for all toxicants, the number of species tested should depend on the toxicant, narcotic chemicals needing a much smaller number of toxicity tests than heavy metals and organic chemicals with a specific mode of action.

The usual measures of variability are the standard deviation and the coefficient of variation. We preferred to use a ratio of percentiles to measure variation instead. Percentiles specify the effect concentration not exceeded by a given percentage of species and play an important role in the laboratory-to-field extrapolation methods referred to above. The standard deviation and coefficient of variation can be related to percentiles only if the underlying distribution is specified. Because distributions may have very different forms (Table 3), we proposed the Sensitivity Ratio, which is directly interpretable in terms of percentiles. For any specific distribution, the $SR_{95:5}$ and other measures of spread can be derived from each other. A lognormal distribution with a standard deviation σ on ln scale has an $SR_{y:x}$ of $\exp\{(z_y - z_x)\sigma\}$, where z_w is the w % point of the normal distribution. A disadvantage of the Sensitivity Ratio is the requirement of a larger dataset (at least 20 in case of a $SR_{95:5}$), and its larger estimation error. Confidence intervals based on resampling techniques (Efron, 1982) indicated that the estimates of the $SR_{95:5}$ were adequate within a factor 10, with the exceptions of parathion ($SR_{95:5}$ could be 20 times larger), zinc, chromium-VI, PCP (could be about 15 times smaller), DDT (30 times smaller) and aniline and mexacarbate (80 times smaller).

Table 3 also presents P-values from the tests on the class-within-phylum component of the statistical model. The lower this value is, the more likely it is that LC_{50}-values of species of the same class are closer than those of different classes. The generally low values for P, with 16 out of 26 chemicals having values less than or equal to 0.10, show clearly that species of the same class have more similar LC_{50}s than species of different classes. This could be caused by associations lower in the taxonomic tree, but the limited amount of data per order or family prohibits a more detailed analysis. Our results are in agreement with earlier findings. Reviewing articles on interspecies toxicity relationships, Slooff *et al.* (1986) concluded that the sensitivities of species depend on their taxonomic position and on the type of chemical eliciting the toxicity. Fletcher *et al.* (1990), in their comparative study with plant species and herbicides, tentatively concluded that variation between taxa plays a larger role than differences between field and greenhouse experiments. Other small scale studies indicated the same (LeBlanc, 1984; Volmer *et al.*, 1988). The taxonomy is a reflection of the phylogenetic relationships between the species, and closely related species may share many characteristics relevant to their sensitivity to chemicals through descent from common ancestors. These properties may be general, like body surface, body volume, composition of ectoderm, or more specific, like certain mechanisms of uptake, toxic action, metabolism, and excretion.

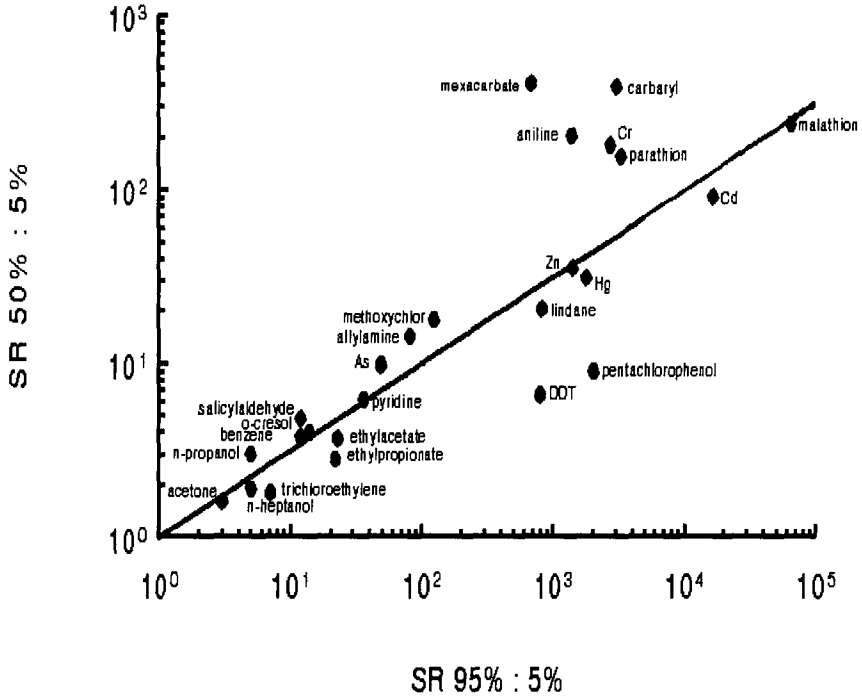

Figure 2. $SR_{50:5}$ plotted against $SR_{95:5}$ for 25 chemicals. Theoretical relation for distributions symmetric on log scale indicated as a solid line.

Three points complicated the interpretation of the results. First, different species were observed for each toxicant, making the Sensitivity Ratios not fully comparable. Hence only a global comparison between classes of chemicals was made. Second, the LC_{50}s may originate from toxicity tests that differed not only in the test species, but also in experimental conditions. The experimental conditions may have affected the bioavailability of the toxicants, particularly in the case of the heavy metals. The Sensitivity Ratio measures the overall variation, including the variation due to differences in experimental setup. However, the clear relation with the taxonomy indicates that at least a substantial part of the variation is among-species variation. The fact that the variation in LC_{50}-values is small for narcotic chemicals and much larger for chemicals with a specific mode of action, points in the same direction. The existence of strongly different life stages, also differently defined between species, is a third complication in comparative ecotoxicology. The small fraction of species represented by more than one stage in our data set and the large differences between LC_{50}s in different

stages, made us treat them as separate organisms in the analysis and interprete the results in terms of among-species variation. This is no ideal solution. Collection and analysis of toxicity data with specific consideration of the ontogeny of sensitivity are needed.

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