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Toxicity of Mixtures of Heavy Metals and Petrochemicals to *Xenopus laevis*

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Environmental pollution situations usually deal with pollutants acting simultaneously. Although the need for the evaluation of joint toxicity has been recognized half a century ago (Bliss 1939), most studies on the aquatic toxicity of chemicals refer to substances tested in isolation to arrive at compound-specific water quality criteria. Based on the information on joint toxicity, scarcely available in the late seventies, EIFAC (1980) suggested that when toxicant concentrations are reduced to their no-effect-levels, their potential for additivity will be also reduced. However, more recent studies indicate that this does not apply for mixtures of compounds with the same mode of action (Hermens et al. 1982, 1984a). In equitoxic mixtures of chemicals attacking the same processes, even minute amounts will add to the overall toxicity. According to Hermens et al. (1984b), depending on the specificity of the test criterion, total additivity can also be observed in testing mixtures of non-related chemicals. Since death can be caused by the distortion of a wide variety of processes, mortality tests often indicate additivity.
Still further information is needed for the estimation of the potential environmental hazard of mixtures. Since effluents of petrochemical industries are containing different groups of non-related organics (Bridié et al. 1979), in this study special attention is given to petrochemical compounds.
Furthermore, petrochemical industries are often located in the vicinity of large water bodies (e.g. River Rhine; Rotterdam) frequently polluted with substantial amounts of heavy metals. Acute tests on mixtures of alcohols, amines, hydrocarbons and halogenated hydrocarbons in combination with some heavy metals are described and discussed. Even in tests involving mixtures of chemicals with an assumed different mode of action at least partial additivity is expected.

MATERIALS AND METHODS

Test solutions were composed of one or more of 33 analytical grade chemicals (Table 1). The mixtures were made of identical fractions (equitoxic mixtures) of the 48 h LC50 values derived from single compound toxicity experiments performed first (Table 1).

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Organic mixture stock solutions were prepared by volumetrically combining the liquid compounds, using density data supplied by the manufacturers and adding the weighed solids. No additional organic solvents were used. Equitoxic amounts of heavy metals, were added to the test vessels separately from an aqueous mixture stock.

Xenopus laevis (clawed toad) was used as test animal since this species is easy to culture and to maintain in the laboratory (Slooff and Baerselman 1980), whereas its susceptibility to toxicants is comparable to that of fish (Slooff et al. 1983). In the experiments 10 randomly selected specimens of 3-4 wk old larvae were exposed in covered all-glass aquaria containing 1 L of Dutch Standard Water (Canton and Slooff 1982) at a temperature of 20 $\pm$ 1°C. Nominal amounts of the test solutions were added only once at the beginning of the experiments. The tests were performed singularly with a minimum of 5 concentration levels having a factorial difference of 1.5. Mortality was recorded after 48 h.

The toxicity of the individual compounds is expressed in mg/L as the median lethal concentration after 48 h (48-h LC 50) which has been calculated as a projection from the least square linear regression on log transformed nominal concentration data and probit transformed percent effect data. The toxicity of mixtures is calculated the same way, but concentration is entered as the sum of the fractions of the LC 50 values of individual compounds present in the test (ΣTU; TU = toxic unit). For each mixture experiment also the mixture toxicity index (MTI) is calculated according to Könemann (1981):

$$MTI = 1 - \frac{\log \Sigma TU}{\log (\Sigma TU / TU_{max})}$$

In equitoxic mixtures this is equivalent to: $1 - \frac{\log \Sigma TU}{\log n}$

where:

$$TU_i = \frac{\text{concentration constituent i}}{\text{LC 50 compound i}}$$

TU_{max} = the maximum value of all TU_i's

$$\Sigma TU = \sum_{i=1}^{n} TU_i$$

n = the number of chemicals present

For equitoxic mixtures involving different groups of chemicals the amount present of each group at the median lethal level is also expressed in relation to the median lethal amount of the group tested solely (group equivalence value; GE). When mixtures of related chemicals are treated as unmixed toxicants yet another

(group) mixture toxicity index (GMTI) can be calculated likewise:

$$GMTI = 1 - \frac{\log \Sigma GE}{\log(\Sigma GE/GE_{max})}$$

For both the MTI and the GMTI, assuming no stochastic variability, the following scale is applicable:

	antagonism	no addition	partial addition	concentration addition	supra addition
MTI:	< 0	0	0-1	1	> 1

Since no replicate testing has been performed, nothing can be stated about the uncertainty in the values of the MTI. However, considering test resolution (factorial difference between test concentrations is 1.5), the expression of toxic effects in the mixtures on a compound by compound base is better reflected by a ΣTU scale:

	antagonism	no addition	partial addition	concentration addition	supra addition
ΣTU:	>nx1.5	nx1.5 to nx0.67	nx0.67 to nx1.5	1.5 to 0.67	< 0.67

For the same reason suggested supra addition of individual compounds can be attributed to the sole action of one or more groups of compounds when at least one group equivalence value is between 0.67 and 1.5. Group antagonism can be concluded when at least one of the group equivalence values is more than 1.5.

RESULTS AND DISCUSSION

In Table 1 the toxicity data on individual compounds are presented. Table 2 gives the data on the mixtures within the groups of chemicals, whereas Table 3 represents the results of the tests on the toxicity of complex mixtures joining the five groups of chemicals in all combinations possible.
The interaction of compounds in simple equitoxic mixtures of heavy metals (#1) and amines (#3) results in an enhanced toxicity, whereas the combined toxicity of a mixture of hydrocarbons (#4) is less than additive. The alcohols (#2) and the halogenated hydrocarbons (#5) are acting more or less additively. On a compound by compound basis most of the complex mixtures are acting additively (#6, 9, 11, 13, 16, 17, 18, 22, 25, 26, 27, 28, 30 and 31; MTI = 0.89 to 1.11).
Of these 14 mixtures six (#13, 16, 25, 26, 27 and 30) show a definite group antagonistic reaction with respect to amine equivalence values varying between 1.69 and 3.70 (GMTI = -1.14 to -26.13). Based on ΣTU, nine mixtures are acting supra additively (#7, 10, 12, 14, 19, 20, 23, 24 and 29); MTI = 1.13 to 1.69).

Table 1. Test compounds and their toxicity to the clawed toad

Compound	48-h LC 50 mg/l	Group
Mercury(II) chloride	0.1	heavy metal
Cadmium nitrate	20.2	"
Copper sulfate	1.7	"
2-Methyl-1-propanol	18.3	alcohol
1-Butanol	1200	"
2-Butanol	1530	"
2-Methyl 2-propanol	2450	"
3-Chloro 1-propanol	581	"
3-Chloro 1,2-propanediol	2750	"
1,2 Ethanediol	326	"
4-Methyl 2-pentanol	656	"
3,5,5-Trimethyl 1-hexanol	13.5	"
1,3-Dichloro 2-propanol	450	"
Bis(2-hydroxyethyl)ether	3065	"
Bis(3-hydroxypropyl)ether	3181	"
3,6-Dioxo 1,8-octanediol	3047	"
Bis(2-propenyl)amine	25.5	amine
Bis(2-hydroxyethyl)amine	1174	"
Bis(2-hydroxypropyl)amine	410	"
2-Propenyl amine	12.4	"
2-Amino ethanol	220	"
1-Amino 2-propanol	420	"
Benzene	190	hydrocarbon
1-Methyl 4(tert)butylbenzene	5.0	"
1,5,9-Cyclododecatriene	1.6	"
1,3,5-Cycloheptatriene	41	"
1,5-Cyclo-octadiene	24	"
1,2-Dimethylbenzene	73	"
3-Bromopropene	0.66	halogenated hydrocarbon
3-Chloropropene	0.34	"
1-Chloro 3-bromopropane	41	"
1,3-Dichloropropane	63	"
3-Chloro 2-methylpropene	10	"

Table 2. Toxicity of mixtures within groups to the clawed toad.

Type of mixture	Group ID#	Number of compounds	ΣTU	MTI
Heavy metals	1	3	0.45	1.73
Alcohols	2	13	0.71	1.13
Amines	3	6	0.14	2.12
Hydrocarbons	4	6	2.64	0.46
Halogenated hydrocarbons	5	5	0.90	1.07

Table 3. Toxicity of complex mixtures to the clawed toad

Type of complex mixture (identified by groep #)	Number of compounds	ΣTU	MTI	GE					ΣGE	GMTI
				# 1	# 2	# 3	# 4	# 5		
1+2 = 6	16	1.03	0.99	0.43	1.18	-	-	-	1.61	-0.53
1+3 = 7	9	0.22	1.69	0.16	-	1.09	-	-	1.25	-0.63
1+4 = 8	9	1.91	0.71	1.41	-	-	0.48	-	1.89	-1.71
1+5 = 9	8	1.03	0.99	0.86	-	-	-	0.72	1.58	0.25
2+3 = 10	19	0.46	1.26	-	0.44	1.08	-	-	1.52	-0.23
2+4 = 11	19	0.79	1.08	-	0.76	-	0.09	-	0.85	2.45
2+5 = 12	18	0.46	1.27	-	0.46	-	-	0.14	0.60	2.92
3+4 = 13	12	1.00	1.00	-	-	3.70	0.19	-	3.89	-26.13
3+5 = 14	11	0.24	1.60	-	-	0.96	-	0.14	1.08	0.35
4+5 = 15	11	5.40	0.30	-	-	-	1.12	2.74	3.86	-2.94
1+2+3 = 16	22	1.23	0.93	0.37	1.02	2.48	-	-	3.87	-2.04
1+2+4 = 17	22	1.07	0.98	0.33	0.89	-	0.11	-	1.33	0.29
1+2+5 = 18	21	1.39	0.89	0.44	1.21	-	-	0.37	2.02	-0.37
1+3+4 = 19	15	0.44	1.30	0.20	-	1.32	0.07	-	1.59	-1.49
1+3+5 = 20	14	0.40	1.34	0.19	-	1.28	-	0.16	1.63	-1.02
1+4+5 = 21	14	1.54	0.84	0.73	-	-	0.25	0.61	1.59	0.40
2+3+4 = 22	25	0.69	1.11	-	0.51	1.23	0.06	-	1.80	-0.54
2+3+5 = 23	24	0.59	1.17	-	0.45	1.09	-	0.14	1.68	-0.20
2+4+5 = 24	24	0.67	1.13	-	0.51	-	0.06	0.16	0.73	1.88
3+4+5 = 25	17	1.22	0.93	-	-	3.19	0.16	0.40	3.75	-7.17
1+2+3+4 = 26	28	1.31	0.92	0.31	0.89	2.09	0.11	-	3.37	-1.54
1+2+3+5 = 27	27	1.14	0.96	0.28	0.77	1.87	-	0.23	3.15	-1.20
1+2+4+5 = 28	27	1.23	0.94	0.30	0.83	-	0.10	0.25	1.48	0.32
1+3+4+5 = 29	20	0.49	1.24	0.16	-	1.09	0.06	0.14	1.46	-0.29
2+3+4+5 = 30	30	1.14	0.96	-	0.69	1.69	0.09	0.21	2.68	-1.14
1+2+3+4+5 = 31	33	0.91	1.03	0.18	0.57	1.23	0.06	0.15	2.19	-0.36

However, only in the mixtures #12 and #24 this supra additivity cannot be attributed to the sole action of the group of amines only. Of the four mixtures partly adding the compound toxicity (#4, 8, 15 and 21; MTI = 0.30 to 0.84), the #15 mixture shows a group antagonistic interaction of the halogenated (GE = 2.74) and non-halogenated (GE = 1.12) hydrocarbons. In conclusion it can be stated that most combinations tested show an addition or a near addition of toxicity, which was expected.
In these tests fractions down to 0.03 of the individual LC 50's contribute to the mixture toxicity, which is in accordance with the findings of Hermens et al. (1984a). The relatively high combined toxicity can be attributed to the similar action of the individual chemicals towards different processes leading to mortality (Hermens et al. 1984b).
Indications for partial additivity only occur in some of the mixtures involving the relatively non-reactive hydrocarbons (#4, 15 and ± 21). This is an unexpected phenomenon, since, of all groups tested, the hydrocarbons are supposed to be the most alike in their action.
The simple mixture of six amines (#3) and, on a compound by compound basis as well as based on group toxicity, the mixtures of alcohols and halogenated hydrocarbons (#12 and ± #24) show supra additive toxicity. The high GE values of amines in complex mixtures suggest a reduced toxic activity of that group in combination with other compounds. Both supra additivity and antagonism cannot chemically be accounted for.

REFERENCES

Bliss CP (1939) The toxicity of poisons applied jointly. Ann Appl Biol 26:585-651

Bridié AL, Wolff CJM, Winter M (1979) The acute toxicity of some petrochemicals to gold fish. Water Res 13:623-626

Canton JH, Slooff W (1982) Toxicity and accumulation studies on cadmium with freshwater organisms of different trophic levels. Ecotoxicol and Environ Safety 6:113-128

EIFAC (1980) Report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water. European Inland Fisheries Advisory Commission, technical paper 37, Food and Agricultural Organisation of the United Nations, Rome

Hermens J, Leeuwangh P (1982) Joint toxicity of mixtures of 8 and 24 chemicals to the guppy. Ecotoxicol and Environ Safety 6:302-310

Hermens J, Leeuwangh P, Musch A (1984a) Quantitative structure-activity relationships and mixture toxicity studies of chloro- and alkylanilines at an acute lethal toxicity level to the guppy. Ecotoxicol and Environ Safety 8:388-394

Hermens J, Canton JH, Janssen P, De Jong R (1984b) Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: Acute lethal and sublethal toxicity to Daphnia magna. Aquatic Toxicol 5: 143-154

Könemann H (1981) Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. Toxicology 19:229-238

Slooff W, Baerselman R (1980) Comparison of the usefulness of the

Mexican axolotl and the clawed toad in toxicological bioassays Bull Environ Contam Toxicol 24:439-443
Slooff W, Canton JH, Hermens J (1983) Comparison of the susceptibility of 22 fresh water species to 15 chemical compounds. I. (sub)acute toxicity tests. Aquatic Toxicol 4:113-128

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