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Development of quantitative structure-activity relationships in toxicity prediction of complex mixtures[1]

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ABSTRACT

AIM: To predict the toxicity of mixtures of halogenated benzenes (narcotics). **METHODS**: Acute toxicity test of single chemicals and mixtures was performed using *Photobacterium phosphoreum*. Partition coefficients of mixtures were calculated by a special equation. Quantitative structure-activity relationship (QSAR) studies were carried out based on results of toxicity tests, *n*-octanol/water partition coefficient (K_{ow}), and partition coefficient of mixtures (K_{mix}). **RESULTS**: 1) There was a good relationship between toxicity and $\lg K_{ow}$ for single compounds. 2) QSAR analysis showed a perfect correlation between the calculated K_{mix} and the mixture toxicity for binary mixtures. 3) Using the QSAR model of binary mixtures, the toxicity of other related mixtures containing different composition and fraction was predicted very well. **CONCLUSION**: The toxicity of halogenated benzene mixtures (narcotics) was predicted by QSAR. This kind of study is helpful for assessing the toxicity of narcotic mixtures.

INTRODUCTION

Halogenated benzenes, which are used extensively as intermediates to synthesize pesticides, herbicides, plastic products and drugs, are being introduced into the environment. Discharges of effluents containing halogenated benzenes have resulted in high levels of persistent organic contaminants in the water column, the biota, and the bottom sediment. Pollution of waters reduces the quantity and quality of available aquatic resources on which our food supply, public recreation, tourism industry, and domestic economy depend. Indeed, toxic pollutants may also have a direct deleterious impact on human health. In most cases, water pollution encompasses complex mixtures of organic and inorganic contaminants, which normally have to be (qualitatively) identified and (semi) quantitatively analyzed in order to be able to accurately assess the associated risk. The assessment of combined effects of substances therefore is of increasing significance to toxicologists and health or environmental officials.

In evaluating the combined toxicity of complex mixtures it can be useful to distinguish between chemicals with similar or with dissimilar modes of action. This distinction allows a simple approach to the estimation of the toxicity of a mixture. The toxicity of mixtures of chemicals with similar modes of action can be predicted theoretically on the basis of the concentration-additivity model. Könemann[1] introduced a mixture toxicity index (MTI), Marking[2] yielded both an additive index (AI) and a toxicity enhancement index (TEI), for comparing results of mixture toxicity experiments. However, these indices can not directly predict the combined toxicity of a mixture. The application of quantitative structure-activity relationships (QSAR) is a valuable part of the toxic effect assessment of single chemicals. Many previous studies have found very successful QSAR based on logarithm of *n*-octanol/water partition coefficient ($\lg K_{ow}$) in the field of aquatic toxicology[3-5]. As to mixtures, only a few QSAR on predicting the toxicity of mixture have been reported in the literature[6,7]. However, there has been a lack of relationship between the partition coefficient of a mixture and the toxicity of mixture.

The purpose of this paper was to develop a toxicity assessment method in complex mixtures of halogenated benzenes. First we worked on the model of the toxicity prediction for single substances to bioluminescence of

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Photobacterium phosphoreum. Second, we deduced an equation of the partition coefficient of a mixture. Subsequently, the partition coefficient of the mixture was correlated with the toxicity of mixture. Finally, the predicted results were illustrated by experimental data from other related mixtures.

MATERIALS AND METHODS

Eight halogenated benzenes were purchased from ACROS Organic Inc, Switzerland. The molecular formulas are given in Tab 1.

Tab 1. Molecular formula, *n*-octanol/water partition coefficient ($\lg K_{ow}$) and toxicity (pEC_{50}) for 8 halogenated benzenes.

No	Compounds	$\lg K_{ow}$	pEC_{50}		
			Expt[a]	Eq2[b]	Difference[c]
1	Benzene	2.13	2.60	2.89	0.29
2	Chlorobenzene	2.84	3.70	3.51	0.19
3	Bromobenzene	2.99	3.77	3.64	0.13
4	1,4-dichlorobenzene	3.55	4.18	4.13	0.05
5	1-bromo-4-chlorobenzene	3.70	4.45	4.26	0.19
6	1,4-dibromobenzene	3.85	4.42	4.40	0.02
7	1,2,3-trichlorobenzene	4.26	4.68	4.75	-0.07
8	1-bromo-2,3-dichlorobenzene	4.41	4.67	4.89	-0.22

[a] Experimental toxicity, [b] Predicted toxicity by Eq2, [c] Experimental toxicity - predicted toxicity.

For testing, separate stock solutions of each chemical were prepared at approximately two folds of the EC_{50} ($mol \cdot L^{-1}$) of chemicals. All stock mixtures were prepared in different toxic concentrations (different fractions of EC_{50}) based on experimental EC_{50} values. The composition of the mixtures is given below. The numbers correspond with that given in Tab 1.

Mixture 1: 1 - 6; Mixture 2: 6 - 3; Mixture 3: 6 - 4; Mixture 4: 6 - 7; Mixture 5: 6 - 2; Mixture 6: 5 - 6; Mixture 7: 6 - 8; Mixture 8: 2 - 5; Mixture 9: 1 - 8; Mixture 10: 2 - 4 - 7; Mixture 11: 4 - 5 - 6; Mixture 12: 2 - 3 - 4 - 6; Mixture 13: 4 - 6 - 5 - 8; Mixture 14: 3 - 4 - 6 - 5 - 7; Mixture 15: 3 - 4 - 5 - 6 - 7 - 8; Mixture 16: 1 up to and including 8.

For mixture partitioning of different partitioning behavior (different $\lg K_{ow}$), the equation derived by Verhaar in 1995[8] has been used to calculate partition coefficients of mixtures, which can be described as following:

$$K_{mix} = \frac{W}{V} \times \frac{\sum_{i=1}^{n} \frac{Q_i}{1 + \frac{W}{VK_i}}}{\sum_{i=1}^{n} Q_i - \sum_{i=1}^{n} \frac{Q_i}{1 + \frac{W}{VK_i}}} \quad (1)$$

Where K_{mix} is the partition coefficient of the mixture, W is the volume of the aqueous phase, V is the volume of the lipid phase, n is the number of compounds in the mixture, Q_i is the total amount of compound i in the system and K_i is the partition coefficient of compound i. In this study, W/V is equal to 6.8×10^5.

The Microtox test: Ecotoxicological descriptors are the concentration values causing 50 % inhibition of bioluminescence of *Photobacterium phosphoreum* after 15 min exposure, expressed as 15 min EC_{50}($mol \cdot L^{-1}$).

The Microtox test instrument (model toxicity analyzer DXY-2) was made by the Institute of Soil Science, Academia Sinica, Nanjing. The 15 min EC_{50} values were performed at 20 ℃ according to the procedures described in the Instrument Manual. The chemicals were dissolved in water with an ultrasonic device. Acetone was used as a cosolvent for compounds slightly soluble in water. The concentration of acetone never exceeded 0.1 $mL \cdot L^{-1}$ of the experimental solution. Five concentrations of each chemical were tested in three replicates. The toxicity of mixutes was determined in the following manner. The concentration of the stock mixtures was assumed one hundred percent (100 %). The stock mixtures were diluted to five concentrations for the toxicity test. They were also measured in three replicates. The unit of 15 min EC_{50} was represented as a percentage (%).

Linear regression analyses were performed using the STATGRAPHICS software (STSC, Inc, 1987). The negative logarithm of EC_{50}(pEC_{50}) acted as the dependent variable, and the quantifier of hydrophobicity acted as the independent variable in QSAR study. Quality of the model was characterized by the number of observation (n), the square of correlation coefficient (r^2), the standard error of estimate (SE), the Fisher criterion (F), and the significance level (P).

RESULTS AND DISCUSSION

QSAR analysis for single compound by regression The determined toxicity values of single compounds have been transformed to the negative logarithm of EC_{50}(pEC_{50}), and are listed in Tab 1. A good correlation equation was obtained:

$$pEC_{50} = 0.876 \lg K_{ow} + 1.022 \quad (2)$$

$n = 8$, $r^2 = 0.934$, $s_{\bar{x}} = 0.194$, $F = 84.525$, $P < 0.05$

It can be seen from equation 2 that with an increase in $\lg K_{ow}$, pEC_{50} increases. This result indicates that the more toxic a halogenated benzene is, the greater the pEC_{50} value is. In addition, the determined pEC_{50} values are very close to the predicted toxicity (Tab 1). In brief, these halogenated benzenes have the same toxic action mode, and the acute toxicity to *Photobacterium phosphoreum* depends on their hydrophobicity.

Calculation of the partition coefficient for mixtures For mixtures of halogenated benzenes that act as narcosis, there is no simple distribution coefficient linking aqueous concentration to the hydrophobic phase concentration. The partition coefficient of mixtures is related not only to the hydrophobicity but also to the fraction of individual compounds in mixtures. The calculated results are given in Tab 2.

Tab 2. The partition coefficient of mixtures.

Mixture number[a]	Ratio	$\lg K_{mix}$	Mixture number[a]	Ratio	$\lg K_{mix}$
1	1:1	3.06	6	1:1	4.47
	1:0.5	2.96		1:0.5	4.44
	0.5:1	3.21		0.5:1	4.49
2	1:1	4.00	7	1:1	4.80
	1:0.5	4.13		1:0.5	4.89
	0.5:1	3.88		0.5:1	4.71
3	1:1	4.36	8	equitoxic	3.94
	1:0.5	4.41	9	equitoxic	3.04
	0.5:1	4.32	10	equitoxic	4.08
4	1:1	4.71	11	equitoxic	4.37
	1:0.5	4.65	12	equitoxic	3.91
	0.5:1	4.78	13	equitoxic	4.55
5	1:1	3.91	14	equitoxic	4.24
	1:0.5	4.06	15	equitoxic	4.36
	0.5:1	3.77	16	equitoxic	3.52

[a] Given in the section of materials and methods.

Prediction of the toxicity of mixtures by QSAR A more theoretical approach begins with the distinction between four types of combined action: simple similar, independent, complex similar, and dependent action[9]. The toxicity of mixtures is often assessed by mixture toxicity index (MTI), additive index (AI), and toxicity enhancement index (TEI). These indices can not directly predict the EC_{50} or LC_{50} value of mixtures. It is therefore very useful for predicting the mixture toxicity to derive the partition coefficient of mixtures. In this paper, we only studied the toxicity of halogenated benzene mixtures.

It has largely been assumed that the toxicity of mixtures of narcotic toxicants is additive[10]. However recent research[11,12] has revealed that toxic additivity does not always occur. Thus, for the toxicity prediction of mixtures, we may not care which toxic action mode these chemicals have in mixtures. We only care whether they belong to the same toxic action class in a single component. The mixture's EC_{50} is defined as EC_{50}^{M}, which can be calculated as (equation 3):

$$EC_{50}^{M} = EC_{50}^{1} + EC_{50}^{2} + \cdots + EC_{50}^{n} = \sum_{i=1}^{n} EC_{50}^{i} \quad (3)$$

EC_{50}^{i} is the concentration value of compound i in a mixture causing 50 % inhibition of bioluminescence of *Photobacterium phosphoreum* after 15 min exposure. Since the determined EC_{50} of mixtures is given as a percentage concentration, EC_{50}^{i} is calculated based on the product of the determined EC_{50} of a mixture and the concentration (C_i) of compound i in a stock mixture. The EC_{50}^{M} values of mixtures have been transformed to the negative logarithm of EC_{50}^{M} (pEC_{50}^{M}), and are listed in Tab 3.

Tab 3. The toxicity of binary mixtures.

Mixture number[a]	Ratio	pEC_{50}^{M} Exptl[b]	pEC_{50}^{M} Eq4[c]	Difference[d]
1	1:1	2.96	3.08	−0.12
	1:0.5	2.86	2.98	−0.12
	0.5:1	3.08	3.21	−0.13
2	1:1	4.02	3.94	0.08
	1:0.5	4.11	4.05	0.06
	0.5:1	4.00	3.83	0.17
3	1:1	4.34	4.26	0.08
	1:0.5	4.32	4.31	0.01
	0.5:1	4.28	4.23	0.05
4	1:1	4.62	4.59	0.03
	1:0.5	4.51	4.53	−0.02
	0.5:1	4.60	4.65	−0.05
5	1:1	3.96	3.85	0.11
	1:0.5	4.01	3.99	0.02
	0.5:1	3.80	3.73	0.07
6	1:1	4.49	4.37	0.12
	1:0.5	4.45	4.34	0.11
	0.5:1	4.49	4.38	0.11
7	1:1	4.45	4.67	−0.21
	1:0.5	4.53	4.75	−0.22
	0.5:1	4.42	4.59	−0.17

[a] Given in the section of materials and methods, [b] Experimental toxicity, [c] Predicted toxicity by Eq4, [d] Experimental toxicity − predicted toxicity.

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Regression analysis of pEC_{50}^{M} versus $\lg K_{mix}$ for binary mixtures resulted in the following QSAR:

$$pEC_{50}^{M} = 0.275 + 0.915 \lg K_{mix} \quad (4)$$
$$n = 21,\ r^2 = 0.951,\ s_{\bar{x}} = 0.121,\ F = 367.469,\ P < 0.05$$

The F and P values of Eq4 show that the correlation is significant. This equation has a good correlation coefficient and small SE, and the experimental toxicity is very close to the predicted toxicity (Tab 3), so the equation can be used to predict toxicity of related mixtures.

The estimated values of toxicity for other related mixtures are given in Tab 4. The calculated pEC_{50}^{M} values are in good agreement with our experimental values. A perfect correlation is obtained between the predicted pEC_{50}^{M} and the observed pEC_{50}^{M} (equation 5).

$$(pEC_{50}^{M})_{pre} = 0.593 + 0.849\ (pEC_{50}^{M})_{obs} \quad (5)$$
$$n = 9,\ r^2 = 0.952,\ s_{\bar{x}} = 0.101,\ F = 139.741,\ P < 0.05$$

Tab 4. The observed and predicted toxicity of other related mixtures.

Mixture number[a]	Ratio	pEC_{50}^{M} Expt[b]	pEC_{50}^{M} Eq4[c]	Difference[d]
8	0.5:1	4.08	3.88	0.20
9	1:0.5	2.86	3.06	−0.20
10	Equitoxic	4.01	4.01	0.00
11	Equitoxic	4.32	4.27	0.05
12	Equitoxic	3.98	3.85	0.13
13	Equitoxic	4.36	4.44	−0.08
14	Equitoxic	4.19	4.16	0.03
15	Equitoxic	4.27	4.26	0.01
16	Equitoxic	3.40	3.50	−0.10

[a] Given in the section of materials and methods, [b] Experimental toxicity, [c] Predicted toxicity by Eq4, [d] Experimental toxicity − predicted toxicity.

According to above results, we can see that despite of the composition of halogenated benzenes or their fraction in a mixture, the toxicity of a mixture can always be predicted accurately by Eq4. Thereby we can use this equation to predict the toxicity of any mixtures containing halogenated benzenes just by calculations instead of experiment action, and hence large amounts of expenses and time can be saved.

In summary, for halogenated benzenes (narcotics), there is a good relationship between pEC_{50} and $\lg K_{ow}$ in single chemicals. The toxicity of a mixture is also perfectly correlated with the hydrophobicity due to quoting the partition coefficient of mixtures (K_{mix}) in our study. The correlation equation is successfully used to predict the toxicity of the different composition or fraction of mixtures. With this study, we have developed QSAR in the toxicity prediction of complex mixtures, which is helpful for assessing the toxicity of mixtures.

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定量结构-活性关系在混合体系毒性预测中的发展[1]

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关键词 毒性；发光细菌属；苯衍生物；构效关系

目的：预测麻醉型化合物卤代苯混合体系的毒性. **方法**：采用发光细菌对卤代苯的单一体系和混合体系进行毒性试验；利用特定的公式计算了混合物的分配系数；建立了混合体系的定量构效关系. **结果**：(1) 建立了单一化合物的正辛醇/水分配系数与毒性的关系. (2) 建立了二元混合体系的分配系数与毒性之间的定量结构模型. (3) 用二元混合体系的 QSAR 模型预测了其它不同组份和不同毒性比例的混合体系毒性. **结论**：QSAR 能很好地预测麻醉型化合物卤代苯混合体系的毒性，并且有助于该类化合物的混合体系毒性作用的评价.

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