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Joint Toxicity of Mixtures of Groups of Organic Aquatic Pollutants to the Guppy (*Poecilia reticulata*)

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In this study acute lethal concentrations (LC_{50}) to the guppy (*Poecilia reticulata*) were determined for mixtures of 4 groups of aquatic pollutants. The groups were composed of 11 nonreactive, nonionized organic chemicals, 11 chloroanilines, 11 chlorophenols, and 9 reactive organic halides. Earlier studies indicated that the joint toxicity within each of these groups was concentration additive, probably because of a similar mode of action. The joint toxicity of combinations of one representative from each group showed a high variance, but generally tended to be partially additive to concentration additive. This high variance is probably caused by the low number of compounds in these mixtures. Experiments with mixtures of whole groups gave more accurate results. The toxicity of a mixture of the first three groups, containing 33 well-known aquatic pollutants, was almost completely concentration additive. Concentrations of 0.04 of the individual LC_{50} values contributed to the toxicity of this mixture.

INTRODUCTION

Rivers such as the Rhine are polluted with a large number of chemicals. Information about joint toxicity of these complex mixtures is rather scarce. Among the pollutants in these mixtures, several groups containing chemicals with similar modes of action can be distinguished. The joint toxicity of mixtures, composed of similarly acting toxicants, can be predicted theoretically with the concentration addition model (Könemann, 1981a; Muska and Weber, 1977; Plackett and Hewlett, 1952; Sprague, 1970). Indications for similarity of mode action can be found in structure–activity relationships (QSARs) (Könemann, 1980).

It is the purpose of this study to determine the joint toxicity of combinations of groups of compounds with different modes of action. Combinations were made of four groups of chemicals, each with probably a different mode of action according to a QSAR with 14-day LC_{50} as toxicity parameter and with a joint toxicity of mixtures of the components of each group conforming to concentration addition (see Table 1). Joint toxicity as presented in Table 1, was evaluated with the mixture toxicity index (MTI) as proposed by Könemann (1981a) (see Table 2). The composition of the four groups is given in Table 3. In order to test the possibility of prediction of this kind of joint action of mixtures of groups from experiments with representatives of these different groups, the joint toxicity of combinations of single representatives of the four groups of Table 1 was also determined.

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TABLE 1

ACUTE LETHALITY TO THE GUPPY OF FOUR MIXTURES OF ORGANIC POLLUTANTS

Group	MTI[d]	M[d]	n[e]
A Nonreactive (chlorinated) hydrocarbons	1.02[a]	0.9	50
B Chloroanilines	0.96[b]	1.1	11
C Chlorophenols	1.00[a]	1.0	11
D Reactive organic halides	1.00[c]	1.0	9

[a] Data drawn from Könemann (1981a).
[b] Data drawn from Hermens *et al.* (1984).
[c] Determined in this study.
[d] Definition of M and MTI are given in Eq. (1).
[e] Number of compounds in the mixtures.

METHODS

Fourteen-day LC_{50} experiments with guppies (*Poecilia reticulata*) were carried out as described earlier by Könemann (1981b). The mixtures were prepared in equitoxic concentration (identical fractions of the LC_{50} values). In experiments with chlorophenols the pH was kept at 7.3 with a buffer solution (Könemann and Musch, 1981). The LC_{50} values of the single compounds are given in Table 3. The joint toxicity was evaluated with the MTI. For equitoxic mixtures this MTI is defined as

$$\mathrm{MTI} = 1 - (\log M/\log n) \quad (1)$$

in which $M = \Sigma\, c/LC_{50}$ at 50% effect in the mixture, and n = total number of compounds in the mixture. Tests with mixtures of one compound per group were carried out four times, each time with different representatives from the groups. Also, the experiments with combinations of the groups were carried out four times. The compositions of the groups of compounds are given in Table 3.

RESULTS AND DISCUSSION

The results of the experiments with mixtures of groups are given in Table 4. Joint toxicity of the mixtures appears to be not much less than concentration

TABLE 2

MIXTURE TOXICITY SCALE[a]

MTI	Classification for toxicity of mixtures (possible types of joint action)
MTI < 0	Antagonism
MTI = 0	No addition (independent action, $r = +1$)[b]
0 < MTI < 1	Partial addition
MTI = 1	Concentration addition (simple similar action)
MTI > 1	Supra addition (potentiation of the toxic actions of one or more of the compounds in the mixture)

[a] MTI calculated after Könemann (1981a).
[b] Positive correlation between susceptibilities of the individual organisms to the single chemicals in a mixture.

TABLE 3

COMPOSITION OF THE GROUPS OF CHEMICALS TOGETHER WITH THE LC_{50} VALUES

No.		log LC_{50}[e]
	Group A: Nonreactive (chlorinated) hydrocarbons[a]	
1	1,3-Dichlorobenzene	1.70
2	1,2,3-Trichlorobenzene	1.11
3	Monochlorobenzene	2.23
4	1,2,3,4-Tetrachlorobenzene	0.57
5	Benzene	2.91
6	Pentachlorobenzene	−0.15
7	Toluene	2.87
8	2,4-Dichlorotoluene	1.46
9	*m*-Xylene	2.55
10	4-Chlorotoluene	1.67
11	Chloroform	2.93
	Group B: Chloroanilines[b]	
12	2-Chloroaniline	1.69
13	3,5-Dichloroaniline	1.38
14	2,3,4-Trichloroaniline	0.85
15	3,4-Dichloroaniline	1.59
16	Aniline	3.13
17	3-Chloroaniline	2.02
18	4-Chloroaniline	2.31
19	2,5-Dichloroaniline	1.01
20	2,4-Dichloroaniline	1.59
21	2,4,5-Trichloroaniline	1.00
22	2,3,4,5-Tetrachloroaniline	0.19
	Group C: Chlorophenols[c]	
23	3-Chlorophenol	1.70
24	2,4-dichlorophenol	1.41
25	3,4,5-Trichlorophenol	0.76
26	2,3,5,6-Tetrachlorophenol	0.77
27	Phenol	2.50
28	2-Chlorophenol	1.94
29	3,5-Dichlorophenol	1.22
30	2,3,5-Trichlorophenol	0.90
31	2,3,6-Trichlorophenol	1.41
32	2,3,4,5-Tetrachlorophenol	0.52
33	Pentachlorophenol	0.15
	Group D: Reactive organic halides[d]	
34	Allylchloride	1.20
35	1,4-Dichloro-2-butene	−0.16
36	Chloroacetone	0.88
37	Benzylchloride	0.49
38	2,3-Dichloropropene	1.01
39	α,α'-Dichloro-*m*-xylene	−0.16
40	1-Chloro-2,4-dinitrobenzene	−0.19
41	2,4-α-Trichlorotoluene	0.08
42	Hexachlorobutadiene	−0.20

[a] LC_{50} values taken from Könemann (1981b).
[b] LC_{50} values taken from Hermens *et al.* (1984).
[c] LC_{50} values taken from Könemann and Musch (1981).
[d] LC_{50} values to be published elsewhere.
[e] LC_{50} in μmol/liter.

TABLE 4

RESULTS OF 14-DAY LC_{50} EXPERIMENT WITH GUPPIES OF MIXTURES OF GROUPS

Combination	n^a	M^b	MTI[c]
Group A–group B	22	1.2	0.94
		1.2	0.94
		1.5	0.87
		1.5	0.87
Group B–group C	22	1.2	0.94
		1.1	0.97
		1.2	0.94
		1.0	1.00
Group A–group C	22	1.3	0.92
		1.5	0.87
		1.2	0.94
		1.4	0.89
Group A–group D	18	1.3	0.91
		1.4	0.88
		1.1	0.97
		1.3	0.91
Group A–group B–group C	33	1.5	0.88
		1.3	0.92
		1.2	0.95
		1.2	0.95

[a] Total number of compounds in mixtures.
[b] Each combination was tested four times. The definition of M is given in Eq. (1).
[c] MTI calculated with Eq. (1).

additive. Such a relatively high joint toxicity was also found in experiments with mixtures of chemicals with diverse modes of action, and can be explained in several ways:

(i) Mortality can be related to the disturbance of several biological systems. Toxicants with different modes of action can lead to disturbance of the same system (a system may be affected at more than one site). Therefore completely independent actions between the toxicants, which will result in a lower joint toxicity, are unlikely in experiments with mortality as overall criterion of effect.

(ii) The joint toxicity between chemicals within each group is concentration additive. This phenomenon enhances the toxicity of combinations of these groups.

(iii) Although different QSARs can be calculated for the four groups, this does not mean that the chemicals have completely different modes of action. If the compounds from the different groups have common toxic actions, their combined effect will be more in the direction of concentration addition.

In the mixture of 33 compounds, the combination of nonreactive (chlorinated) hydrocarbons, the chloroanilines and the chlorophenols, concentrations of 0.04 of the LC_{50} values do contribute to the toxicity of the mixture, and the joint toxicity

TABLE 5

RESULTS OF 14-DAY LC_{50} EXPERIMENTS WITH GUPPIES OF MIXTURES COMPOSED OF ONE COMPOUND PER GROUP

Combination[a]	M^b	MTI[b]	Combination[a]	M^b	MTI[b]
Group A–group B			Group B–group C		
1–12	1.3	0.62	12–24	1.4	0.51
2–13	0.7	1.51	13–23	1.5	0.42
3–14	0.9	1.15	14–26	1.5	0.42
4–15	1.2	0.74	15–25	1.5	0.42
Group A–group C			Group A–group D		
1–23	1.2	0.74	1–34	1.1	0.86
2–24	1.5	0.42	2–35	1.8	0.15
3–25	1.2	0.74	3–36	0.8	1.32
4–26	1.4	0.51	4–37	0.9	1.15
Group A–group B–group C					
1–12–23	1.5	0.63			
2–13–24	1.5	0.63			
3–14–25	1.6	0.57			
4–15–26	1.4	0.69			

[a] Numbers correspond with those from Table 3.
[b] Definitions of M and MTI are given in Eq. (1).

is almost completely concentration additive (MTI = 0.93 ± 0.02). This means that in mixtures of this kind of aquatic pollutants, also at concentrations far below the unobserved lethal concentrations, the joint effect is much higher than is to be expected from the influence based on a single compound.

The results of the experiments with mixtures composed of one compound per group are given in Table 5. The joint toxicity of most of the mixtures is partially additive (0 < MTI < 1). Within a combination, for instance between groups A and B, the MTI values show a high variance (MTI varies from 0.62 to 1.51). A possible explanation for this high variance is that the results of mixture toxicity experiments with only a few compounds are less reliable compared to mixtures of many chemicals (Könemann, 1981a). Because of this high variance in the experiments with mixtures of two or three chemicals, a comparison of the joint toxicity of combinations of one representative from each group with the joint toxicity of mixtures of whole groups is not to be recommended.

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