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QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS AND TOXICITY STUDIES OF MIXTURES OF CHEMICALS WITH ANAESTHETIC POTENCY: ACUTE LETHAL AND SUBLETHAL TOXICITY TO *DAPHNIA MAGNA*

JOOP HERMENS¹, HANS CANTON², PETER JANSSEN and ROB DE JONG

¹*Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, Biltstraat 172, 3572 BP Utrecht,* ²*National Institute of Public Health, P.O. Box 1, 3720 BA Bilthoven, The Netherlands*

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In this study quantitative structure–activity relationships (QSARs) were calculated between hydrophobicity of a group of organic chemicals with anaesthetic potency and toxicity (immobilization, mortality and inhibition of reproduction) to *Daphnia magna*. Differences in slopes of the high quality QSARs might be explained in terms of possible different sites of action for the three criteria of effect.

The combined effects of mixtures of 5–50 chemicals on immobilization and mortality did not deviate from additivity, while the effect on reproduction deviated somewhat from it.

Key words: structure–activity relationships; mixtures; toxicity; *Daphnia magna*

INTRODUCTION

Polluted surface water generally contains hundreds to thousands of chemicals. Little is known, however, about the possible combined effects of such complex mixtures. Most studies of the toxicity of chemicals to aquatic organisms refer to substances tested singly or in mixtures of a limited number of compounds. Only a few data are available on the combined effect of toxicants on the sublethal level. Reviews discussing these studies are given by EIFAC (1980) and Sprague (1970).

In a study by Muska and Weber (1977) on the guppy, a mixture of Cu and Ni was concentration additive with regard to both lethal and sublethal responses. In binary mixtures of Cr/HCN/NH₃, Broderius and Smith (1979) mainly found no interaction when growth of the fathead minnow was studied, while combined effects were found on acute lethal level. The acute toxicity of a mixture of Cu/Zn/Cd on fathead minnow was slightly more than concentration additive based on mortality, while the chronic toxicity (effect on reproduction) of the trimetal mixture was little if any more toxic than its Zn component alone (Eaton, 1973). In a study by Sprague

(1965) on salmon, a mixture of Cu and Zn was concentration additive at the lethal level, while mixtures of these two metals showed modest potentiation in avoidance reactions. From these studies the authors of the EIFAC report (1980) suggest that when the concentrations of toxicants are reduced to their no effect levels, their potential for addition will be reduced also. This suggestion seems plausible for mixtures of chemicals with different modes of action but not so for mixtures of compounds with the same mode of action.

In this study we determined the combined toxicity of mixtures of 5–50 chemicals with the same anticipated mode of action to Daphnids in a short term test (48-h immobilization) and in 16-day experiments (mortality and reproduction). The results of these experiments were evaluated using the mixture toxicity index (MTI) and toxicity scale (Table I) of Könemann (1981a).

The chemicals belong to a group of more or less lipophilic nonreactive organic compounds with unspecific acute toxicity (related in action to the volatile anaesthetics and characterized by central nervous system depressions, probably due to membrane perturbation). Indications that these chemicals act 'simple similar' (Plackett and Hewlett, 1952) were given earlier by Könemann (1981b) and Slooff et al. (1983) who calculated quantitative structure–activity relationships (QSARs) of a good quality between toxicity to diverse aquatic organisms and partition coefficients between octanol and water (P_{oct}).

Before the tests with the mixtures were started QSARs were calculated for a limited number of the chemicals involved. With these QSARs we calculated the toxicity of the other toxicants in about the same log P_{oct} range, and for which we expected a similar reaction to the tested compounds. We restricted the choice of substances to those chemicals that were used earlier by Könemann (1981b) in the study with guppies, which showed that the toxicity was predictable from the QSAR. The advantage of this procedure is that it allowed us to extend the number of toxicants in the mixtures.

MATERIALS AND METHODS

The criteria and conditions of the experiments are summarized in Table II. A

TABLE I

Könemann mixture toxicity scale (1981a).

Classification for toxicity of mixtures (possible type of joint action)	
MTI < 0	Antagonism
MTI = 0	No addition (independent action, $r = 1^a$)
$0 < \text{MTI} < 1$	Partial addition
MTI = 1	Concentration addition (simple similar action)
MTI > 1	Supra addition (potentiation of the toxic action(s) of one or more of the compounds in the mixture)

^aPositive correlation between susceptibilities of the individual organisms to the single toxicants.

TABLE II

Criteria and conditions during the toxicological experiments with *Daphnia*.

Criteria	Age	Exposure time	Number of organisms per group ^a	Test volume per group (l)	Food	Temp. (°C)	Test medium	Hardness (mmol/l)	Dosing and ratio of concentrations	Re-nesting rate
50% Immobilization	< 2 days	48 h	25	1	None	22 ± 1	DSW ^b	ca. 1	static 1.8	–
48-h IC50										
50% Mortality	< 1 day	16 days	15 ^c	1	<i>Chlorella spec.</i>	19 ± 1	DSW ^b	ca. 1	static 3.2	3 times a week
16-day LC50										
50% Re-production	< 1 day	ca. 16 ^c days	15 ^c	1	<i>Chlorella spec.</i>	19 ± 1	DSW ^b	ca. 1	static	3 times a week
16-day EC50		(3–4 broods)							3.2	

^aAll tests were carried out in duplicate.^bDutch standard water (Canton and Slooff, 1982).^cDeviating from Concept NEN 6502.

more detailed description of the tests with *Daphnia magna* is given by the concept NEN reports (6501, 6502).

IC50-, LC50- and EC50-values (see Table II) are based on the quantities of chemicals added at the start of the experiments. Gaschromatographic analysis during EC50 experiments showed that more than 70% of the added quantities were present at the start of the experiments, both when chemicals were tested singly and in mixtures. The decrease in concentration during the tests, till renewing the solutions, was maximally 20%. IC50-values were calculated by logit transformation according to Brown (1978), while EC50- and LC50-values were determined by log/probit plots.

Relationships between toxicity and P_{oct} were calculated with a computer program, based on the method of least squares.

The P_{oct} of the compounds was calculated according to Rekker (1977). This method, used earlier by Könemann (1981b) and Könemann and Musch (1981), among others, was found to give quite satisfactory P_{oct} values for application in QSARs. The experiments with mixtures were carried out under the same conditions as the experiments with the separate chemicals. All mixtures were prepared in equitoxic concentrations (identical fraction of IC50 or EC50). The substances tested were the same as used earlier by Könemann (1981b) in a study with guppies.

RESULTS

I. QSAR studies

The slopes of concentration response curves were steep. In reproduction experiments (ratio between concentrations factor 3.2) often no partial effect was observed.

The results of the IC₅₀, LC₅₀ and EC₅₀ determinations are summarized in Tables III and IV, together with the P_{oct} values.

The following equation describes the relationship between the 48-h IC₅₀ and log P_{oct} for 19 compounds in Table III.

QSAR 1. 48-h IC₅₀ (immobilization); 19 compounds in Table III, 1-19:

$$\log \frac{1}{\text{IC}_{50}} = (0.91 \pm 0.03) \log P_{\text{oct}} - 4.72$$

TABLE III

48-h IC₅₀ (immobilization) to *Daphnia magna* and P_{oct} of the substances tested.

Substance	Log IC ₅₀ exp. ^a	Log IC ₅₀ calc. ^b	Log P_{oct} ^c
1. benzene	2.86	2.78	2.13
2. monochlorobenzene	2.36	2.16	2.81
3. 1,2-dichlorobenzene	1.41	1.51	3.53
4. 1,2,4-trichlorobenzene	1.17	0.90	4.20
5. 1,2,3,4-tetrachlorobenzene	0.40	0.22	4.94
6. pentachlorobenzene	-0.31	-0.46	5.69
7. toluene	2.21	2.36	2.59
8. 4-chlorotoluene	1.45	1.71	3.31
9. 2,4-dichlorotoluene	0.58	1.10	3.98
10. 2,4,5-trichlorotoluene	0.45	0.42	4.72
11. <i>m</i> -xylene	2.13	1.91	3.09
12. 1,2-dichloropropane	2.60	2.75	2.16
13. 1,2,3-trichloropropane	2.38	2.33	2.63
14. 1,1,2-trichloroethene	2.20	2.72	2.20
15. diethylether	4.27	3.92	0.88
16. acetone	5.02	4.99	-0.30
17. ethanol	5.07	4.96	-0.26
18. 2-ethoxyethanol	4.93	4.91	-0.21
19. ethanediol	5.91	5.95	-1.35
20. 1,3-dichlorobenzene		1.51	3.53
21. 1,4-dichlorobenzene		1.51	3.53
22. 1,2,3-trichlorobenzene		0.90	4.20
23. 1,3,5-trichlorobenzene		0.90	4.20
24. 1,2,3,5-tetrachlorobenzene		0.22	4.94
25. 1,2,4,5-tetrachlorobenzene		0.22	4.94
26. 3-chlorotoluene		1.71	3.31

TABLE III continued

27. 3,4-dichlorotoluene	1.10	3.98
28. <i>o</i> -xylene	1.91	3.09
29. <i>p</i> -xylene	1.91	3.09
30. dichloromethane	3.35	1.51
31. chloroform	2.88	2.02
32. tetrachloromethane	2.18	2.79
33. 1,1-dichloroethane	2.97	1.92
34. 1,2-dichloroethane	3.12	1.76
35. 1,1,1-trichloroethane	2.45	2.49
36. 1,1,2-trichloroethane	2.55	2.38
37. 1,1,2,2-tetrachloroethane	1.98	3.01
38. pentachloroethane	1.46	3.58
39. tetrachloroethene	2.04	2.95
40. 1,3-dichloropropane	3.16	1.71
41. 1-chlorobutane	2.58	2.35
42. 2-methoxyethanol	5.39	-0.74
43. 2-butoxyethanol	3.95	0.85
44. 2-isopropoxyethanol	4.54	0.20
45. digol	5.90	-1.30
46. trigol	5.85	-1.24
47. butyldigol	3.89	0.91
48. propanol-2	4.58	0.15
49. 2-methylpropanol-2	4.02	0.77
50. pentanol-3	3.62	1.21

^aExperimentally determined 48-h IC₅₀; IC₅₀ in $\mu\text{mol/l}$.

^bCalculated 48-h IC₅₀ with QSAR 1.

^cLog P_{oct} values calculated according to Rekker (1977).

Figures in parentheses are standard errors; $n = 19$; $r = 0.992$; $s = 0.24$ (n = number of compounds; r = correlation coefficient; s = standard error of estimate). The relationships between 16-day LC₅₀, 16-day EC₅₀ and log P_{oct} for compounds in Table IV are given in QSAR 2 and 3.

QSAR 2. 16-day LC₅₀ (mortality); 5 compounds in Table IV, 1-5:

$$\log \frac{1}{\text{LC}_{50}} = (0.64 \pm 0.04) \log P_{\text{oct}} - 3.27$$

$n = 5$; $r = 0.995$; $s = 0.08$.

QSAR 3. 16-day EC₅₀ (reproduction); 5 compounds in Table IV, 1-5:

$$\log \frac{1}{\text{EC}_{50}} = (0.72 \pm 0.06) \log P_{\text{oct}} - 3.05$$

TABLE IV

16-day LC50 (mortality), 16-day EC50 (reproduction) to *Daphnia magna* and P_{ocf} values of the substances tested.

Substance	log LC50 exp. ^a	log LC50 calc. ^b	log EC50 exp. ^c	log EC50 calc. ^d	log P_{ocf} ^e
1. monochlorobenzene	1.55	1.47	0.99	1.03	2.81
2. 4-chlorotoluene	1.10	1.15	0.66	0.67	3.31
3. 1,2,4-trichlorobenzene	0.49	0.58	0.17	0.03	4.20
4. 1,2,3,4-tetrachlorobenzene	0.17	0.11	-0.70	-0.51	4.94
5. pentachlorobenzene	-0.36	-0.37	-1.00	-1.05	5.69
6. benzene		1.91		1.52	2.13
7. toluene		1.61		1.19	2.59
8. 1,2-dichlorobenzene		1.01		0.51	3.53
9. 2,4-dichlorotoluene		0.72		0.18	3.98
10. 2,4,5-trichlorotoluene		0.25		-0.35	4.72
11. <i>m</i> -xylene		1.29		0.83	3.09
12. 1,2,3-trichloropropane		1.59		1.16	2.63
13. tetrachloromethane		1.48		1.04	2.79
14. 1,1,2-trichloroethane		1.75		1.34	2.38
15. 1,1,2,2-tetrachloroethane		1.34		0.88	3.01
16. pentachloroethane		0.98		0.47	3.58
17. tetrachloroethene		1.38		0.93	2.95
18. 1,3-dichloropropane		2.18		1.82	1.71
19. 1-chlorobutane		1.77		1.36	2.35
20. trichloroethene		1.86		1.47	2.20
21. 1,4-dichlorobenzene		1.01		0.51	3.53
22. 1,3,5-trichlorobenzene		0.58		0.03	4.20
23. 1,2,4,5-tetrachlorobenzene		0.11		-0.51	4.94
24. 3-chlorotoluene		1.15		0.67	3.31
25. 3,4-dichlorotoluene		0.72		0.18	3.98

^aExperimentally determined 16-day LC50, LC50 in $\mu\text{mol/l}$.

^bCalculated 16-day LC50 with QSAR 2.

^cExperimentally determined 16-day EC50; EC50 in $\mu\text{mol/l}$.

^dCalculated 16-day EC50 with QSAR 3.

^eLog P_{ocf} values calculated according to Rekker (1977).

$n = 5$; $r = 0.990$; $s = 0.14$. A graphical presentation of the QSARs is given in Fig. 1.

II. Toxicity studies of mixtures

The results of the toxicity experiments with mixtures using the 48-h IC50 as a criterium are given in Table VII. The mixtures were prepared in equitoxic concentrations based on 48-h IC50 values from Table III. The composition of the mixtures is given in Table V. Mixture 1 is composed of chlorobenzenes and chlorotoluenes, while mixture 2 contains chemicals with more diverse structures. All 48-h IC50 values of chemicals in mixtures 1 and 2 are experimentally determined, while mixture 3 is composed of chemicals for which IC50 values are calculated. Mixture 4 con-

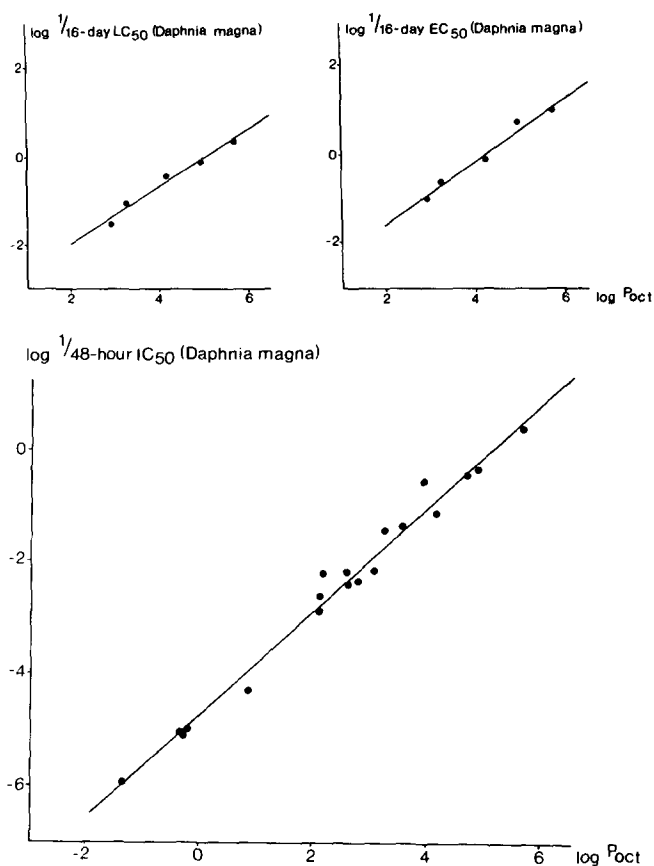


Fig. 1. Correlations between 48-h IC₅₀, 16-day LC₅₀ and 16-day EC₅₀ to *Daphnia magna* and P_{oct} of compounds in Tables III and IV.

tains all chemicals from Table III.

The results of the toxicity experiments with mixtures using the 16-day EC₅₀ and 16-day LC₅₀ as criteria are summarized in Tables VIII and IX. The mixtures were prepared in equitoxic concentrations based on 16-day EC₅₀s. Because 16-day EC₅₀

TABLE V

Composition of mixtures at 48-h IC₅₀ experiments.

Mixture	n^a	Composition ^b									
1	10	1	2	3	4	5	6	7	8	9	10
2	10	1	3	6	7	9	10	14	15	16	17
3	10	21 - 23 - 25 - 26 - 28 - 31 - 32 - 42 - 45 - 48									
4	50	1 up to and including 50									

^a n = number of compounds in mixture.

^bNumbers correspond with numbers from Table III.

and LC50 of the mixtures were determined in the same experiment, the concentrations of the chemicals in Table IX (16-day LC50) deviate little from equitoxicity. The composition of the mixtures is presented in Table VI. Mixture 5 contains 5 of the chemicals in Table IV, mixture 6 contains 10 chemicals, while mixture 7 is composed of all the chemicals in Table IV. The standard deviations in MTI are calculated with an estimated standard deviation in log IC50, log EC50 or log LC50 of 0.10. Estimates of the errors in 48-h IC50 are based on results from a study by Canton and Adema (1978), while the errors in 16-day EC50 and 16-day LC50 are estimates based partly on the standard errors found in the QSARs.

TABLE VI

Composition of mixtures at 16-day EC50 and LC50 experiments.

Mixture	n^a	Composition ^b
5	5	1 2 3 4 5
6	10	1 2 3 4 5 6 7 8 9 10
7	25	1 up to and including 25

^a n = Number of compounds in mixtures.

^bNumbers correspond with those from Table IV.

TABLE VII

Results of mixture toxicity experiments with 48-h IC50 (immobilization) as criterium.

	Mixture			
	1	2	3	4
Number of chemicals (n)	10	10	10	50
M^a	1.0	1.1	1.1	1.2
MTI ^b	1.00	0.96	0.96	0.95
sMTI ^c	0.10	0.10	0.10	0.06

^a M = sum of concentrations, expressed as fractions of the IC50, LC50 or EC50, at standard response.

^bMTI calculated after Könemann (1981a).

^cStandard deviation in MTI calculated after Könemann (1981a) with an estimated error in log IC50, log EC50 or log LC50 of 0.10.

TABLE VIII

Results of mixture toxicity experiments with 16-day EC50 (reproduction) as criterium.

	Mixture		
	5	6	7
Number of chemicals (n)	5	10	25
M^a	2.0	2.0	1.5
MTI ^a	0.57	0.70	0.87
sMTI ^a	0.17	0.11	0.07

^aSee Table VII.

TABLE IX

Results of mixture toxicity experiments with 16-day LC50 (mortality) as criterium.

	Mixture		
	5	6	7
Number of chemicals (<i>n</i>)	5	10	25
<i>M</i> ^a	1.0	0.9	1.5
MTI ^a	1.00	1.05	0.87
sMTI ^a	0.14	0.10	0.07

^aSee Table VII.

DISCUSSION

I. QSAR studies

According to the high correlation coefficients and low standard deviations in QSAR 1, 2 and 3 the quality of the correlations is good.

The slopes of QSAR 2 and 3 (16-day experiments) are lower than the slope of QSAR 1 (48-h experiment). This might be explained in two ways. (1) QSAR 1 is calculated with compounds varying in log *P* from -1.30 to 5.69 , while in QSAR 2 and 3 log *P* values are in the range 2.81 – 5.69 . At higher log *P* values deviation from linearity often occurs and parabolic functions often describe QSARs better than linear ones do (Hansch, 1971). In parabolic relationships between log *P* and toxicity the slope at low values of log *P* is higher than at high log *P*. Nonlinearity of the relations between toxicity and *P*_{oct}, however, was not observed in our experiments (see Fig. 1). (2) The QSARs with low slopes (0.72 and 0.64) occur in the longer term (16-day) experiments, while the QSAR with a rather high slope (0.92) is found in a short term (48-h) experiment. In an extensive study of 137 QSARs, Hansch (1972, 1978) observed that the slopes tended to be grouped around 1.0 and 0.7 . A first group of 71 QSARs had a mean slope of 0.66 ± 0.12 and occurred in correlations involving proteins, enzymes and bacteria. The mean of a second group of 57 examples was 1.0 ± 0.13 and was found in interactions of organic compounds with nerve or erythrocyte membranes. This mean value of about 1.0 was also found in a study of Slooff et al. (1983) who calculated QSARs with 48-h LC50 to diverse aquatic organisms of 5 chemicals assumed to have the same mode of action as the chemicals used in this study. Although the distribution of log *P* values in this study was not optimal the slopes were rather constant. On the basis of 14 examples a mean value of 0.97 ± 0.07 was calculated. The differences in slopes of QSAR 1 versus 2 and 3 may be related to a difference in primary site of action of the tested chemicals between short term and longer term exposure. In short term experiments probably only the membrane perturbing mechanism resulting in anaesthesia of these chemicals dominates, while in longer term experiments toxicity may be due to other mechanisms. The calculated QSARs estimates can be made for the IC50-, EC50-

and LC50 values of untested chemicals from the extensive group of nonreactive, non-ionized organic compounds with unspecific toxicity. These estimates can be used, for instance, as alternatives for range finding experiments or as a selection criterium for composing lists with priority chemicals. As long as no definitive testing results are available these estimates can be used in the setting of provisional water quality criteria.

In the development of water quality criteria 'no observed effect concentrations' (NOEC) are of more interest than EC50 or LC50 values. In the 16-day experiments, in addition to EC50 and LC50, also NOECs on reproduction and mortality were determined (Table X). QSARs with these criteria were not calculated because they are not reliable, due to the high ratio between concentration steps (factor 3.2).

II. Toxicity studies of mixtures

The results shown in Tables VII and IX indicate that the toxicity of mixtures of chemicals using 48-h IC50 and 16-day LC50 as criteria does not deviate from concentration addition (MTI = 1.0). We also expected concentration addition because the chemicals in the mixtures probably act similar, as indicated by the high quality QSARs. Also Könemann (1981a) found a MTI of about 1.0 with mixtures of the same chemicals in his experiments with guppies. The toxicity of mixtures containing 5 or 10 chemicals (mixtures 5 and 6 from Table VIII) deviates from concentration addition. This deviation, which is small, is difficult to explain.

The joint toxicity is not reduced when concentrations of the toxicants become lower and reach their levels of no effect, as suggested in the EIFAC report (1980). If the potential for addition falls, the MTI has to decrease when the number of chemicals increases. The MTI values in Tables VII-IX do not show a systematic decrease with increasing number of chemicals. Conclusions based on toxicity experiments with mixtures of the 50 chemicals in Tables VII and X or 25 chemicals in Tables VIII and IX must be regarded with some prudence, because the toxicity of the chemicals was partly calculated.

From these studies it can be concluded that joint effects are also present on the

TABLE X

Summary of the results of the 16-day experiments with *Daphnia magna* (in mg/l).

Substances	16-day EC50	16-day NOEC ^a	16-day LC50	16-day NOEC ^b
monochlorobenzene	1.1	0.32	4.0	1.0
4-chlorotoluene	0.58	0.32	1.6	1.0
1,2,4-trichlorobenzene	0.27	0.10	0.56	0.32
1,2,3,4-tetrachlorobenzene	0.043	0.010	0.32	0.10
pentachlorobenzene	0.025	0.010	0.11	0.10

^aNo observed effect concentration on reproduction.

^bNo observed effect concentration on mortality.

sublethal level. The toxicity of mixtures of the tested chemicals, which probably acted 'simple similar', as was demonstrated with QSAR studies, was concentration additive or very near to it. It is difficult to conclude if the small deviation from concentration addition on a sublethal level, such as inhibition of reproduction, is systematic because little information is available.

The calculated QSARs, with P_{oct} as hydrophobicity character, were of excellent quality and probably predict a minimum toxicity of nonreactive, nonionized organic chemicals with anaesthetic potency. This idea of a minimum toxic effect was discussed earlier by Könemann (1981b) in a study with puppies.

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