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General Toxicity Prediction Approach for Mixtures Containing Polar Narcotic Chemicals

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It has been widely recognized that aquatic organisms in the environment are usually exposed to multiple rather than single chemicals, and prediction of mixture toxicity is becoming the hottest research interest of environmental scientists. But so far, most of their studies predict the mixture toxicity from the toxicity of individual chemicals, and this prediction is carried out only if that all individual chemicals are known (Verhaar et al. 1995). A general approach for prediction of the toxicity of an unknown mixture is therefore necessary and it will help risk assessment and establishment of ecological criteria.

Introduced by pharmacologists, the QSAR approach has emerged as one of the most promising methods of evaluating the toxicity for single chemicals provided that the adequate physicochemical parameters of single chemicals (such as K_{OW}, α, β, E_{LUMO}, E_{HOMO} and so on) are known. Similarly, if these physicochemical parameters of mixture can be obtained, development of QSAR in the field of mixture will provide a good way to predict the toxicity of the mixture, even without knowing the individual chemicals in it.

In 1995, Verhaar et al. firstly extended K_{OW}, the octanol-water partition coefficient, to the filed of mixture and he described the partition coefficient of a mixture as follows:

$$K_{MD} = \frac{W}{V} \times \frac{\sum_{i=1}^{n} \frac{Q^0_{water,i}}{1 + \frac{W}{VK_{SDi}}}}{\sum_{i=1}^{n} Q^0_{water,i} - \sum_{i=1}^{n} \frac{Q^0_{water,i}}{1 + \frac{W}{VK_{SDi}}}} \qquad (1)$$

Here K_{MD} is the C_{18}-Empore™ disk/water partition coefficient for a mixture. K_{SDi} is the partition coefficient of the individual chemical *i*. W is the volume of solution. V is the volume of hydrophobic phase. Q^0_{water} is the initial amount of chemical *i* in water. *n* is the total number of individual chemicals in the mixture. The value of W/V is suggested as 6.8×10^5.

According to the K_{MD} calculated from Eq.1, a K_{MD}-based approach on mixture

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toxicity prediction was sucessfully proposed in our previous study (Lin et al. 2002). Although this approach can predict the toxicity for an unknown mixture (Lin et al. 2003a), it was only suitable for mixtures containing nonpolar narcotic chemicals. Apparently, this approach can not directly predict the toxicity of mixture pollutants in the real environment because mixture pollutants may also contain polar narcotic chemicals, such as phenol, aniline and so on. Kamlet et al. (1986a) observed that the toxicity of individual polar narcotic chemicals is greater than that of nonpolar narcotic chemicals provided that partition coefficients is the same, and he concluded that the increased toxicity is related to the hydrogen bond donor activity of these structures. Therefore, Kamlet et al. (1986b, 1988) proposed hydrogen donor acidity (α) and hydrogen acceptor (β) in an aim to quantify this increased toxicity, but great difficulty is found in obtaining α and β through laboratory work. Fortunately, Feng et al. (1996) proposed a simplified method to obtain α and β through measurement of Lewis acidity (A) and basicity (B) in Eq.2~3, as strong correlation had been found between (A and B) and (α and β) in Eq.4 and Eq.5,

$$A = \lg K_{bw} - \lg K_{cw} \qquad (2)$$

$$B = \lg K_{chw} - \lg K_{tw} \qquad (3)$$

$$A = 2.78\alpha + 0.23 \qquad (4)$$
$$n=45,\ r=0.980,\ SE=0.139$$

$$B = 0.84\,\alpha + 1.59\,\beta - 0.01 \qquad (5)$$
$$n=45,\ r=0.981,\ SE=0.07$$

K_{bw} is the di-n-butyl ether/water partition coefficients. K_{cw} is the cyclohexane-water partition coefficients. K_{chw} is the chloroform-water partition coefficients. K_{tw} is the carbon tetrachloride-water partition coefficients.

Lin et al. (2003b) pointed out that, for mixtures containing polar narcotic chemicals, the joint effect of toxicity contributed by the hydrogen bond of individual chemicals is concentration additive. Therefore this joint effect of the hydrogen bond in mixtures could be quantitatively determined, provided that the partition coefficients (K_{bw}, K_{cw}, K_{chw}, K_{tw}) in Eq.2 and Eq.3 can be extended to the field of mixtures according to the relationship described by Eq.1.

The purposes of this study are: 1) to determine the toxicity of mixtures containing polar narcotic chemicals to *Vibrio fischeri*, 2) to calculate the K_{MD} for the mixture, and 3) to quantify the joint effect of the hydrogen bond in mixtures by determining K_{bw}, K_{cw}, K_{chw}, K_{tw} of individual chemicals, thus proposing a general approach to predict toxicity of mixtures containing polar narcotic chemicals.

MATERIALS AND METHODS

Five polar narcotic chemicals (Table 1) were purchased in the highest available purity from ACROS Organics Inc, and they are 4-choloro-phenol, phenol, resorcinol, aniline and 3-chloro-aniline. The stock solutions of these chemicals

were prepared in HPLC-grade acetone, stored at –20℃ and used throughout the study. For single chemical toxicity experiment, the stock solutions were redissolved in 3% NaCl solution. For mixture toxicity experiment, the stock solutions of each individual chemical were added in 3% NaCl solution at proportions of their respective EC_{50} values. In this study, binary mixtures were tested at three proportions 0.5:1 1:1 1:0.5 (identical fraction of EC_{50}), ternary mixtures were tested at 0.5:0.5:0.5, quadruple mixtures were tested at 0.5:0.5:0.5:0.5 and quintuple mixtures were tested at 0.4:0.4:0.4:0.4:0.4. The compositions of mixtures are given in Table 2.

The freeze-dried marine bacterium, *Vibrio fischeri* (T_3 mutation), was supplied by the Institute of Soil Science, Academic Sciences, Nanjing PRC. It was reconstituted and maintained on agar slants at 4℃. The bioluminescence assays were performed using the diluted bacteria that had been cultured at 20℃ in yeast-tryptone-salts-gycerol broth for 12~14 h. The toxic test instrument (toxicity analyzer DXY-2) was made by the Institute of Soil Science, Academic Sciences, Nanjing PRC.

Toxicity was measured (with DXY-2) by quantifying the decrease in light emission from the bacteria as a result of exposure to 3% NaCl solution containing the test chemicals for 15 min. The decrease in light emission was measured at six different concentrations and each was tested in triplicate. Based on the decrease in light emission, the median effective concentration (EC_{50}) was calculated using the probit model (Finney 1971). The toxicity of 5 single chemicals was measured and reported as log $1/EC_{50}$ $mol \cdot L^{-1}$ (Table 1). The mixture toxicity tests were conducted in a similar manner as the single chemical tests. Assumed that the initial concentration of the mixture was 100%, the decrease in light emission was measured at six different concentrations, 10%, 18%, 32%, 56%, 80%, 100%, and therefore the median effective concentration was calculated in the unit of percentage (%). The mixture toxicity was quantitatively described by Eq.6 (Preston *et.al* 2000) and the results are listed in Table 2.

$$EC_{50M} = \frac{C_M}{\frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}} + \cdots} \qquad (6)$$

The EC_{50} is the effective concentration required to bring about a 50% decrease in light output. C is the concentration of an individual chemical, and subscripts A, B, ····· and M are the individual chemicals and mixture, respectively. Because the joint effect of these polar narcotic chemicals are concentration additive, the concentration of the individual chemical (C_A, C_B, ·····) could be calculated according to the median effective concentration in the unit of percentage (%), that is C_A, C_B, ····· = percentage % × the initial concentration of individual chemical.

The octanol-water (K_{ow}), di-n-butyl ether/water (K_{bw}), cyclohexane-water (K_{cw}), cholorform-water (K_{chw}) and carbon tetrachloride-water (K_{tw}) partition coefficients were measured by the shake-flask method as described in the OECD Guideline for Testing of Chemicals (1981), followed by centrifugation and analysis of the chemical in the aqueous phase with a UV spectrophotometer against water as the

blank. In addition, based on the PK_a of the single chemicals (Table 1), KH_2PO_4/Na_2HPO_4 (1/30: 1/30, mol·L^{-1}) buffers were used at pH 6.7 so that only the neutral form of the solutes was presented in solution. The results are listed in Table 1. According to Verhaar et al. (1995), the correlation between log K_{OW} and log K_{SD} (C_{18}-Empore™ disk/water partition coefficient of single chemicals) was described by Eq.7 (n=18, r^2=0.93, SE=0.24).

$$\log K_{SD} = 0.995 \log K_{OW} + 0.70 \qquad (7)$$

The $\log K_{SD}$ of the single chemicals studied were calculated using Eq.7 (Table 1). Furthermore, $\log K_{MD}$ $\log K_{mbw}$, $\log K_{mcw}$, $\log K_{mchw}$ and $\log K_{mtw}$ values were calculated using Eq.1 (Table 2). In addition, the joint effect of the hydrogen bond in mixtures were calculated by using Eq.2~3 and are reported as A^{MH}, B^{MH} in Table 2.

Statistical analyses were performed using the SPSS 9.0 software (SPSS Inc.). The coefficient of determination (r^2), standard error (SE), F-ratio, and P value were taken into consideration in testing the quality of the regression.

RESULTS AND DISCUSSION

Relationships of log $1/EC_{50M}$ versus log K_{MD} are derived in the following approach for mixtures containing polar narcotic chemicals,

$$\log 1/EC_{50M} = -0.045 + 1.498 \log K_{MD} \qquad (8)$$

n=35, r^2=0.730, SE=0.224, F=89.671,P=0.000

However, the weak correlation in Eq.8 (r^2=0.730) indicates that this K_{MD}-based approach is not adequate to describe the toxicity of mixtures containing polar narcotic chemicals. As revealed in our previous study (Lin et al. 2003b), for these mixtures, not only their partition coefficients (K_{MD}) but also the joint effect of hydrogen bond in individual chemicals contribute to their mixture toxicity. Therefore, introduction of the A^{MH} and B^{MH} into Eq.8 will improve the quality of the approach.

$$\log 1/EC_{50M} = 4.052 + 0.559 \log K_{MD} - 0.377 A^{MH} - 2.024 B^{MH} \qquad (9)$$

n=35, r^2=0.933, SE=0.115,F=143.825,P=0.000

The value of r^2 (0.933) in Eq.9 indicates that, the log $1/EC_{50M}$ is significantly correlated with both the partition coefficient (K_{MD}) and the hydrogen bond in individual chemicals. However, this correlation is free from the range difference of the hydrophobicity, the hydrogen bond, the ratio or the number of the individual chemicals. Whether the mixtures consist of high hydrophobicity individual chemicals (such as 1#, 4-choloro-phenol, log K_{OW}=2.17) or those with low partition-coefficients (such as 3#, resorcinol, log K_{OW}=0.78), the toxicity of the mixtures can be described by Eq.9. Furthermore, although the effect of the hydrogen bond in resorcinol (3#) is strong (A=4.44, B=1.77) while that in 3-choloro-aniline (5#) is weak (A=0.98, B=0.78), the significant correlation in Eq.9 is also found. In addition, when the toxicity of the binary mixture was tested in variant ratios (0.5:1 1:1 1:0.5, as the ratio of the EC_{50} value), the log EC_{50M} is always highly correlated with both K_{MD} and A^{MH}. This significant correlation is also found for mixtures containing 2~5 individual chemicals.

Table 1. Toxicity and partition coefficients for single chemicals

No.	Single chemicals	$\log 1/EC_{50}$ mol·L^{-1}	$\log K_{ow}$	$\log K_{SD}$	$\log K_{cw}$	$\log K_{bw}$	$\log K_{chw}$	$\log K_{tw}$	A^H	B^H	PKa
1	4-choloro-phenol	4.38	2.17	2.86	0.06	1.57	1.38	0.86	1.51	0.52	9.37[a]
2	phenol	2.95	1.46	2.15	-0.93	1.12	0.37	-0.36	2.05	0.73	9.99[a]
3	resorcinol	3.14	0.78	1.48	-3.79	0.55	-1.34	-3.11	4.44	1.77	10.00[a]
4	aniline	2.29	0.92	1.62	-0.05	0.64	1.42	0.25	0.69	1.17	4.60[b]
5	3-choloro-aniline	3.57	1.63	2.32	0.69	1.67	2.08	1.30	0.98	0.78	3.52[b]

[a]From Dean (1985), [b]From Gao (1997)

Table 2. Toxicity and partition coefficients for mixtures

No.	Individual chemicals in mixtures	xEC_{50}:yEC_{50}	$\log K_{mcw}$	$\log K_{mbw}$	$\log K_{mchw}$	$\log K_{mtw}$	A^{MH}	B^{MH}	$\log K_{MD}$	$\lg 1/EC_{50M}$[mol·L^{-1}]		
										Obs.	Pre.	Diff.
1	1:2	1.0:0.5	-0.72	1.17	0.58	-0.04	1.89	0.62	2.26	3.30	3.35	0.05
2	1:2	1.0:1.0	-0.81	1.15	0.49	-0.17	1.96	0.66	2.21	3.22	3.21	-0.01
3	1:2	0.5:1.0	-0.87	1.13	0.44	-0.25	2.00	0.69	2.18	3.11	3.12	0.01
4	1:3	1.0:0.5	-0.93	0.84	0.40	-0.13	1.77	0.53	2.01	3.35	3.44	0.09
5	1:3	0.5:1.0	-1.49	0.65	-0.15	-0.69	2.14	0.54	1.70	3.28	3.10	-0.18
6	1:4	1.0:0.5	-0.05	0.69	1.42	0.27	0.74	1.15	1.72	2.48	2.41	-0.07
7	1:4	1.0:1.0	-0.05	0.67	1.42	0.26	0.72	1.16	1.67	2.42	2.37	-0.05
8	1:4	0.5:1.0	-0.05	0.65	1.42	0.26	0.70	1.16	1.65	2.41	2.36	-0.05
9	1:5	1.0:1.0	0.64	1.66	2.03	1.26	1.02	0.77	2.44	3.72	3.47	-0.25
10	1:5	0.5:1.0	0.67	1.66	2.05	1.28	0.99	0.77	2.39	3.65	3.46	-0.19
11	2:3	1.0:0.5	-1.05	1.03	0.25	-0.48	2.08	0.73	2.06	2.98	2.94	-0.04
12	2:3	0.5:1.0	-1.29	0.89	0.02	-0.72	2.18	0.74	1.90	2.83	2.79	-0.04
13	2:4	1.0:1.0	-0.12	0.77	1.34	0.19	0.89	1.15	1.77	2.33	2.38	0.05
14	2:4	0.5:1.0	-0.09	0.72	1.38	0.22	0.81	1.16	1.71	2.35	2.35	0.00
15	2:5	1.0:0.5	-0.20	1.22	1.18	0.40	1.42	0.78	2.17	3.10	3.15	0.05
16	2:5	1.0:1.0	-0.02	1.29	1.40	0.62	1.31	0.78	2.19	3.25	3.20	-0.05

Table 2. Continued

No.	Individual chemicals in mixtures	xEC_{50}:yEC_{50}	log K_{mcw}	log K_{mbw}	log K_{mchw}	log K_{mtw}	A^{MH}	B^{MH}	log K_{MD}	$lg1/EC_{50M}[mol \cdot L^{-1}]$		
										Obs.	Pre.	Diff.
17	3:4	1.0:0.5	-0.16	0.62	1.31	0.14	0.78	1.17	1.59	2.38	2.28	-0.10
18	3:4	1.0:1.0	-0.11	0.63	1.36	0.19	0.74	1.17	1.60	2.32	2.30	-0.02
19	3:4	0.5:1.0	-0.08	0.63	1.39	0.22	0.71	1.17	1.61	2.44	2.32	-0.12
20	3:5	1.0:0.5	-0.11	1.01	1.28	0.50	1.12	0.78	1.76	3.20	3.03	-0.17
21	3:5	0.5:1.0	0.32	1.34	1.71	0.93	1.02	0.78	2.03	3.12	3.22	0.10
22	4:5	1.0:0.5	-0.00	0.74	1.46	0.35	0.74	1.11	1.66	2.52	2.45	-0.07
23	4:5	0.5:1.0	0.10	0.92	1.55	0.54	0.82	1.01	1.76	2.52	2.68	0.16
24	1:2:4	0.5:0.5:0.5	-0.12	0.79	1.34	0.20	0.91	1.14	1.81	2.44	2.41	-0.03
25	1:2:5	0.5:0.5:0.5	0.02	1.31	1.40	0.63	1.29	0.77	2.23	3.20	3.25	0.05
26	1:3:4	0.5:0.5:0.5	-0.11	0.65	1.36	0.20	0.76	1.16	1.65	2.48	2.34	-0.14
27	1:3:5	0.5:0.5:0.5	0.12	1.21	1.51	0.74	1.09	0.77	2.01	3.12	3.21	0.09
28	2:3:4	0.5:0.5:0.5	-0.17	0.76	1.29	0.14	0.93	1.15	1.75	2.28	2.35	0.07
29	2:3:5	0.5:0.5:0.5	-0.16	1.15	1.22	0.44	1.31	0.78	2.05	2.93	3.13	0.20
30	2:4:5	0.5:0.5:0.5	-0.03	0.88	1.42	0.36	0.91	1.06	1.82	2.44	2.58	0.14
31	3:4:5	0.5:0.5:0.5	-0.02	0.79	1.44	0.37	0.81	1.07	1.68	2.33	2.52	0.19
32	1:2:3:5	0.5:0.5:0.5:0.5	-0.16	1.16	1.22	0.46	1.32	0.76	2.09	3.06	3.18	0.12
33	1:2:4:5	0.5:0.5:0.5:0.5	-0.03	0.89	1.42	0.37	0.92	1.05	1.84	2.54	2.61	0.07
34	2:3:4:5	0.5:0.5:0.5:0.5	-0.08	0.86	1.37	0.32	0.94	1.05	1.79	2.50	2.57	0.07
35	1:2:3:4:5	0.4:0.4:0.4:0.4:0.4	-0.08	0.87	1.37	0.32	0.95	1.05	1.82	2.54	2.59	0.05

Table 3. Observed and predicted toxicity of 10 other related mixtures

No.	Individual chemicals in mixtures	xEC_{50}:yEC_{50}	log K_{mcw}	log K_{mbw}	log K_{mchw}	log K_{mtw}	A^{MH}	B^{MH}	log K_{MD}	log $1/EC_{50M}$[mol·L^{-1}]		
										Obs.	Pre.	Diff.
1	1:3	1.0:1.0	-1.20	0.73	0.13	-0.40	1.93	0.53	1.83	3.30	3.27	-0.03
2	1:5	1.0:0.5	0.60	1.65	1.99	1.22	1.05	0.77	2.52	3.60	3.51	-0.09
3	2:4	1.0:0.5	-0.18	0.85	1.28	0.14	1.03	1.14	1.86	2.50	2.40	-0.10
4	2:5	0.5:1.0	0.22	1.38	1.61	0.83	1.16	0.78	2.21	3.11	3.27	0.16
5	3:5	1.0:1.0	0.12	1.18	1.51	0.73	1.06	0.78	1.90	3.22	3.14	-0.08
6	4:5	1.0:1.0	0.04	0.81	1.49	0.43	0.77	1.06	1.70	2.58	2.57	-0.01
7	1:2:3	0.5:0.5:0.5	-1.02	1.00	0.29	-0.37	2.02	0.66	2.04	2.98	3.09	0.11
8	1:4:5	0.5:0.5:0.5	0.04	0.83	1.49	0.43	0.79	1.06	1.74	2.44	2.58	0.14
9	1:2:3:4	0.5:0.5:0.5:0.5	-0.17	0.77	1.30	0.15	0.94	1.15	1.78	2.38	2.37	-0.01
10	1:3:4:5	0.5:0.5:0.5:0.5	-0.02	0.80	1.44	0.38	0.82	1.06	1.72	2.48	2.56	0.08

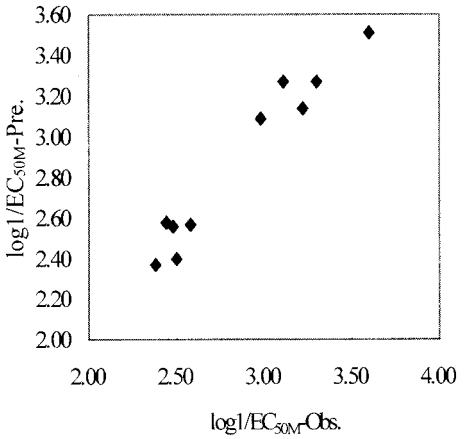

Figure 1. Observed and predicted $lg1/EC_{50M}$ of 10 other related mixtures

The predictive capability of the approach and the statistical validity of the modeling are confirmed by application of the 10 other related mixtures to the approach (Table 3). The predicted $log1/EC_{50M}$ are plotted against the observed ones in Figure 1, which shows a good consistency between them, with r^2=0.949, SE=0.104 and F=150.266 at a level of significance p=0.000. Since the 10 mixtures are randomly composed of the 5 chemicals, this consistency is satisfactory and it proves the predictive capability of the approach.

It is suggested by this study that this approach may be applied to an unknown mixture. For unknown organic micropollutants, Verhaar et al. (1995) and van Loon et al. (1996, 1997) successfully quantitatively determined their Bioconcentration Factor (BCF) in wastewater with Vapour Pressure Osmometry (VPO). Similarly, if the partition coefficients ($logK_{MD}$, $logK_{mbw}$, $logK_{mcw}$, $logK_{mchw}$ and $logK_{mtw}$) of an unknown mixture can be determined by VPO, it proves that the approach is capable of predicting the toxicity for the unknown mixture containing polar narcotic chemicals. This is being studied in our lab.

However, the approach is derived from the mixture pollutants containing polar narcotic chemicals. But in the real environment, there may be some other specific-acting chemicals and they will lead to the greater toxicity. Further study will be carried out by introduction of some other proper physicochemical parameter to describe this greater toxicity.

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