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QSARs for the Chronic Toxicity of Halogenated Benzenes to Bacteria in Natural Waters

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The Yangtze River Delta is one of the most densely urbanized areas in China, with a series of big, medium and small cities and towns along the river. In recent years, water pollution in the lower reaches of the Yangtze River has become more serious due to the release of industrial and agricultural pollutants.

Halogenated aromatic compounds are widely used as solvents, herbicides, antiseptics, and pesticides, and have a high environmental persistence. They have been reported to be present in the water and/or sediment of the Yangtze River, but the concentration of these compounds is still considered to be relatively low (Jiang et al. 2000). At present, most of the information about the toxic effects of these compounds on aquatic organisms comes from acute toxicity tests (Zhao and Wang 1995; Zhang et al. 2000). In order to investigate the effect of pollutants at low concentrations on aquatic organisms, we have determined the chronic toxicity values of 17 halogenated benzenes to mixed bacteria from the Yangtze River and developed quantitative structure-activity relationship (QSAR) models described in this paper.

MATERIALS AND METHODS

The bacterial growth inhibition test was used to determine the chronic toxicity of the 17 halogenated benzenes (Alsop et al. 1980). Chlorinated chemicals were purchased from Shanghai Chemical Reagent Co., China (analytical grade). A water sample was gathered from the Nanjing section in the Yangtze River (Jiangsu Province, China). The sample was obtained at a depth of 0.5 m and 50 m away from the bank. There were no large industry enterprises or new pollutant sources in the vicinity of this section of the river. The pollutants from the upper reaches of the river have been admixed equably, and the concentration of most halogenated aromatic hydrocarbons is at the $ng \cdot L^{-1}$ level (Jiang et al. 2000). The temperature of the water sample was 15°C, the pH was 6.5 and the dissolved oxygen (DO) was 7.4 $mg \cdot L^{-1}$. The water sample was stored at 4 °C when

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not being used. Bacterial counts were determined by standard plate count techniques (Wang 1988) and was determined to be 6.1×10^3 CFU/ml. The composition of the medium contained: beef extract, 3 g; peptone, 10 g; agar, 20 g; distilled water, 1 L. The pH of the culture medium was adjusted to 7.4-7.6, and then the culture was sterilized for 20 min at 121 ℃. A 1 ml diluted water sample was cultivated in 15 ml of the above medium at 31 ˚C for 24 hr, and the number of colonies was enumerated as bacterial counts.

The culture was maintained in liquid medium containing beef extract, 3 g; peptone, 10 g; NaCl, 5 g; distilled water, 1 L. The pH of the culture medium was adjusted to 7.2-7.4 and the medium was sterilized for 20 min at 121℃. Each compound was dissolved in 95% ethanol, and 8-10 concentrations were tested with a logarithmic difference of 0.05-0.1 $M{\cdot}L^{-1}$. 1 mL of test chemical solution and 1 mL of river water were added to 50 mL of culture medium in 250 mL conical flasks. 1 mL of 95% ethanol solution and 1 mL of sterilized river water added to 50 mL of culture medium was used as a blank control, and 1 mL of river water and 1 mL of 95% ethanol solution added to 50 mL of culture medium was used as a seed control. For each concentration and control, experiments were performed in triplicate. All samples were incubated for 7 d at 20±1℃.

The turbidities were measured using a spectrophotometer (UV-1201) at 530 nm against a blank control. The concentration-response curve of the logarithm of the toxicant concentration against the absorbance rate is shown in Figure 1.

The no observed effect concentration (NOEC, $M{\cdot}L^{-1}$) and the lowest observed effect concentration (LOEC, $M{\cdot}L^{-1}$) values were calculated using the concentration-response curves. The toxicity of the chemicals was expressed as the negative logarithm values of NOEC and LOEC (see Table 1).

The energy of the lowest unoccupied molecular orbital (E_{LUMO}) was calculated by the quantum chemical MOPAC (ver. 6.0, http: //ftp.osc.edu) program. Each molecule was geometrically optimized using the AM1 Hamiltonian in MOPAC. The logarithm of n-octanol/water partition coefficient (log P) was obtained from ClogP for Windows software (ver. 3.55, Biobyte Company, Claremont, CA, USA). The parameter values of the studied chemicals are listed in Table 1. The linear regression analyses were carried out using the SPSS statistical package (ver. 10.0, SPSS Company, Chicago, IL, USA).

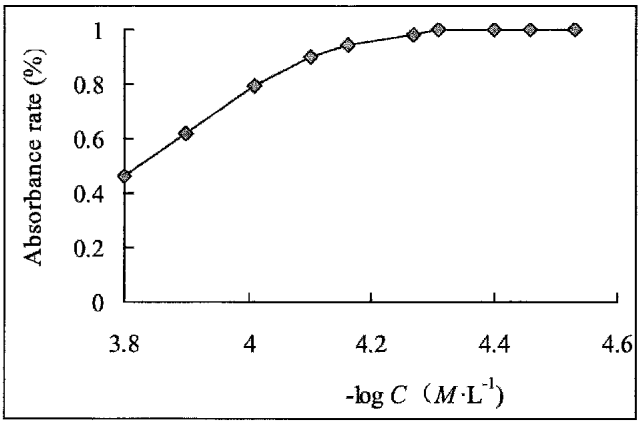

Figure 1. Concentration-response curve for chlorobenzene

Table 1. Structural parameters and chronic toxicity data

No.	Compounds	log *P*	$-E_{HOMO}$	$-E_{LUMO}$	Toxicity ($M \cdot L^{-1}$)			
			eV	eV	Obs.[a]	Obs.[b]	$-\log IC_{50}$[c]	Cmiz[d]
1	Chlorobenzene	2.84	9.82	0.065	4.27	4.31	3.86	3.23
2	1,2-Dichlorobenzene	3.43	9.83	0.302	4.77	4.82	4.38	4.13
3	1,3-Dichlorobenzene	3.53	9.85	0.289	4.70	4.75	4.24	
4	1,4-Dichlorobenzene	3.44	9.73	0.370	4.80	4.85	4.39	4.13
5	2-Chlorotoluene	3.42	9.67	0.078	4.45	4.52		
6	4-Chlorotoluene	3.33	9.56	0.129	4.40	4.48	3.88	
7	1,2,4-Trichlorobenzene	4.02	9.78	0.572	5.06	5.11	4.50	4.80
8	Bromobenzene	2.99	9.87	0.137	4.37	4.42	3.78	3.60
9	1,2-Dibromobenzene	3.64	9.93	0.417	4.63	4.71		
10	4-Bromotoluene	3.42	9.63	0.200	4.50	4.60		
11	4-Bromoaniline	1.97	8.97	0.113	4.32	4.40	3.92	
12	2-Chloroaniline	1.90	9.04	0.066	4.41	4.47		
13	3-Chloroaniline	1.88	9.79	0.159	4.45	4.50		3.91
14	4-Chloroaniline	1.83	9.66	0.212	4.41	4.45	3.57	3.91
15	2-Chlorophenol	2.15	9.78	0.185	4.69	4.79	4.14	3.54
16	4-Chlorophenol	2.39	9.37	0.104	4.76	4.80	4.48	3.74
17	2,4-Dichlorophenol	3.06	9.41	0.315	5.00	5.05	4.45	4.64

a. Observed -log (LOEC); b. Observed -log (NOEC); c. Acute toxicity for 15-min to *V. fischeri* from Zhao and Wang (1995); d. Concentration of minimum inhibition zone to yeast *S. cerevisiae* from Liao and Wang (1996).

RESULTS AND DISCUSSION

The experimental results in Table 1 show that the -log (NOEC) values ranged from 5.11 for 1,2,4-trichlorobenzene to 4.31 for chlorobenzene, whereas the -log (LOEC) values ranged from 5.06 for 1,2,4-trichlorobenzene to 4.27 for chlorobenzene. The two toxicity descriptors gave consistent results for the seventeen chemicals studied.

When comparing the toxic effect of compounds on mixed bacteria to that on *Vibrio fischeri* (Zhao and Wang 1995) and yeast *Saccharomyces cerevisiae* (Liao and Wang 1996), shown in Table 1, the following results were obtained:

$$-\log(\text{NOEC}) = -0.747 \log IC_{50} + 1.601,\ n=12,\ r=0.90,\ s=0.12 \quad (1)$$

$$-\log(\text{LOEC}) = -0.466 \log \text{Cmiz} + 2.811,\ n=10,\ r=0.83,\ s=0.16 \quad (2)$$

Data in Table 1 indicate that all the chemicals tested exhibit higher toxicity to mixed bacteria than to yeast. Thus, the mixed bacteria assay is more sensitive than the yeast assay.

It is well known that non-specific toxicity of chemicals can be described by two kinds of actions: non-polar narcosis (type I narcosis) and polar narcosis (type II narcosis). Non-polar narcotic chemicals are considered baseline toxicants. Their toxicity is proportional to their concentrations at the site of action and is caused solely by membrane perturbation (Schultz et al. 1986; Veith and Broderius 1990). Polar narcotic chemicals, typified by most phenols and anilines, exhibit toxic potency higher than that estimated by their hydrophobicity due to the existence of polar substituents in the molecules (Kamlet et al. 1986). The addition of an electronic parameter can improve the predication of a log octanol/water partition coefficient (log P) dependent model (Schultz et al. 1989).

Log P-dependent QSARs for non-polar and polar narcotic toxicity to bacteria are established and shown below.

Non-polar narcosis

$$-\log(\text{NOEC}) = 0.652 (\log P) + 2.437 \quad (3)$$

$$n=10,\ r^2=0.790,\ r^2_{cv}=0.782,\ s=0.116,\ F=30.0,\ Pr>F=0.001$$

Polar narcosis

$$-\log(\text{NOEC}) = 0.517 (\log P) + 3.516 \quad (4)$$

$$n=7,\ r^2=0.860,\ r^2_{cv}=0.859,\ s=0.100,\ F=30.7,\ Pr>F=0.003$$

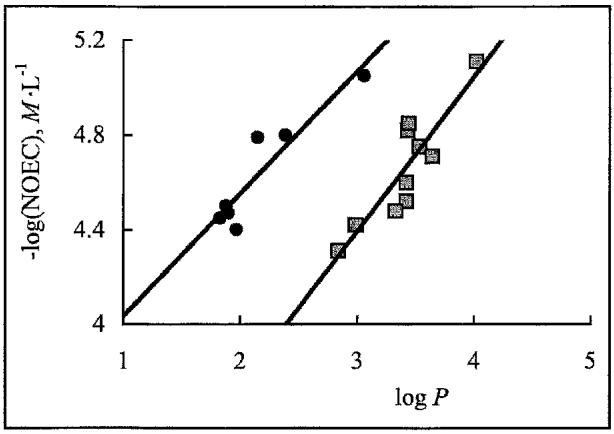

Figure 2. Observed toxicity to river mixture bacteria versus hydrophobicity ■: nonpolar narcotics; ●: polar narcotics

Where n is the number of observations; r^2 is the square of the correlation coefficient; r^2_{cv} is the cross-validated r^2 value using a leave-one-out cross-validation method; s is the standard error; F is the mean square ratio and Pr is the probability greater than the F value.

A plot of toxicity versus hydrophobicity (log P) for 17 assay compounds is displayed in Figure 2. The seventeen compounds form two groups according to their mechanism of action. Alkyl halogenated benzenes form a non-polar narcotic group based on their baseline toxicity to bacteria. Polar narcotic compounds halogenated phenols and anilines form another group and show toxicity higher than their corresponding baseline toxicities. Furthermore, the polar narcosis QSAR (Eq. 3) has a lower slope and a higher intercept than the non-polar narcosis QSAR (Eq. 4).

Ren et al. (2004) observed the toxicity of twenty narcotic compounds to the activated sludge bacteria, and found the toxicity of the polar narcotics was higher than the corresponding baseline toxicity. They developed log P-dependent QSARs for non-polar narcotics and polar narcotics, respectively. The quality of model fit and prediction of the two equations was similar with comparable r^2, r^2_{cv} and RMSE (root mean square error) values.

E_{LUMO} describes how susceptible the molecule is to interactions with a nucleophile and thus is directly related to electron affinity. Among all the compounds studied in this paper, the algebraic value of E_{LUMO} of 1,2,4-trichlorobenzene is the lowest (-0.572 eV), and its toxicity is the highest (NOEC=7.76×10^{-6} $M \cdot L^{-1}$), whereas toxicity of

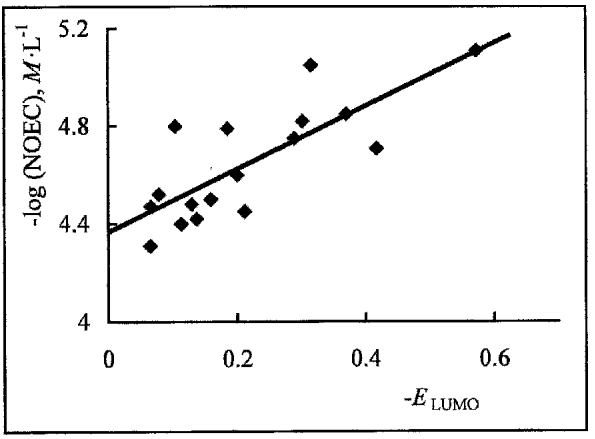

Figure 3. A plot of chronic toxicity versus E_{LUMO}

chlorobenzene is the lowest (NOEC=4.90×10^{-5} $M\cdot L^{-1}$), and its E_{LUMO} is -0.065 eV.

A plot of toxicity versus E_{LUMO} for the 17 narcotic chemicals is displayed in Figure 3. Apparently, there is negative correlation between E_{LUMO} and the chronic toxicity. This conclusion is consistent with the results from acute toxicity tests (Cronin and Schultz 1996; Lu et al. 2003).

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