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COMPARISON OF THE SUSCEPTIBILITY OF 22 FRESHWATER SPECIES TO 15 CHEMICAL COMPOUNDS. I. (SUB)ACUTE TOXICITY TESTS

W. SLOOFF[1], J.H. CANTON[2] and J.L.M. HERMENS[3]

[1]*National Institute for Water Supply, Chemical Biological Division, 2260 AD Leidschendam,*

[2]*National Institute of Public Health, Bilthoven,*

and

[3] *Department of Veterinary Pharmacology and Toxicology, University of Utrecht, The Netherlands*

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The relative susceptibility of 22 taxonomically different species (bacteria, algae, protozoans, crustaceans, insects, coelenterates, molluscs, fishes and amphibians) to chemicals was determined by comparing the (sub)acute toxicity data of 15 test substances. Marked differences were observed in the susceptibility among the species with respect to the individual test compounds (up to a factor 9000). In contrast to this, differences were small (less than a factor 7) when the relative susceptibilities based on toxicity data on all compounds were taken into account. This tendency was also shown by comparing the susceptibilities based on quantitative structure–activity relationships. Thus, there is no such thing as the most susceptible species to chemicals. As the susceptibility of the species is highly pollutant-specific, at least a set of tests on different species will be required for toxicity screening. However, results indicated that a standard test combination with representative species of green algae, crustaceans and fishes failed to indicate the full toxic potential of chemicals. Since incorporation of a *Microcystis aeruginosa* test improved the combination in estimating acute toxic levels of chemicals, it is recommended to include this test in both national and international ecotoxicological testing programmes.

Key words: species susceptibility; freshwater organisms; (sub)acute toxicity tests; quantitative structure–acitvity relationships

INTRODUCTION

Recently, several organizations have addressed themselves to the complex problem of overall hazard assessment of both existing and new chemicals. Concerning the information on aquatic toxicity it is generally accepted that results from acute toxicity tests are required as an initial range-finding step in a comprehensive testing programme. However, a consideration of these results may lead to the decision to terminate the ecotoxicological testing, e.g. if the $LC_{50}$is >1 mg·l^{-1} (EPA, 1978) or <0.01 mg·l^{-1} (BCW, 1981). Therefore, the ultimate choice of test species as representatives of aquatic ecosystems is one of extreme importance. Although several hundreds of aquatic species have been reported as study objects (Blanck et

al., 1978), the number of test species to be chosen for hazard assessment has to be limited. The EC-directive 67/548/EEC (1979) dictates the use of a macroinvertebrate and a fish species only, whereas the Organization for Economic Cooperation and Development recommendations includes the use of an algae species as well (OECD, 1979). However, the reliability of such a set of tests in predicting the toxic potential of chemicals to aquatic life is not known. In the present paper the problem of species differences to toxicants is studied by an attempt to compare and relate sets of (sub)acute toxicity data on taxonomically diverse species.

MATERIALS AND METHODS

Test species and test protocols

Part of the toxicity data presented in this paper have been published elsewhere (Bringmann and Kühn, 1977, 1978,1980; Bringmann, 1978; Bringmann et al., 1980; Kühn and Canton, 1979; Slooff and Baerselman, 1980). Although these data were obtained by four different laboratories (National Institute for Water Supply, National Institute of Public Health, Central Laboratory TNO, The Netherlands and Institut für Wasser-, Boden- und Lufthygiene, F.R.G.), the results can be compared since they were based on experiments equal in performance as a result of coordination between the laboratories.

Additional short-term tests were carried out on algae (*Selenastrum capricornutum, Chlorella pyrenoïdosa* and *Scenedesmus pannonicus*), insects (*Aedes aegypti* and *Culex pipiens*), coelenterates (*Hydra oligactis*), molluscs (*Lymnaea stagnalis*) and fishes (*Salmo gairdneri, Oryzias latipes* and *Pimephales promelas*). All organisms tested were obtained from standardized laboratory cultures. The criteria and test conditions during the experiments are summarized in Table I. During the experiments (minimally in duplicate) the actual concentrations of the compounds were not measured. The NOL(E)C* and LC_{50} values were based on the quantities added to the water at the start of the experiments and were determined using the log-probit model.

Test compounds

The following chemicals were tested: mercury(II)chloride, cadmium nitrate, *n*-propanol, *n*-heptanol, ethylacetate, ethylpropionate, acetone, trichloroethylene, allylamine, benzene, pyridine, *o*-cresol, salicylaldehyde and pentachlorophenol. All test compounds were of the purest grade available (purity > 98%).

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* The highest test concentration at which no effect (NOEC) or lethality (NOLC) was observed.

Comparison of the data

Although all tests performed provide information about the (sub)acute short-term effects of toxicants, it is recognized that the toxicity of a chemical involves a range of responses of a particular group of organisms to the chemical at a given time and circumstance. As many factors may affect organism susceptibility to toxicants, all applied test procedures were standardized by adopting test protocols that are appropriate to meet the environmental requirements of the test species as well as to enable the measurement of an ecologically important response. As a result, the test criteria and test circumstances were sometimes different, which makes a comparison of the susceptibility of different species to toxicants difficult; for instance, as for microorganisms, death is difficult to determine, the data are expressed as effective concentration (EC) instead of lethal concentration (LC). These EC values are often lower than LC values because impairment of function occurs before mortality. Also the duration of the test is species-dependant and varies from 6 to 192 h, whereas it is generally known that in most cases toxicity increases with duration of exposure. Although, therefore, a comparison of L(E)C values obtained from different tests is not scientifically defensible, it should be realized that information generated from such tests is used by legislators involved in fashioning policy decisions concerning the control of environmental pollution. These decisions take place when a substance has a $L(E)C_{50}$ below or above a certain threshold, independently from the test species for which the toxicity value has been obtained. Thus, from a regulatory point of view, a comparison of the susceptibility of different species – or, more precisely, a comparison of the sensitivity of toxicity tests – may be justified.

In this respect attention has been given to: 1. The susceptibility of the test species per test compound by simply comparing the NOL(E)C and LC_{50} values. 2. The relative susceptibility of the test species for all test compounds. Hereto the average NOL(E)C value was determined for each compound and subsequently for all species the ratios NOL(E)C/average NOL(E)C were calculated per compound. As a measure for the relative susceptibility of a species the geometric mean of these ratios was divided by that of the most susceptible species. 3. The relative susceptibility of the test species based on quantitative structure–activity relationships (QSAR). Recently Könemann (1981) found an excellent quantitative structure–activity relationship for 50 more-or-less lipophilic, non-reactive organic compounds. The unspecific toxicity of these compounds leading to anaesthesia (membrane perturbation) was highly correlated to the partition coefficient octanol–water (Poct). Since many chemical water pollutants are of this kind, the susceptibilities to such chemicals were also compared based on QSARs. Hereto the QSARs were calculated for the unspecific toxicants tested in this study (Table II), according to the equation $\log (1/c) = a \log \text{Poct} - b$. Assuming equal or slightly varying slopes (a), the intercepts b represent the relative susceptibilities of the species to any compound with this mode of action. 4. The relative sensitivity of sets of recommended or obligatory

TABLE I

Test organisms, experimental conditions and toxicological criteria used in this study (previously published data are referred to).

Test organisms	Age/stage	Exposure time (h)	No. of organisms/group	Test volume/group(l)	Temperature (°C)	Test medium	Criteria	References
P. putida	log phase	6	ext.TE/F = 10	0.1	25	Bringmann	Cell multiplication	Bringmann and Kühn (1977)
M. aeruginosa	log phase	192	ext.TE/F = 20	0.01	27	Bringmann	Cell multiplication	Bringmann and Kühn (1978)
C. pyrenoïdosa	log phase	48	10^4 cells/ml	0.15	25	M_1[a]	Growth	–
S. pannonicus	log phase	48	10^4 cells/ml	0.15	25	M_1	Growth	–
S. pannonicus	log phase	192	ext.TE/F = 20	0.01	27	Bringmann	Cell multiplication	Bringmann and Kühn (1978)
S. capricornutum	log phase	96	5×10^4 cells/ml	0.15	26	M_1	Growth	–
E. sulcatum	log phase	72	1.5×10^3 cells/ml	0.02	25	Bringmann	Cell multiplication	Bringmann (1978)
Uronema parduczi	log phase	20	1.5×10^3 cells/ml	0.02	25	Bringmann	Cell multiplication	Bringmann and Kühn (1980)
C. paramaecium	log phase	48	1.5×10^3 cells/ml	0.02	20	Bringmann	Cell multiplication	Bringmann et al. (1980)
D. magna	≤24 h	48	25	1	19	SRW[b]	Mortality	Canton and Adema (1978)
D. pulex	≤24 h	48	25	1	19	SRW	Mortality	Canton and Adema (1978)

D. cucculata	≤24 h	48	25	1	19	SRW	Mortality	Canton and Adema (1978)
A. aegypti	3rd instar	48	10	0.05	26	DSW[c]	Mortality	–
C. pipiens		48	10	0.05	26	DSW	Mortality	–
H. oligactis	Budless	48	10	0.05	17	DSW	Mortality	–
L. stagnalis	3–4 wk	48	10	1	20	DSW	Mortality	–
Leuciscus idus	5–7 cm	48	10	10	20	Tap water[d]	Mortality	Juhnke and Lüdemann (1978)
S. gairdneri	5–8 wk	48	10	10	15	Tap water[e]	Mortality	–
P. reticulata	3–4 wk	48	10	1	24	A.A.[f]	Mortality	Kühn and Canton (1979)
O. latipes	4–5 wk	48	10	1	24	DSW	Mortality	–
P. promelas	3–4 wk	48	10	1	20	DSW	Mortality	–
X. laevis	3–4 wk	48	10	1	20	DSW	Mortality	Slooff and Baerselman (1980)
Ambystoma mexicanum	3–4 wk	48	10	1	20	DSW	Mortality	Slooff and Baerselman (1980)

[a]Canton and Slooff (1982).
[b]Standard Reference Water (Freeman and Fowler, 1953).
[c]Dutch Standard Water (Canton and Slooff, 1982).
[d]Tap water: pH 7–8, hardness 16°d.H.
[e]Tap water: pH 7–8, hardness 5.5°d.H.
[f]Alabaster and Abraham (1965): hard water.

test species. To determine to what extent the acute toxicity levels as determined by exploring recommended or obligatory test species in general cover the acute toxicity levels for other aquatic organisms, the lowest L(E)C values determined for three different species as obligatory or recommended in the first or second step in the ecotoxicological testing programmes an alga, a crustacean, a fish) (EPA, 1978; EC, 1979; OECD, 1979) were compared with those derived for other species. Therefore, *S. capricornutum, Daphnia magna* and *Poecilia reticulata* were compared with organisms of the same group with respect to their susceptibility to the test substances. Furthermore, the lowest NOL(E)C and LC_{50} values obtained from this set of tests were compared with those from tests with the other species.

RESULTS

Susceptibility of test species per test compound

The basic data on the (sub)acute toxicity of the test compounds to the aquatic microorganisms, macroinvertebrates and vertebrates are summarized in Tables III–V, respectively. Marked differences in species susceptibility can be observed. Based on the ratio highest L(E)C/lowest L(E)C as a measure for the difference in species susceptibility for each compound, a ratio of >10 can be determined for 14 compounds; for 8 compounds this ratio is >100, whereas for 4 chemicals the ratio exceeds a factor 1000. The highest ratio for the individual compounds was found to be almost 9000.

As a group the microorganisms showed to be generally insensitive for ethylacetate, trichloroethylene and benzene, whereas within this group (Table III) marked differences in susceptibility occurred (up to a factor 9000 for allylamine). The bacteria *Pseudomonas putida* appeared to be insensitive for allylamine and salicylaldehyde, but showed a relatively high susceptibility to benzene. The cyanobacterium *Microcystis aeruginosa* was much more sensitive; for 8 out of the 14 compounds tested, this species belonged to one of the most susceptible microorganisms. Among the green algae the susceptibility to the test compounds

TABLE II

Log Poct values of the compounds used in QSAR calculations.

Compounds	Log Poct[a]
n-Propanol	0.27
n-Heptanol	2.39
Acetone	−0.30
Trichloroethylene	2.20
Benzene	2.13

[a]Log Poct calculated after Rekker (1977).

TABLE III

NOEC-values ($mg \cdot l^{-1}$) of 14 chemical compounds for several microorganisms. The exposure time varied from 6 h for *P. putida* to 192 h for *M. aeruginosa* and *S. pannonicus*.

Compounds	Bacteria		Algae				Protozoa		
	P. putida[a]	*M. aeruginosa*[a]	*C. pyrenoidosa*	*S. pannonicus*[a,b]	*S. pannonicus*[c]	*S. capricornutum*	*E. sulcatum*[a]	*U. parduczi*[a]	*C. paramecium*[a]
Mercury(II)chloride	0.012	0.006	1.3	0.1	0.2	0.08	0.03	0.09	0.015
Cadmium nitrate	0.24	0.2	3.1	0.9	0.9	0.7	0.7	0.07	0.16
n-Propanol	2700	255	1150	3100	2900	2000	91	570	175
n-Heptanol	67	4	18	17	46	35	55	17	115
Ethylacetate	650	550	>1000	15	>1000	2000	890	1620	3250
Ethylpropionate	270	14	320	14	570	140	1140	665	1220
Acetone	1700	530	3400	7500	4740	7000	72	1710	3520
Trichloroethylene	65	63	–	>1000	–	175	390	>1000	> 400
Allylamine	700	0.35	16	2	17	13	23	3140	7.7
Aniline	130	0.16	11	8	16	10	24	90	250
Benzene	92	>1400	–	>1400	–	600	>1400	490	440
Pyridine	340	28	150	120	280	50	11	180	3.9
o-Cresol	33	7	34	11	36	65	34	31	132
Salicylaldehyde	10	1.6	10	5	4.8	5.5	1.4	5.5	3.3

[a]Toxicity threshold (TGK) at which a test compound causes initial inhibition of cell multiplication (Bringmann and Kühn, 1980). This value is considered more or less equal (within a factor 2) to the NOEC as it lies between the highest concentration at which no effect was observed and the lowest concentration at which inhibition of cell multiplication occurred, whereas the test concentrations differed by a factor 2.

[b]Exposure time of 192 h (Bringmann and Kühn, 1978).

[c]Exposure time of 48 h (see Table I).

TABLE IV

NOLC and LC_{50} 48-h values ($mg \cdot l^{-1}$) of 15 chemical compounds for several macroinvertebrates.

Test compounds	Crustaceans						Insects				Coelenterates		Molluscs	
	D. magna		*D. pulex*		*D. cucullata*		*A. aegypti*		*C. pipiens*		*H. oligactis*		*L. stagnalis*	
	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}
Mercury(II)chloride	0.0026	0.005	0.0013	0.003	–	0.0032	0.6	5.6	0.45	1.05	0.064	0.076	0.2	0.6
Cadmium nitrate	0.025	0.046	0.08	0.14	–	0.2	5.6	11	0.5	2.1	1.2	1.6	1.1	1.6
n-Propanol	4100	6300	2300	3025	–	5820	3200	4400	3600	4800	5100	6800	4000	6500
n-Heptanol	50	65	29	49	–	84	100	160	70	123	118	160	13	40
Ethylacetate	370	590	103	262	–	164	160	350	2400	3950	1120	1350	560	1100
Ethylpropionate	140	250	48	70	–	45	180	350	>1000	>1000	235	340	100	170
Acetone	8500	15800	5800	8800	–	7635	3500	15000	8000	17000	11500	13500	3500	7000
Trichloroethylene	54	94	25	45	–	57	32	48	29	55	62	75	32	56
Allylamine	28	39	23	34	–	28	36	120	125	171	12	17.5	4	5
Aniline	0.34	0.64	0.07	0.1	–	0.68	75	155	58	94	235	406	560	800
Benzene	240	400	196	305	–	373	170	200	40	71	24	34	120	230
Pyridine	700	1080	265	575	–	2470	55	130	38	66	940	1150	250	350
o-Cresol	2.9	9.5	5.2	9.6	–	16.4	65	80	31	46	63	75	56	160
Salicylaldehyde	4.3	5.8	3.5	5.4	–	5.5	8	16	38	54	5.6	7.1	2.1	6.5
Pentachlorophenol	0.16	0.48	1.2	2.0	–	1.5	1.8	7.2	24	34	0.43	0.73	0.20	0.56

TABLE V

NOLC and LC_{50} 48-h values ($mg \cdot l^{-1}$) of 15 chemical compounds for several vertebrates.

Test compounds	Fishes										Amphibians			
	L. idus		*S. gairdneri*		*P. reticulata*		*O. latipes*		*P. promelas*		*X. laevis*		*A. mexicanum*	
	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}	NOLC	LC_{50}
Mercury(II)chloride	–	0.75	0.46	0.65	0.25	0.41	0.43	0.59	0.025	0.05	0.07	0.1	0.27	0.35
Cadmium nitrate	–	12	0.08	0.15	70	115	45	73	1.4	2.2	23	32	1.1	1.3
n-Propanol	–	4830	2000	3200	6200	6700	4400	5900	2600	5000	3100	4000	3200	4000
n-Heptanol	–	32	36	43	51	64	41	48	25	34	40	44	37	52
Ethylacetate	–	270	240	260	160	210	100	125	180	270	100	180	100	145
Ethylpropionate	–	77	39	56	90	125	46	58	56	70	40	56	33	54
Acetone	–	9880	5700	7400	6700	9600	9500	14300	12000	15000	20000	24000	12000	20000
Trichloroethylene	–	213	33	42	120	182	220	270	36	47	41	45	29	48
Allylamine	–	52	12	15	9	12	13	16	1.1	2.1	3.7	5	0.9	1.8
Aniline	–	49	36	43	52	100	100	165	45	65	390	560	360	440
Benzene	–	132	40	56	265	420	126	250	54	84	105	190	120	370
Pyridine	–	196	460	560	1100	1390	1420	1560	82	115	1150	1400	700	950
o-Cresol	–	18	3.8	13	27	38	32	41	30	34	24	38	32	40
Salicylaldehyde	–	3.3	1.15	1.35	4.0	5.2	3.6	4.2	3.6	4.2	6.2	7.7	5.6	7.6
Pentachlorophenol	–	–	0.17	0.2	0.62	0.85	0.93	1.1	0.17	0.21	0.21	0.26	0.13	0.3

differed more than a factor 100 maximally (see ethylacetate). However, also different NOEC values were recorded for the same species (*S. pannonicus*) when tested by different laboratories (see ethylacetate and ethylpropionate). *S. capricornutum* and *S. pannonicus* showed a similar response to the test compounds. Relative to these algae *C. pyrenoïdosa* exhibited a low susceptibility to the chemicals, with the exception of *n*-propanol and *n*-heptanol. Among the protozoans also different susceptibilities were observed (up to a factor 50, see acetone, allylamine and pyridine). *Entosiphon sulcatum* showed to be susceptible for *n*-propanol, acetone and salicylaldehyde, but insensitive for allylamine.

Chilomonas paramecium was less susceptible for *n*-heptanol, ethylacetate, ethylpropionate and *o*-cresol, whereas pyridine showed to be relatively toxic to this protozoan.

Concerning the macroinvertebrates (Table IV) the crustaceans seemed to be more sensitive than the other macroinvertebrates tested (up to a factor 8000, see mer-

TABLE VI

The relative susceptibility of the test species based on toxicity data on all test compounds.

Test species	Relative susceptibility based on NOL(E)C values (see text)
P. putida	4.0
M. aeruginosa	1.2
C. pyrenoïdosa	4.2
S. pannonicus (a)	4.2
S. pannonicus (b)	2.0
S. capricornutum	3.5
E. sulcatum	2.0
U. parduczi	5.3
C. paramecium	3.2
D. magna	1.4
D. pulex	1.0
D. cucullata	1.2[a]
A. aegypti	4.6
C. pipiens	6.3
L. stagnalis	2.9
H. oligactis	4.6
L. idus	3.5[b]
S. gairdneri	1.7
P. reticulata	5.3
O. latipes	5.3
P. promelas	1.8
X. laevis	3.7
A. mexicanum	2.7

[a]Derived from LC_{50} values for daphnids.
[b]Derived from LC_{50} values for fish.

cury(II)chloride, cadmium nitrate, aniline). For 6 out of the 15 compounds tested, the daphnids belonged to the most sensitive test organisms. Although *Daphnia pulex* was generally the most sensitive daphnia species tested, the mutual differences in the susceptibility of *D. magna, D. pulex* and *Daphnia cucculata* were rather small (a factor 7 maximally).

The larvae of both *A. aegypti* and *C. pipiens* showed to be insensitive for mercury(II)chloride, *n*-heptanol and salicylaldehyde, whereas only *C. pipiens* tolerated high concentration levels of ethylacetate, ethylpropionate and pentachlorophenol. The mollusc *L. stagnalis* and the coelenterate *Hydra oligactis* showed a similar susceptibility (differences less than a factor 7). In comparison with the other species *L. stagnalis* was less sensitive for aniline, whereas *H. oligactis* was more sensitive for benzene. As a group the vertebrates (Table V) showed a low susceptibility for pyridine in comparison with the other test organisms. The differences in susceptibility of the species within this group were small, although there are exceptions (e.g. cadmium nitrate). Generally, *S. gairdneri* and *P. promelas* were somewhat more sensitive than the other fish species and the amphibians.

Relative susceptibility of test species for all test compounds

The relative susceptibility of the test species for all compounds is shown in Table VI. From this table it can be derived that, although marked differences in species susceptibilities with respect to the individual toxicants do occur, these differences become rather small when data on several compounds are taken into consideration together (a factor 6.5 maximally).

Relative susceptibility of test species based on QSARs

Although the log Poct values are not optimally distributed (Table II), the results show that the quality of most QSARs is good (Table VII). However, with protozoa the slopes were aberrant and with algae some compounds (benzene and trichloroethylene) did not fit in the equation. Therefore the data on these organisms were excluded in the comparison of the intercepts (*b*) of the QSARs as a measure for the susceptibilities of the species to these compounds as a group. Differences up to a factor 2.2 and 4.2 were found based on QSARs with respectively LC_{50} and NOL(E)C values as parameters.

Relative sensitivity of sets of recommended or obligatory test species

The results are given in Table VIII. It is shown that the standard test organisms fail to cover the acute toxicity level of all compounds for other aquatic organisms; for 4 out of 15 compounds the difference in NOL(E)C and LC_{50} values was more than a factor 10.

As can be derived from Table VI, *D. pulex* and *P. promelas* were generally the most susceptible species in this study. The influence of replacing the standard tests by these more sensitive organisms with respect to covering the acute toxicity levels is given in Table IX. As expected, each replacement contributes to a decrease in the ratios of the concerned effect values. However, only a marked reduction in these ratios is achieved when *M. aeruginosa* is included in the standard test combination.

DISCUSSION

In literature it is not uncommon to find extremely varying acute toxicity levels for

TABLE VII

QSARs for the test organisms, based on LC_{50} and NOL(E)C values derived for the compounds shown in Table II.

Test organisms	QSAR equations $\log(1/c) = a \log \text{Poct} - b$ (c in $\mu\text{mol}\cdot l^{-1}$)							
	Based on LC_{50} values				Based on NOL(E)C values			
	a[a]	b	r	s	a	b	r	s
P. putida	–	–	–	–	0.74	4.51	0.967	0.28
M. aeruginosa[b]	–	–	–	–	0.74	3.80	0.925	0.04
C. pyrenoïdosa[c]	–	–	–	–	0.97	4.51	0.999	0.51
S. pannonicus (a)[c]	–	–	–	–	1.12	4.88	0.997	0.17
S. pannonicus (b)[c]	–	–	–	–	0.89	4.77	0.994	0.20
S. capricornutum	–	–	–	–	0.76	4.83	0.909	0.50
E. sulcatum[b]	–	–	–	–	0.03	3.14	0.141	0.40
U. parduczi[d]	–	–	–	–	0.59	4.27	0.792	0.75
C. paramecium[d]	–	–	–	–	0.40	4.20	0.716	0.64
D. magna	0.95	5.22	0.967	0.36	0.93	4.99	0.967	0.36
D. pulex	0.91	4.94	0.952	0.42	0.94	4.78	0.950	0.45
D. cucculata	0.88	5.03	0.947	0.43	–	–	–	–
A. aegypti	0.93	5.12	0.965	0.36	0.81	4.72	0.942	0.42
C. pipiens	1.02	5.16	0.987	0.24	1.02	4.92	0.983	0.28
L. stagnalis	0.95	5.03	0.961	0.40	0.99	4.77	0.953	0.46
H. oligactis	1.02	5.15	0.974	0.34	1.03	5.06	0.975	0.34
L. idus	0.94	5.06	0.979	0.29	–	–	–	–
S. gairdneri	1.00	4.89	0.995	0.15	0.98	4.73	0.996	0.13
P. reticulata	0.86	5.12	0.963	0.35	0.88	5.01	0.996	0.34
O. latipes	0.92	5.19	0.978	0.29	0.91	5.03	0.985	0.23
P. promelas	1.10	5.15	0.994	0.18	1.09	4.97	0.996	0.14
X. laevis	1.08	5.23	0.977	0.34	1.08	5.13	0.988	0.25
A. mexicanum	1.00	5.19	0.955	0.45	1.05	5.02	0.979	0.32

[a] a = slope; b = intercept; r = correlation coefficient and s = SE of estimate.
[b] QSAR calculated without benzene.
[c] QSAR calculated without benzene and trichloroethylene.
[d] QSAR calculated without trichloroethylene.

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one toxicant. Mostly it is not clear to what extent this scatter is due to differences in methodology or to differences in susceptibility. In this study a proper evaluation of the observed differences in L(E)C values is also difficult to make. Inherent to the needs and testing possibilities of the species, the performance of the tests was sometimes different. The organisms have been tested in specific stages of growth and development or under specific circumstances, in which cases they may exhibit a lower or higher susceptibility. This is clearly indicated in Table III, showing a marked discrepancy in some of the test results when *S. pannonicus* is tested by two different laboratories under different conditions (e.g. see ethylacetate, ethylpropionate).

Also the results of interlaboratory testing programmes by the International Standardization Organization (ISO) and European Community (EC) showed that differences in the experimental protocols (including type of strains) are likely to interfere significantly in the scatter of the results. In the ISO collaborative study on algae, extreme variations in the results were found for each species (a factor 1000), as the test methods differed in almost every technical aspect (Hanstveit, 1980). Using a more standardized experimental protocol, the variation was significantly reduced (up to a factor 40; Hanstveit and Oldersma, 1981). Following strict test protocols, as prescribed in the EC-ring tests with *D. magna* and *Brachydanio rerio*, results in even less variation in the test results (maximally a factor 10; Cabridenc, 1979a, b). When comparative tests are carried out with tests completely standardized in both technical and biological aspects, almost no differences can be observed

TABLE VIII

Differences in susceptibility to the test compounds between standard test species and the other tested aquatic species.

Ratio L(E)C values	No. of test substances observed within the intervals, indicative for the factor in which the (lowest) L(E)C value found for the standard test species deviates from that observed for other tested species				
	≤3.2	3.2–10	10–32	32–100	>100
NOEC value *S. capricornutum*/Lowest NOEC value other microorganisms[a]	1	5	5	3	–
LC_{50} value *D. magna*/Lowest LC_{50} value other macroinvertebrates	9	4	2	–	–
LC_{50} value *P. reticulata*/Lowest LC_{50} value other vertebrates	7	6	1	–	1
Lowest NOL(E)C value standard organisms/Lowest NOL(E)C value all other organisms	7	3	4	1	–

[a]Because of marked difference in test results, data on the toxicity of ethylpropionate and ethylacetate to *S. pannonicus* are considered as unreliable and are therefore excluded.

in test results (mostly less than a factor 2; Canton and Adema, 1978; Juhnke and Lüdemann, 1978; Adema et al., 1981). Although these results clearly indicate that complete standardization is required for comparison of test results of one species, it will remain unclear what conditions have to be fulfilled to allow comparison of results obtained for different species.

In this study distinct differences were observed in the susceptibility of the species with respect to the test compounds separately (Tables III, IV and V; up to a factor 9000). In contrast to this, these differences were considerably smaller (Table VI: less than a factor 7) when the relative susceptibilities derived from the data on all compounds tested were taken into account, and no differences could be found between the lowest relative susceptibilities determined for the microorganisms, macroinvertebrates and vertebrates. These results indicate that if toxicity data of more compounds are considered, the differences in the relative susceptibility will become smaller.

Also, a comparison of the QSARs showed less variability in the susceptibility of the test species. The average value of the slopes (0.97) based on 14 QSARs calculated for the macroorganisms is in agreement with those usually found in animal studies and is indicative for interactions with membrane functions (Hansch, 1978). The lower value (0.74) found for *P. putida* corresponds with those found for bacteria (Hansch, 1978). The poor quality of the QSARs for protozoa indicates that the mechanism of toxic action of the compounds tested is probably different for these organisms. As this may result in a relatively high susceptibility (e.g. *E. sulcatum: n*-propanol, acetone) some caution must be exercised in using QSARs for toxicity estimates.

TABLE IX

Differences in covering the acute toxicity levels between several test combinations.

Ratio lowest NOL(E)C values	No. of test substances observed within the intervals, indicative for the factor in which the lowest NOL(E)C value found for different test combinations deviates from that observed for all other tested species				
	≤3.2	3.2–10	10–32	32–100	>100
Combination I[b]/All other organisms[a]	7	3	4	1	–
Combination II[c]/All other organisms	12	2	1	–	–
Combination III[d]/All other organisms	9	2	3	1	–
Combination IV[e]/All other organisms	8	3	3	1	–
Combination V[f]/All other organisms	13	2	–	–	–

[a]Excluding toxicity data on ethylpropionate and ethylacetate for *S. pannonicus* (see Table VIII).
[b]Combination I: *S. capricornutum, D. magna, P. reticulata.*
[c]Combination II: *M. aeruginosa, D. magna, P. reticulata.*
[d]Combination III: *S. capricornatum, P. pulex, P. reticulata.*
[e]Combination IV: *S. capricornutum, D. magna, P. promelas.*
[f]Combination V: *M. aeruginosa, D. pulex, P. promelas.*

Thus, in spite of the small differences in the relative susceptibilities of taxonomically diverse species, the susceptibility is distinctly pollutant-specific. From this it can be concluded that it is not possible (1) to select test species based on their relatively high susceptibility to toxicants, nor (2) to predict responses of one species from the results of experiments with another. As a consequence, at least a set of tests should be carried out with different test species. However, this study even showed that the standard test combination with *S. capricornutum, D. magna* and *P. reticulata* did not cover the acute toxicity level of all test compounds within one log concentration interval (Table VIII). Thus, this test combination may fail to indicate the potential toxicity of a compound, which may ultimately lead to incorrect decisions with respect to the question whether or not a compound is on the pass or fail side of an arbitrary line. Although expansion of the terms in the regulations by demanding other tests will impose additional costs, this study indicates that incorporation of a test with *M. aeruginosa* improves the ability of the test combination to estimate acute toxicity levels of substances for aquatic life (Table IX). The observation that *M. aeruginosa* is relatively highly susceptible to toxicants is confirmed by other recent comparative toxicological studies (Bringmann and Kühn, 1980a; Adema et al., 1981; Canton and Slooff, 1982). Therefore, it may be recommendable to incorporate a test with *M. aeruginosa* next to those with daphnids and fishes in the first step of both national and international ecotoxicological testing programmes.

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