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Use of Model Parameter Estimations from Standard Fish Toxicity Tests To Indicate Toxic Mechanisms

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It is presently widely accepted that the prediction of environmental hazards and risks should be based on both exposure and effect assessment. In practice the effect assessment is largely based on laboratory ecotoxicity data. Usually however, no information on the toxic mechanisms is derived from these standard tests, although this would be valuable for ecological risk assessment since it is obvious that it is impossible to carry out detailed mechanistic studies for all chemicals.

In order to predict the toxicity of mixtures of chemicals it is necessary to be able to group chemicals according to their toxic mode of action. Also for predictive methods using QSAR's knowledge about the toxic mode of action is useful, because it is well known that these relations often have limitations related to specific groups of chemicals (e.g. Veith et al., 1990). Thus for various reasons it is important to obtain information on the toxic mode of action of compounds. Also other authors have suggested that the prediction of ecological risk is enhanced by improving the interpretation of standard toxicity test results, (e.g. McKim et al., 1987, Bradbury and Lipnick, 1990, Shirazi and Vaughn Lowrie, 1990, Newman and Aplin, 1992). From the results of ecotoxicological tests usually only simple endpoints are used, such as the LC_{50} after 96 hours of exposure to fish. It is important to realize however, that these tests produce more information about the time-concentration-response relationship.

In this paper an attempt is made to use this additional information to indicate differences in toxic responses. The method is a statistical approach to indicate different toxic response characteristics by deriving parameters from modelling the concentration time-response-surfaces of standard acute toxicity tests with *Brachydanio rerio* HAMB.-BUCH. (zebrafish).

MATERIALS AND METHODS

The chemicals with a narcotic mode of action (Broderius, 1985, Shirazi and Vaughn Lowrie, 1990) were: N,N-dimethylaniline (Fluka, purity: >99.5%), 1-octanol (Fluka, 99.5%), 1-hexanol (Aldrich, 98%). The electrophiles studied were: ethylacrylate (Fluka, >99%), 1-butenal (Aldrich, >99%) and acrylamide (Janssen Chimica >99%). The acetylcholinesterase inhibitors tested were malathion (Synthalyse, 98%), parathion (Synthalyse, 99%) and diazinon (Synthalyse, 99%). The potassium bichromate was from Baker (p.a.).

For each chemical a standard acute toxicity test was carried out with *B. rerio* according to the EEC guideline (1989). Test solutions were prepared with Dutch Standard Water (pH=8, bicarbonate hardness 1.4 meq/l). Test conditions were semi-static, the test solutions of each chemical were freshly prepared at least once in two days.

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solutions of the electrophiles were freshly prepared each day. To minimize volatilization of the narcotics the aquaria were covered with a glass-plate and the solutions were not aerated. Total Organic Carbon analyses showed a recovery of 1-hexanol of 96.4% after 46 hours, and a recovery of 1-octanol of 92.2% after 24 hours. Mortality was scored at least three times a day.

The following multinomial model was applied on the mortality data of the acute toxicity tests:

$K_1 = P_2 - P_1$ with

$$P_2 = \frac{\exp\left(\frac{\frac{a}{t_2} + b - {}^{10}\log c}{\beta}\right)}{1 + \exp\left(\frac{\frac{a}{t_2} + b - {}^{10}\log c}{\beta}\right)} \qquad (1)$$

and

K_1 = probability of mortality in the period of time t_2-t_1
P_2 = probability of survival until time t_2
c = concentration (mg/l)
t = time (hr).

In this model for each concentration $\sum_i K_i = 1$.

Model (1) is a logistic quantal response model that is applied on data of acute toxicity tests (Kooijman, 1981) combined with an empirical based relationship between LC_{50} and time (Ericksen Jones, 1964):

$$^{10}\log LC_{50}(t_2) = \frac{a}{t_2} + b \qquad (2)$$

The parameters in model (1) correspond with the following toxicological characteristics:

a = decrease of the LC_{50} with time
b = $\log\{LC_{50}(\infty)\}$
β = parameter of the decrease of the probability of survival with increasing concentration.

The model was fitted on the mortality data with a maximum-likelihood method. A weighing factor for a multinomial distribution was applied. By fitting the model on the experimental mortality data, the parameters **a**, **b** and **β** were estimated. The parameters **a** and **β** were tested for their potential to indicate the mode of toxic action.

The parameters of model [1] were estimated for the chemicals with a known mode of action, viz. narcotics, electrophiles and acetylcholinesterase inhibitors. At first we used a t-test to test whether estimated values of **a** and **β** for single electrophiles and acetylcholinesterase inhibitors differed significantly from the means of **a** and **β** for the group of narcotics. Secondly we tested whether the three groups of chemicals could be distin-

guished on the basis of the estimated values of **a** and **β**, using a discriminant analysis (SAS®, PROC DISCRIM, SAS, 1990). The input of a discriminant analysis is an a priori classification into groups and the estimated values of one or more parameters of the elements of the given groups. In the analysis a classification criterium is generated for the given groups, based on the input parameter values. Based on this classification criterium the elements are then again categorized into one of the given groups. Comparing this classification result with the a priori classification, one can evaluate the potency of the parameters to discriminate between the groups.

RESULTS AND DISCUSSION

The reproducibility of the parameter estimations was checked for potassium bichromate by analysing the results of four identical standard acute toxicity tests carried out with different populations of *B. rerio* at different periods in time. The results presented in Table 1 show that the values of parameter **a** show less variation than parameter **β**, but both parameters are within a relatively limited range for all four tests.

Table 1. Parameter estimations with standard deviations of acute toxicity tests with potassium bichromate. The tests have been carried out at different periods in time with different populations of *B. rerio*.

test	b (mg/l)	a (mg*hr/l)	β
1	2.14 (0.10)	8.02 (2.89)	0.134 (0.042)
2	2.33 (0.09)	7.98 (3.21)	0.116 (0.039)
3	1.99 (0.08)	6.55 (2.54)	0.087 (0.031)
4	1.99 (0.08)	6.16 (2.30)	0.082 (0.030)

The data obtained in standard acute toxicity tests with the three groups of compounds with *B. rerio* were used to estimate the parameters in model [1]. The basic test results are given in Table 2, the results of the parameter estimations from these tests are summarized in Table 3.
To evaluate the results, at first a t-test was used to distinguish between narcotic and non-narcotic compounds using the two parameters **a** and **β**. The results are presented in Table 4.
The results show that using the parameter **β** estimations not all narcotics could be distinguished from the non-narcotics group, especially the grouping of acetylcholinesterase inhibitors was not consistent. Parameter **a** however, showed for all non-narcotic compounds significant differences from the narcotics group. The results were not improved by using both parameters **a** and **β**. Parameter **a** represents the relationship between response and time of response and indicates the rapidity of change of LC_{50} during the course of the test. Compounds with a low value for **a** (such as narcotics) show a relatively fast response with an LC_{50} value that rapidly after the start of the test already approaches the LC_{50} at t_∞. In fact for these compounds there is no need to prolong the test for 4 days to determine the acute LC_{50} value accurately enough.

Subsequently a discriminant analysis was used to evaluate the potential of the parameters **a** and **β** to categorize the compounds into the three different groups. The results are presented in Tables 5 and 6. Again parameter **β** was not able to discriminate between these groups in all cases, but parameter **a** properly distinguished the compounds into the three groups with different toxic modes of action.

Table 2. Results of the acute toxicity tests with *B. rerio* indicating the number of surviving fish (7 fish treated) at different times (h, vertically) and concentrations (mg/l, horizontally).

1-hexanol

	0	60	89.6	134	200
0	7	7	7	7	7
2	7	7	7	7	0
24	7	7	7	7	0
48	7	7	7	7'	0
72	7	7	7	7	0
96	7	7	7	7	0

1-octanol

	0	15	22.4	33.5	50
0	7	7	7	7	7
2	7	7	7	0	0
24	7	7	0	0	0
48	7	7	0	0	0
72	7	7	0	0	0
96	7	7	0	0	0

N,N-dimethylaniline

	0	40	78.3	153	300
0	7	7	7	7	7
2	7	7	6	0	0
24	7	7	3	0	0
48	7	7	0	0	0
72	7	7	0	0	0
96	7	7	0	0	0

ethylacrylaat

	0	3.16	4.64	6.81	10
0	7	7	7	7	7
2	7	7	7	7	7
24	7	7	7	7	7
48	7	7	7	0	0
72	7	7	0	0	0
96	7	6	0	0	0

acrylamide

	0	70	107	164	250
0	7	7	7	7	7
2	7	7	7	7	7
24	7	7	7	7	7
48	7	7	7	5	0
72	7	7	7	0	0
96	7	7	2	0	0

1-butenal

	0	0.6	1.08	1.94	3.5
0	7	7	7	7	7
2	7	7	7	7	7
24	7	7	6	7	0
48	7	7	6	0	0
72	7	7	6	0	0
96	7	7	0	0	0

parathion

	0	0.36	0.61	1.03	1.76	3
0	7	7	7	7	7	7
2	7	7	7	7	7	7
24	7	7	7	7	7	3
48	7	7	7	7	7	0
72	7	7	7	7	6	0
96	7	7	7	7	6	0

malathion

	0	1.59	2.99	5.64	10.6	20
0	7	7	7	7	7	7
2	7	7	7	7	7	7
24	7	7	7	7	3	0
48	7	7	7	7	3	0
72	7	7	7	7	3	0
96	7	7	7	7	3	0

diazinon

	0	5	8.43	14.23	24
0	7	7	7	7	7
4	7	7	7	7	4
24	7	7	7	7	0
48	7	7	7	7	0
71	7	7	7	3	0
96	7	7	6	0	0

Table 3. Parameter values obtained by modelling the test results of *B. rerio* for three groups of compounds.

test compound	b* (mg/l)	a** (mg*hr/l)	β***	LC_{50}(96) (mg/l)
narcotics				
1-hexanol	2.16 (0.33)	0.051 (0.044)	0.011 (0.019)	145
1-octanol	1.24 ($5.76*10^{-8}$)	0.386 ($5.42*10^{-8}$)	0.008 ($5.12*10^{-8}$)	17.5
N,N-dimethyl aniline	1.70 (0.065)	0.777 (0.159)	0.094 (0.023)	51.1
electrophylic compounds				
ethyl-acrylate	0.425 (0.136)	13.6 (4.96)	0.180 (0.068)	3.69
acrylamide	1.88 (0.15)	17.5 (7.79)	0.235 (0.104)	115
1-butenal	-0.144 (0.106)	11.4 (3.32)	0.155 (0.043)	0.944
acetylcholinesterase inhibitors				
parathion	0.307 (0.063)	4.49 (2.19)	0.080 (0.030)	2.26
malathion	0.987 (0.028)	1.70 (0.19)	0.074 (0.028)	10.1
diazinon	1.166 (0.085)	2.17 (1.71)	0.189 (0.027)	15.4

b* $\log[LC_{50}(\infty)]$
a** decrease of the LC_{50} with time
β*** parameter of the decrease of the probability of survival with increasing concentration.

Summarizing, the results show that parameter **a** (decrease of LC_{50} with time) successfully categorized the nine tested chemicals into three groups of different toxic modes of action. This parameter therefore shows a potential to indicate chemicals with unknown toxic mode of action.

Tabel 4. Results of the t-test for parameters a and β for narcotics and non-narcotics (right sided test, level of significance 0.95).

test compound	a	t-test a	β	t-test β
malathion	1.70	+	0.074	-
parathion	4.49	+	0.080	-
diazinon	2.17	+	0.189	+
acrylamide	17.5	+	0.235	+
1-butenal	11.4	+	0.155	+
ethylacrylaat	13.6	+	0.180	+

+ = significantly different from the mean of the narcotics group
- = not significantly different.

Table 5. Results of the discriminant analysis with posterior probabilities (SAS, Proc DISCRIM) for three groups of compounds, using parameter a.

test compound	a priori group	classified in group	posterior probability membership group 1	2	3
1-hexanol	1	1	0.93	0.00	0.07
1-octanol	1	1	0.94	0.00	0.06
N,N-dimethylaniline	1	1	0.86	0.00	0.14
ethylacrylate	2	2	0.00	1.00	0.00
acrylamide	2	2	0.00	1.00	0.00
1-butenal	2	2	0.00	1.00	0.00
malathion	3	3	0.01	0.00	0.99
parathion	3	3	0.00	0.01	0.99
diazinon	3	3	0.00	0.00	1.00

Group 1 = narcotics, group 2 = electrophylic compounds, group 3 = acetylcholinesterase inhibitors.

In this paper, it was demonstrated that a parameter derived from modelling the concentration-time-response relationship of acute fish toxicity tests, corresponding with the time-course of mortality may be indicative to the toxic mode of action of the tested chemicals. Using this parameter, reactive chemicals could be distinguished from narcotic chemicals. This parameter was also able to discriminate between chemicals with a narcotic and an electrophylic toxic mode of action and acetylcholinesterase inhibitors. In this study no distinction could be made between narcotics and polar narcotics. Narcotics could not be distinguished from all tested reactive chemicals using a parameter corresponding with the slope of the concentration-response curve. The indicative value of this parameter therefore seems to be limited.

Table 6. Results of the discriminant analysis with posterior probabilities (SAS, Proc STEPDISC) for three groups of compounds, using parameter β.

test compound	a priori group	classified in group	posterior probability membership group 1	2	3
1-hexanol	1	1	0.86	0.00	0.14
1-octanol	1	1	0.87	0.00	0.13
N,N-dimethylaniline	1	3*	0.34	0.11	0.55
ethylacrylaat	2	2	0.02	0.67	0.31
acrylamide	2	2	0.00	0.91	0.09
1-butenal	2	2	0.05	0.49	0.46
malathion	3	1*	0.49	0.05	0.46
parathion	3	3	0.44	0.07	0.49
diazinon	3	2*	0.01	0.73	0.26

Group 1 = narcotics, group 2 = electrophylic compounds, group 3 = acetylcholinesterase inhibitors, * = misclassified observation.

Some authors suggest that in standard acute toxicity tests a short time interval to mortality is an indication that the chemical tested has a narcotic mode of action (e.g. Broderius and Kahl, 1985). Our experiments support these observations. A steep slope of the concentration-response curve has also been considered to indicate a narcotic mode of action (e.g. Broderius, 1985). In our experiments the chemicals with a narcotic mode of action also showed a relatively steep slope of the concentration-response curve, but the steepness was not exclusively characteristic for this mode of action.

The results of our experiments are also in accordance with a study of Shirazi and Vaughn Lowrie (1990). In their study, the four parameters of a deterministic model were graphically determined from a fitted surface. Two parameters corresponded with the form of the surface and two parameters with the scale of the surface. A calculated 'form-parameter for time-response' of a sub-group of 44 narcotics differed significantly from the form-parameter of 470 organic chemicals. A calculated 'form-parameter for dose-response' of the sub-group of narcotics differed not significantly.
In the study presented in this paper a stochastic model was used, based on the multinomial distribution of the probability of mortality of individuals in specified periods of time (Kooijman, 1981). This model is expected to be more appropriate to describe mortality in quantal response tests than a deterministic model, such as applied by Shirazi and Vaughn Lowrie (1990). Furthermore in the maximum-likelihood estimation of the parameters used in this study, a correct weighing factor was applied. Another advantage of the estimation method applied here is that it also provides a standard deviation of the estimated parameters that can be used in the evaluation of the results.

Another method to distinguish between narcotics and non-narcotics could be the use of QSAR's as an indicator of the toxic mode of action, comparing it to an $LC_{50}(t)$-value calculated from a QSAR for narcotics (Veith and Broderius, 1990). This is based on the assumption that all organic compounds produce a minimum toxicity related to the log K_{ow} (e.g. Ferguson, 1939, Könemann, 1980, Hermens and Leeuwangh, 1982, Veith et al., 1983, Veith and Broderius, 1990) and that an additional toxicity indicates reactive compounds. The method, however, is not applicable to chemicals for which a log K_{ow} is not available, such as surfactants. In that case an alternative approach is needed.

Preliminary results with the method presented here for some chemicals with unknown toxic mode of action showed that potassium bichromate and some ethyleneamines were classified as non narcotics. Several aminoxide surfactants were classified as narcotics, a mono-and a dialkyl quaternary ammonium surfactant as non-narcotics.

The results presented in this paper suggest the potential use of a statistical modelling approach to indicate toxic modes of action as was also indicated by Shirazi and Vaughn Lowrie (1990). It was also emphasized that standard toxicity tests produce more information than is mostly used. It must be realized however, that the method presented is a correlative indication and does not represent a causal relationship. In reality parameter **a** can be considered as the result of all processes leading to a toxic response, including uptake, elimination and biotransformation kinetics. For instance the elimination rates have shown to be important in determining the time to reach a toxic response for chemicals with a high log K_{ow} (e.g. Opperhuizen and Sijm, 1990).
This also stresses that the potential use of this method in practice should be further evaluated by testing more chemicals within the 3 groups, by testing chemicals with other toxic modes of action and eventually by testing with other species and involving the uptake and elimination kinetics.

REFERENCES

Bradbury SP, Lipnick RL (1990) Introduction: structural properties for determining mechanisms of toxic action. Environ Health Persp 87:181-182.

Broderius S, Kahl M (1985): Acute toxicity of organic chemical mixtures to the fathead minnow. Aquat Tox 6:307-322.

European Economic Communities (1989): Methods for the determination of ecotoxicity C1 Acute toxicity for fish (Updated Version 11/1989). EEC Directive 79/831, Annex V Part C. Brussels Belgium

Erickson Jones JR (1964): Fish and river pollution. Butterworths, London.

Ferguson J (1939): The use of chemical potentials as indices of toxicity. Proc R Soc London (Ser B) 127:387-404.

Hermens J, Leeuwangh P (1982): Joint toxicity of mixtures of 8 and 24 chemicals to the Guppy (*Poecilia reticulata*). Ecotox Environ Saf 6:302-310.

Kooijman SALM (1981): Parametric analyses of mortality rates in bioassays. Water Res 15:107-119.

Könemann H (1980): Structure- activity relationships and additivity in fish toxicities of environmental pollutants. Ecotox Environ Saf 4:415-421.

McKim JM, Bradbury SP, Niemi GJ (1987): Fish acute toxicity syndromes and their use in the QSAR approach to hazard assessment. Environ Health Persp 171-186.

Newman MC, Aplin MS (1992): Enhancing toxicity data interpretation and prediction of ecological risk with survival time modelling: an illustration using sodium chloride toxicity to mosquitofish (*Gambusia holbrooki*). Aquat Toxicol 23:85-96.

Opperhuizen A, Sijm DTHM (1990): Bioaccumulation and biotransformation of polychlorinated dibenzo-p-dioxins and dibenzofurans in fish. Envir Toxicol Chem 9: 175-186.

SAS Institute Inc (1990): SAS® User's Guide Version 6. Fourth Edition Volume 2. Cary, NC., 846 pp.

Shirazi MA, Vaughn Lowrie L (1990): A probabilistic statement of the structure activity relationship for environmental risk analysis. Arch Environ Contam Toxicol 19:597-602.

Veith GD, Call DJ, Brooke LT (1983): Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can J Fish Aquat Sci 40:743-748.

Veith GD, Broderius SJ (1990): Rules for distinguishing toxicants that cause type I and type II narcosis syndromes. Environ Health Persp 87:207-211.