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QSARS for Acute Toxicity of Halogenated Benzenes to Bacteria in Natural Waters[1]

GUANG-HUA LU[*], CHAO WANG[*,2], AND YU-MEI LI[†]

[*]*Key Laboratory for Integrated Regulation and Resources Exploitation on Shallow Lakes, Ministry of Education, College of Environmental Science and Engineering, Hohai University, Nanjing 210098, Jiangsu, China;*
[†]*School of Energy and Environmental Engineering, Inner Mongolia University of Science and Technology, Baotou 014010, Inner Mongolia, China*

Objective To measure the acute toxicity of halogenated benzenes to bacteria in natural waters and to study quantitative relationships between the structure and activity of chemicals. **Methods** The concentration values causing 50% inhibition of bacteria growth (24h-IC_{50}) were determined according to the bacterial growth inhibition test method. The energy of the lowest unoccupied molecular orbital and the net charge of carbon atom of 20 halogenated benzenes were calculated by the quantum chemical MOPAC program. **Results** The log1/IC_{50} values ranged from 4.79 for 2,4-dinitrochlorobenzene to 3.65 for chlorobenzene. A quantitative structure-activity relationship model was derived from the toxicity and structural parameters: log1/IC_{50} =-0.531(E_{LUMO})+1.693(Q_C)+0.163(log$P$) +3.375. This equation was found to fit well (r^2=0.860, s=0.106), and the average percentage error was only 1.98%. **Conclusion** Halogenated benzenes and alkyl halogenated benzenes are non-polar narcotics, and have hydrophobicity-dependent toxicity. The halogenated phenols and anilines exhibit a higher toxic potency than their hydrophobicity, whereas 2,4-dinitrochlorobenzene is electrophile with the halogen acting as the leaving group.

Key words: River bacteria; Acute toxicity; QSARs; Prediction model

INTRODUCTION

The Yangtze River Delta is one of the most densely populated and highly urbanized areas in China, with a series of large, medium and small-sized cities and towns located 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 hydrocarbons 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[1]. Information on the extent of organic pollutant toxicity is important for risk assessment of chemicals in the environment and for regulating their manufacture and use. To this end, we determined the acute toxicity of a number of halogenated benzenes to mixed bacteria from the Yangtze River in an attempt develop a quantitative structure and toxicity relationship model.

MATERIALS AND METHODS

The bacterial growth inhibition test was used to determine the acute toxicity of 20 halogenated benzenes[2]. Chlorinated chemicals were purchased from Shanghai Chemical Reagent Co., China (analytical grade). A water sample was gathered from the Nanjing section of 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 were admixed equably, and the concentration of most halogenated aromatic hydrocarbons was at the ng/L

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[2]Correspondence should be addressed to Chao WANG.

Biographical note of the first author: Guang-Hua LU, born in 1969, Ph. D., Professor, majoring in aquatic environmental toxicology. E-mail: ghlucc@sina.com

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level as previously described[1]. The temperature of the water sample was 15℃, the pH was 6.5 and the dissolved oxygen (DO) was 7.4 mg/L. The water sample was stored at 4℃ when not being used. Bacterial counts were 6.1×10^3 CFU/mL determined by standard plate count techniques[3].

The culture was maintained in liquid medium containing 3 g beef extract; 10 g peptone; 5 g NaCl, and 1 L distilled water. 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 5-7 concentrations were tested with a logarithmic difference of 0.2 mol/L. One mL of test chemical solution and 10 mL of river water were added to 40 mL of culture medium in 250 mL conical flasks. One mL of 95% ethanol solution and 10 mL of sterilized river water were added to 40 mL of culture medium and used as a blank control; 1 mL of 95% ethanol solution and 10 mL of river water were added to 40 mL of culture medium and used as a seed control. For each concentration and control, experiments were performed in triplicate. All samples were incubated for 24 h at 20℃±1℃.

The turbidities were measured using a UV-spectrophotometer (UV-1201) at 530 nm against a blank control. The absorbance values of the toxicant-amended mixtures were calculated as a percentage of the control using the simple relationship as follow:

$$\text{Absorbance rate}=\frac{\text{Absorbance of test bottle}}{\text{Absorbance of seed control}}\times100\%$$

The concentrations causing 50% growth inhibitory (IC_{50}, mol/L) were calculated using concentration-response curves (Fig. 1). The toxicity of the chemicals was expressed as the negative logarithm values of IC_{50} (Table 1).

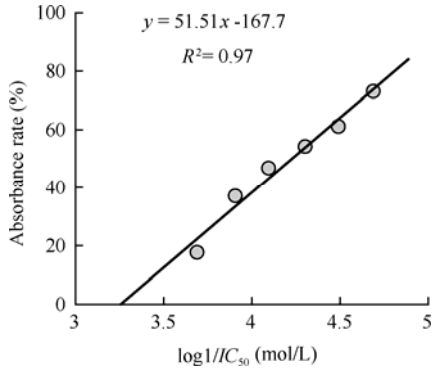

FIG .1. Concentration-response curve for 2-chlorophenol.

The energy of the lowest unoccupied molecular orbital (E_{LUMO}) and the net charge of carbon atom in benzene ring conjoined with the halogen atom (Q_C) of compounds were calculated by the quantum chemical MOPAC (ver. 6.0, http://ftp.osc.edu) program. The logarithm of n-octanol/water partition coefficient (log*P*) was obtained by ClogP for Window software (ver. 3.55, Biobyte Company, Claremont, CA, USA). The parameter values of studied chemicals are listed in Table 1.

RESULTS

The experimental results in Table 1 showed that the most toxic compound was 2,4-dinitrochlorobenzene (log1/IC_{50} was 4.79), whereas the least toxic one was chlorobenzene (log1/IC_{50} was 3.65). The values of E_{LUMO} ranged from -1.762 for dinitrochlorobenzene to -0.065 for chlorobenzene. The values of Q_C ranged from -0.121 for bromobenzene to 0.107 for 2,4-dichlorophenol. The log*P* values ranged from 1.83 for 4-chloroaniline to 4.02 for 1,2,4-trichlorobenzene.

When the toxicity of 10 compounds on bacteria was compared with those from *V. fischeri* (Table 1), all the chemicals tested exhibited a higher toxicity to *V. fischeri* than to river bacteria. Their correlation equation was log1/EC_{50}=1.18(log1/IC_{50})-0.52, and the correlation coefficient was 0.90.

DISCUSSION

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[5-6]. Polar narcotic chemicals characterized by most phenols and anilines exhibit a higher toxic potency than their hydrophobicity due to the existence of polar substitutes in the molecules[7]. The addition of an electronic parameter can improve the predication of a log octanol/water partition coefficient (log*P*)-dependent model[8].

Log*P*-dependent QSAR for non-polar toxicity to bacteria is established and shown below.

$$\log1/IC_{50}=0.565(\log P)+2.000 \quad (1)$$

n =10, r^2 = 0.887, r^2_{adj} = 0.873, s =0.070, F =62.6, $Pr > F$ =0.000.

Where n is the number of observations (i.e. compound numbers 1-10 in Table 1), r^2 is the square of the correlation coefficient, r^2_{adj} is the adjusted r^2 value, s is the standard error, F is the mean square ratio, and Pr is the probability greater than the F value.

TABLE 1

Structural Parameters and Toxicity Data of Bacteria Used in This Study

No.	Compounds	log*P*	$-E_{LUMO}$ eV	Q_C	Toxicity		
					Exp.[a]	Pre.[b]	log1/EC_{50}[c]
1	Chlorobenzene	2.84	0.065	-0.095	3.65	3.71	3.86
2	1,2-Dichlorobenzene	3.43	0.302	-0.088	4.01	3.94	4.38
3	1,3-Dichlorobenzene	3.53	0.289	-0.083	3.96	3.96	4.24
4	1,4-Dichlorobenzene	3.44	0.370	-0.087	3.87	3.98	−−−
5	2-Chlorotoluene	3.42	0.078	-0.064	3.82	3.86	−−−
6	4-Chlorotoluene	3.33	0.129	-0.070	3.85	3.87	3.88
7	1,2,4-Trichlorobenzene	4.02	0.572	-0.076	4.32	4.20	4.50
8	Bromobenzene	2.99	0.137	-0.121	3.73	3.73	3.78
9	4-Bromotoluene	3.42	0.200	-0.055	3.89	3.94	−−−
10	1,2-Dibromobenzene	3.64	0.417	-0.116	4.13	3.99	−−−
11	4-Bromoaniline	2.26	0.113	0.070	3.87	3.92	3.92
12	2-Chloroaniline	1.90	0.066	-0.054	3.68	3.63	−−−
13	3-Chloroaniline	1.88	0.159	-0.009	3.83	3.75	−−−
14	4-Chloroaniline	1.83	0.212	-0.010	3.90	3.77	−−−
15	2-Chlorophenol	2.15	0.185	0.054	3.80	3.91	4.14
16	3-Chlorophenol	2.50	0.186	0.054	3.84	3.97	−−−
17	4-Chlorophenol	2.39	0.104	0.080	4.11	3.95	4.48
18	2,4-Dichlorophenol	3.06	0.315	0.107	4.22	4.22	4.45
19	4-Bromonitrobenzene	2.55	0.872	-0.080	3.93	4.12	−−−
20	2,4-Dinitrochlorobenzene	2.17	1.762	0.049	4.79	4.75	−−−

Note. [a]Exp. is the experimental toxicity to bacteria; [b]Pre. is the calculated toxicity from equation (4); [c]log1/EC_{50} is the experimental toxicity to *V. fischeri* from Zhao and Wang[4]. Linear regression analyses were carried out using the SPSS statistical package (version 10.0, SPSS company, Chicago, IL, USA).

A plot of toxicity versus hydrophobicity (log*P*) for 20 studied compounds is displayed in Fig. 2. These 20 compounds form three groups according to their mechanisms 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 higher toxicity than their corresponding baseline toxicity. 2,4-dinitrochlorobenzene itself forms an individual group with the strongest toxicity in these compounds studied.

Ren *et al.*[9] observed toxicity of the twenty narcotic compounds (including eleven non-polar narcotics and nine polar narcotics) to the activated sludge bacteria, and found that 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 and prediction of the two equations were similar with comparable r^2 and RMSE (root mean square error) values.

Based on the others' work, E_{LUMO}, Q_C, and log*P* were selected as the structural parameters to establish QSARs for the twenty compounds studied. The following models were obtained from multivariable regression analyses:

$$\log 1/IC_{50} = -0.548(E_{LUMO}) + 3.781 \quad (2)$$

$n = 20$, $r^2 = 0.679$, $r^2_{adj} = 0.661$, $s = 0.151$, $F = 38.1$, $Pr > F = 0.000$

$$\log 1/IC_{50} = -0.533(E_{LUMO}) + 0.853(Q_C) + 3.811 \quad (3)$$

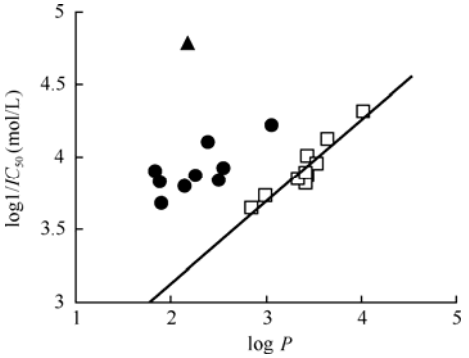

FIG. 2. Observed toxicity to river bacteria *versus* hydrophobicity. □: nonpolar narcotics; ●: polar narcotics; ▲: reactive compound.

$n = 20$, $r^2 = 0.735$, $r^2_{adj} = 0.704$, $s = 0.141$, $F = 23.6$, $Pr > F = 0.000$

$$\log 1/IC_{50} = -0.531(E_{LUMO}) + 1.693(Q_C) + 0.163(\log P) + 3.375 \quad (4)$$

$n = 20$, $r^2 = 0.860$, $r^2_{adj} = 0.832$, $s = 0.106$, $F = 32.8$, $Pr > F = 0.000$

Eq. (4) was used to predict the toxicity in our study due to its higher r^2 and lower standard error. The predicted values are listed in Table 1. Most compounds fitted very well, the greatest percentage error was 4.78% for 4-bromonitrobenzene, and the average was only 1.98%.

The obtained models have revealed that the toxicity of halogenated benzenes to bacteria is related mainly to electronic properties and hydrophobicity. E_{LUMO} describes how susceptible the molecule is to interactions with a nucleophile and thus is directly related to electron affinity. There is an obvious negative correlation between toxicity and E_{LUMO}. Among all the compounds studied in this paper, the algebraic value of E_{LUMO} of 2,4-dinitrochlorobenzene is the lowest (-1.762 eV), and its toxicity is the highest (log1/IC_{50}=4.79 mol/L), whereas toxicity of chlorobenzene is the lowest (log1/IC_{50}=3.65 mol/L), and its E_{LUMO} is the highest (-0.065 eV). Q_C is the net charge of carbon atom of most deficient electron, conjoined with the halogen atom, which is defined as the difference between the valence electron number and electron density of atom. Q_C can reflect the leaving potential of halogen. log$P$ is a hydrophobicity parameter. The higher the log$P$, the stronger the hydrophobicity and the easier the compounds are bio-concentrated in an organism.

Although the chemicals examined in this study all belong to halogenated benzenes, different mechanisms of toxic action are presented. Halogenated-and alkyl-halogenated benzenes are non-polar narcotics, and have hydrophobicity-dependent toxicity. The halogenated phenols and anilines can result in polar narcosis and exhibit a higher toxic potency than their hydrophobicity. 2,4-dinitrochlorobenzene is electrophile with the halogen acting as the leaving group.

CONCLUSION

The acute toxicity of halogenated benzenes to bacteria in the Yangtze River can be determined using the bacterial growth inhibition test, and the log1/IC_{50} values range from 4.79 for 2,4-dinitrochlorobenzene to 3.65 for chlorobenzene. QSAR model log1/IC_{50} = -0.531(E_{LUMO})+1.693(Q_C)+0.163(log$P$)+3.375 can fit well (r^2=0.860, s=0.106), and the average percentage error is only 1.98%. The twenty compounds form three groups according to their mechanisms of action. Halogenated benzenes and alkyl-halogenated benzenes are non-polar narcotics, and have hydrophobicity-dependent toxicity. The toxicity of polar narcotics-halogenated phenols and anilines is higher than the corresponding baseline toxicity, whereas 2,4-dinitrochlorobenzene is a reactive compound with the halogen acting as the leaving group.

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