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Structure–Activity Relationships and Additivity in Fish Toxicities of Environmental Pollutants[1]

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The first part of this paper will deal with the relationships between physicochemical properties and toxicity to fish, for two groups of chemicals. The results of this part will be used in discussing the toxicity of mixtures of aquatic pollutants to fish. The toxicity studies used in this paper were LC50 determinations with 2- to 3-month-old guppies (*Poecilia reticulata*), which were exposed to the toxicants in a static system, with daily renewal of the water, without aeration. Most experiments lasted 14 days, a number were restricted to 7 days (Könemann, 1979).

QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS

The relationship between physicochemical properties and toxicity has been studied with the Hansch approach (Hansch, 1971). He used an empirical equation with several variables to describe quantitative Structure–activity relationships (QSARs). His general equation is

$$\log 1/C = k_1 \log P_{\text{oct}} + k_2(\log P_{\text{oct}})^2 + k_3 \text{p}K_a + k_4 E_s + \ldots + k_5, \qquad (1)$$

where in this equation C is the concentration to produce the standard response (e.g., LC50); P_{oct}, the partition coefficient of a chemical for the distribution between n-octanol and water; K_a, the acid dissociation constant; E_s, a steric parameter; the coefficients k_n are obtained by fitting the equation to the experimental data.

P_{oct} is the dominating parameter in QSAR research. In this work we have always used P_{oct} values which are calculated after Rekker (1977), using his hydrophobic fragmental constants. In our opinion these values are better than experimental ones for calculated log P_{oct} values >4 (Könemann *et al.*, 1979).

The first LC50s determined in this study were those of benzene and its chloro derivatives. The most simple QSAR gave a good description of their toxicity:

$$\log 1/\text{LC50} = 0.845 \log P_{\text{oct}} - 4.63, \quad n = 12, r = 0.980, s = 0.133, \qquad (2)$$

where in this and the following QSARs n is the number of chemicals for which the equation has been calculated, r is the correlation coefficient, and s is the standard deviation, indicating the distribution around the line.

Hexachlorobenzene has been left out of the calculation, because it did not produce any mortality, even at very high concentrations. This QSAR appeared to

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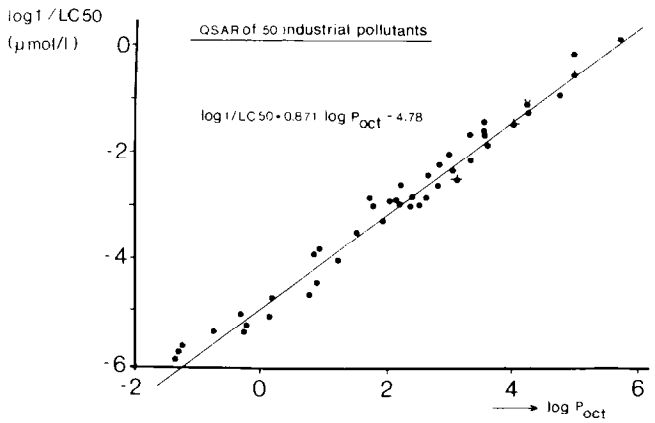

FIG. 1. Quantitative structure–activity relationship for 50 industrial pollutants.

give good predictions of the toxicity of some other compounds we were testing, like acetone and diethylether, suggesting a wide applicability of this QSAR. To determine the range of applicability, the following compounds were tested:

11 (chloro) toluenes
19 aliphatic chlorohydrocarbons (C_1–C_5)
4 alcohols (C_2–C_5)
9 glycolderivatives
5 other related compounds

Structurally these chemicals do not have much in common. However, all of them are rather stable, nonionizable chemicals, and well-known environmental pollutants. Pesticides were excluded from this selection. For 38 of the 48 additionally tested compounds a good agreement was found between the predicted LC50 and the experimental value. Together with the chlorobenzenes they form the next QSAR:

$$\log 1/\mathrm{LC50} = 0.871 \log P_{\mathrm{oct}} - 4.87, \quad n = 50, r = 0.988, s = 0.237. \quad (3)$$

The coefficients of this equation have hardly changed with this expansion. A graphical representation is given in Fig. 1.

The range of toxicities of these chemicals is very large, varying from less than 1 μmol/liter for pentachlorobenzene ($\log P_{\mathrm{oct}} = 5.69$) to 0.5 mol/liter for diethyleneglycol ($\log P_{\mathrm{oct}} = -1.30$). Of course it was not possible to calculate the LC50 for every chemical using this QSAR. One exception has already been mentioned: hexachlorobenzene, which did not produce mortality in concentrations far above its maximal water solubility. Therefore it is probably an example of cutoff, a phenomenon which is well known and has been described by Ferguson (1939). Some other exceptions are toluenes, which are chlorinated at the methyl group, and partially unsaturated chlorohydrocarbons, which are all considerably more toxic than calculated with Eq. (1). Cyclohexane was the only chemical which was significantly less toxic than calculated.

This QSAR probably predicts a minimum toxicity. With perhaps a few exceptions every hydrophobic chemical in the investigated $\log P_{\mathrm{oct}}$ range (−1 to 6),

which is nonionizable and fairly stable, should be at least as toxic as predicted by Eq. (2). The toxic action is nonspecific and may be related to membrane perturbation (Seeman, 1972). If hydrophobic chemicals are more toxic, this nonspecific toxicity is masked by another effect, which emerges at lower concentrations. It is reduced to a secondary site of action. Or, in other words, every hydrophobic chemical has a site of action for which the toxic response is quantitatively described by this QSAR, but it is not always the primary site of action. In fact the success of this simple QSAR and its wide applicability is not very surprising, for this nonspecific toxicity of hydrophobic compounds has already drawn the attention of Meyer (1899) and Overton (1899), Ferguson (1939), and several others.

To study the influence of ionization on QSARs, the LC50s of phenol and 10 chlorophenols were determined. It is beyond the scope of this paper to discuss this subject in detail and we will, therefore, only give the QSARs at two different pH values (Könemann, 1979).

$$\text{pH 7.8: } \log 1/\text{LC50} = 1.12 \log P_{\text{oct}} + 0.43\ \text{p}K_a - 8.35, \quad r = 0.981,\ s = 0.102; \tag{4}$$

$$\text{pH 6.1: } \log 1/\text{LC50} = 0.71 \log P_{\text{oct}} - 0.03\ \text{p}K_a - 3.20, \quad r = 0.992,\ s = 0.101. \tag{5}$$

A clear difference is found between the QSARs at the two pH values and the difference in $\text{p}K_a$ coefficient is particularly striking.

MIXTURE TOXICITY

It is obvious that the toxicity of polluted waters, in which thousands of chemicals occur, cannot be described completely by the toxicity of each separate chemical. Studying the toxicity of mixtures is therefore a necessity, if we want to understand and fight the water pollution problem. Studies on the toxicity of mixtures, however, are usually restricted to combinations of two chemicals. In this section a few aspects of toxicity experiments using mixtures of many chemicals have been discussed and some experimental results are given.

Many terms and definitions are used in this field and this often leads to confusion. We will begin with a short introduction to the concepts used here, starting with the classification of Plackett and Hewlett (1952). They divide the joint action of two toxicants into four classes. First, the action is called similar if the toxicants have the same site of action, or dissimilar if the sites of action differ. Second, a division has been made into interactive and noninteractive joint actions. When a joint action is interactive, this means that a chemical affects the amount of another chemical reaching its site of action, or the changes induced by the other chemical at the site of action.

These types of joint action form Plackett and Hewlett's starting point for their mathematical treatment of dose–response relationships with combinations of two chemicals. The advantage of their classification, compared to other approaches, is that it indicates the type of joint action, not the result. We will discuss the types of multiple mixtures for which the toxicity can be predicted.

	Similar joint action	Dissimilar joint action
Interaction absent	Simple similar action	Independent action
Interaction present	Complex similar action	Dependent action

FIG. 2. Plackett and Hewlett's classification of joint toxic action, with respect to quantal responses.

SIMPLE SIMILAR ACTION

The chemicals have the same site of action and do not show interaction as described above. These conditions can be theoretically fulfilled for mixtures of many chemicals. The joint action is quantitatively characterized by the ability to replace a certain proportion of the LC50 of a chemical, without changing the response. So 50% mortality occurs when

$$\sum_{i=1}^{n} C_i/\mathrm{LC50}_i = 1. \tag{6}$$

This procedure is called concentration addition (Anderson and Weber, 1975) and implies that every fraction of a chemical, however small it may be, can contribute to the toxicity of the mixture. This conflicts with the idea of no-effect levels.

INDEPENDENT ACTION

The chemicals have different sites of action and do not affect each other's action in any way. A characteristic of this model is that effects only have to be summed. The corresponding procedure is therefore called response addition (Anderson and Weber, 1975). Response addition actually represents a variety of procedures, because an additional variable influences its result, namely the possibility of correlation between the susceptibilities of the individual animals (the tolerance correlation) with respect to the differently acting chemicals (Plackett and Hewlett, 1948). For mixtures of many chemicals this correlation can vary from completely positive to zero ($r = +1$ to 0). Positive correlation means that the individual animals of a population which are most susceptible to one of the chemicals in the mixture, are also more susceptible to the other chemicals. When $r = 0$, such a correlation is absent, of course. The two extreme cases, $r = 0$ and $r = +1$, will be discussed.

In the case of response addition with $r = 0$, the probability of survival after exposure to a mixture, Q_{mix}, can be obtained by multiplying the probabilities of survival for each of the separate compounds in the mixture Q_i (Finney, 1971):

$$Q_{\mathrm{mix}} = Q_1 \times Q_2 \times Q_3 \times \ldots \times Q_n. \tag{7}$$

The probability of survival is used in this equation instead of the usual probability of mortality, because this leads to a more conveniently ordered equation. One example may illustrate this formula: When a mixture, consisting of 100 chemicals with identical Q_i values, produces 50% mortality in a population of fishes ($Q_{\mathrm{mix}} = 0.5$), the probability of survival after exposure to the separate chemcials will be 0.993 ($\approx$0.7% mortality). This corresponds with a concentration of about 0.6 × LC50, at concentration response curves with a usual slope. However, the reliability of concentration–response curves is very poor in this region, making it very difficult to test this model in practice. For response addition with $r = +1$ the

toxicity is determined by the chemical, which is present in the most toxic concentration. This special case of response addition will be called no addition in this paper.

All forms of response addition have one thing in common: if a chemical does not produce any response when it is present alone in a certain concentration, that concentration does not contribute to the toxicity of a mixture. Consequently this model does not affect the value of no-effect levels of separate chemicals, applied to mixtures, in contrast to simple similar action. Therefore independent action (of which no addition is a particular case) is not a very problematic type of joint action.

COMPLEX SIMILAR ACTION AND INDEPENDENT ACTION

Even for pairs of chemicals these interactive types of joint action cannot be described in simple formulas. As the interactions between different pairs will also differ, the results of these types of joint action cannot be calculated theoretically for mixtures of many chemicals in which interactive joint action occurs.

From this discussion of Plackett and Hewlett's classification it appears that the toxicity can be predicted for only two types of joint action, i.e., for simple similar action and for independent action ($r = 1$), with the procedures concentration addition and no addition, respectively. In the mixtures of our experiments, all chemicals were present in equal fractions of their LC50s (for short called equitoxic mixtures). The sum of their fractions in a mixture giving 50% mortality is of course 1 for concentration addition and is n for no addition (where n is the number of chemicals in the mixture), for in that case the concentration of one of the chemicals must be equal to its LC50 and therefore all are. Thus the next scale is formed, which can be used as a reference, when discussing the results of mixture toxicity experiments:

	no addition	concentration addition
$\sum C_i/LC50_i \leftarrow$	n	1

This scale can be replaced by a more sophisticated one (H. Könemann, 1979), but that will not be necessary in this paper. In our experimental work most attention has been paid to mixtures which are expected to give concentration addition. One mixture, however, was composed using chemicals with very diverse modes of action. It consisted of:

K^+ (KCl)	1,2,4-trichlorobenzene
Cu^{2+} ($CuCl_2$)	α,α'-dichloro-*m*-xylene
decamethrin OMS-1998	2,4-dichloroaniline
triphenyltinchloride	hexachlorobutadiene
3,4,5-trichlorophenol	

It is impossible to classify this mixture in Plackett and Hewlett's scheme, thus the toxicity of this mixture cannot be predicted. The next toxicity was found:

	no addition		concentration addition	
$\sum C_i/LC50_i \leftarrow$	9		2.5	1
		this mixture	↑	

This mixture is clearly more toxic than follows from no addition, but it is less toxic

TABLE 1

RESULTS OF SOME MIXTURE TOXICITY EXPERIMENTS

	$\sum C_i/LC50_i$	$C_i/LC50_i$
11 Chlorophenols	1.0	0.09
10 Chlorobenzenes	1.5	0.15
50 Chemicals of Eq. (2)	0.9	0.02

than follows from concentration addition. The same trend is observed in an experiment of the British Water Pollution Research Laboratory (Brown, 1968) with a mixture of Cu^{2+}, Zn^{2+}, NH_3/NH_4^+, CN^-, and phenol. More experiments have to be done to verify whether this result is typical for mixtures of differently acting chemicals. I would not be surprised if it is, if the mixtures consist of a sufficiently large number of chemicals (>10).

To examine the validity of the concentration addition model, mixtures were selected containing putative simple similarly acting chemicals. Although limited information is available about the mode of action of chemicals toward fish, it was not difficult to find chemicals with simple similar action: groups of chemicals of which the toxicity can be described with a good QSAR do probably act simple similarly. The results of toxicity experiments with three of such mixtures are presented in Table 1. The first one consisted of the 11 phenols of Eq. (5) (at pH 6.1); the second one, of 10 chlorobenzenes; and the third one, of the 50 compounds, for which the QSAR plot has been shown (Fig. 1).

In Table 1 the last column indicates the concentrations of the separate chemicals, giving 50% mortality in the mixture. The agreement of the first and third mixture with the expected result for concentration addition ($\sum C_i/LC50i = 1$) is almost perfect and the second one does not deviate much. Therefore it can be concluded that the model of concentration addition is completely valid for mixtures of which the chemicals form a QSAR of acceptable quality. Consequently, no-effect levels of separate chemicals loose their meaning for mixtures. Therefore no-effect levels have to be established for groups of chemicals, when they act simple similarly. The size of these groups is therefore important. The number of phenols for which the toxicity can be predicted with our QSARs is certainly not restricted to 11, at least other phenol derivatives are possible members of this group. The QSAR of the 50 chemicals has a particularly wide applicability, as already mentioned. Even hydrophobic chemicals, which are considerably more toxic than predicted from this QSAR, can, by means of a common secondary site of action, contribute to the toxicity of such a mixture, or, to put it more strongly, every hydrophobic chemical can contribute in any concentration to the toxicity of a mixture with respect to the nonspecific common site of action to which the QSAR of Eq. (2) refers.

Summarizing:

1. Good predictions can be made of the toxicity of a group of phenols and of a group of 50 chemicals of a more varying nature, by means of QSARs. This second QSAR has a very wide applicability.

2. The toxicity of mixtures of many chemicals can only theoretically be predicted when all chemicals act independently, or when all chemicals have a simple similar action.

3. QSARs can be used to select chemicals with a simple similar action. In our

experiments the toxicity of these mixtures follows the concentration addition procedure, which makes no-effect levels for separate chemicals inapplicable for mixtures.

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