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Quantitative structure-activity relationships for the Toxicity of Substituted Benzenes to *Cyprinus carpio*[1]

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Objective To measure the 96h-LC_{50} values of 32 substituted benzenes to the carp and to study the relationship between quantitative structure-activity and structural parameters of chemicals. **Methods** The acute toxicity values of 32 substituted benzenes to the carp were determined in a semistatic test. The energy of the lowest unoccupied molecular orbital, and the highest occupied molecular orbital, the dipole moment and the molecular weight of substituted benzenes were calculated by the quantum chemical method MOPAC6.0. **Results** The range of the toxicity of studied compounds was broad, and the most toxic compound was pentachlorophenol, while the least toxic compound was 4-methylaniline. By the stepwise regression analyses, a series of Quantitative structure-activity relationships (QSAR) equations were derived from all compounds and subclasses. The equation $\log 1/LC_{50}=0.759\log P+2.222$ (R^2 (adj)=0.818) was found to fit well and the average predicted percentage error was 6.16%. **Conclusion** The toxicity of anilines and phenols to the carp could be modeled well by log*P* alone, whereas the toxicity of the halogenated benzenes and nitrobenznes not containing hydroxyl or amino group can be controlled by hydrophobic and electronic factors.

Key words: 96h-LC_{50}; *Cyprinus carpio*; Hydrophobicity; Orbital energy

INTRODUCTION

With rapid development of economy and industrialization, organic pollution of river water is more and more serious in China. The transformation and ecotoxicological effects of these organic pollutants in aquatic environment, especially toxic compounds, have aroused more and more attentions. Information regarding the extent of toxicity of organic pollutants is very important in risk assessment of the chemicals in the environment. Quantitative structure-activity relationships (QSARs) are powerful tools in predicting toxicology.

The carp (*Cyprinus carpio*) is a major kind of fish in China and one of the five test organisms recommended by OECD. In view of this, we measured the 96 h-LC_{50} values of 32 substituted benzenes to the carp and developed QSAR models.

MATERIALS AND METHODS

One-year-old carps supplied by the Changchun Aquatic Institute were used in the experiment. The test water was dechlorinated tap water. Temperature of the test water was 15℃-18℃, dissolved oxygen was 6.35 mg/L, and pH was 7.0-7.5. Test aquaria were 60 L glass tanks. Each contained 20 L of test water and ten randomly selected fish. During the 96 h experiment, the test water was replaced twice a day and 10 L each time. The aquaria with the same number of carps served as controls. For each compound at least five concentration gradients were planned with the same logarithmic difference in concentration, and there were two replicates at each concentration and control. Fifty percent lethal concentration (96 h-LC_{50}) was obtained by the one variable regression analysis of the logarithm of compound concentrations and the mortality rates after 96 h.

The energy of the lowest unoccupied molecular orbital (E_{LUMO}), and the highest occupied molecular orbital (E_{HOMO}), the dipole moment (μ) and molecular weight (M_W) of substituted benzenes were calculated by the quantum chemical method MOPAC (ver. 6.0, http://ftp.osc.edu) program. Each molecule was geometry optimized using the AM1 Hamiltonian in MOPAC. The logarithm of n-octanol/water partition coefficient (log*P*) was obtained from SRC-logK_{OW}

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for Windows software (ver. 1.54, SRC Company, UK). The parameter values of studied chemicals are listed in Table 1. Linear regression analysis was performed using the SPSS statistical package (ver. 9.0, SPSS Company, USA).

RESULTS

The experimental results in Table 1 showed that the range of the toxicity of studied compounds was broad, and the most toxic compound was pentachlorphenol ($\log 1/LC_{50}$ was up to 6.29), while the least toxic compound was 4-methylaniline ($\log 1/LC_{50}$ was 2.91). log*P* was 1.03 for aniline and 5.04 for pentachlorophenol, E_{LUMO} was 0.76 for aniline and -1.52 for 3,4-dichloronitrobenzene, and E_{HOMO} was -8.36 for 4-methylaniline and -10.46 for 4-nitrobenzaldehyde.

The parameters log*P*, E_{LUMO}, E_{HOMO}, M_W and μ were selected as structural descriptors to establish the

TABLE 1

Toxicity Data and Parameters of Studied Chemicals

No.	Compounds	E_{LUMO} eV	log*P*	Mw	$-E_{HOMO}$ eV	μ D	$\log 1/LC_{50}$ (mol/L)		
							Exp.	Pre.	Er%
1	Aniline	0.76	0.90	93.13	8.52	1.54	2.95	2.91	1.36
2	2-Chloroaniline	0.19	1.90	127.50	8.63	1.76	3.78	3.66	3.17
3	2,3-Dichloroaniline	-0.35	2.82	162.02	9.92	1.21	4.37	4.36	0.23
4	2,4-Dichloroaniline	-0.43	2.78	162.02	10.15	0.59	4.65	4.33	6.88
5	2,5-Dichloroaniline	-0.46	2.75	162.02	10.18	1.87	4.43	4.31	2.71
6	4-Bromoaniline	-0.11	2.26	172.03	8.97	3.64	3.71	3.94	6.20
7	3-Methylaniline	0.60	1.40	107.15	8.45	1.52	3.06	3.28	7.19
8	4-Methylaniline	0.61	1.39	107.15	8.36	1.41	2.91	3.28	12.7
9	Phenol	0.40	1.46	94.11	9.12	2.93	3.70	3.33	10.0
10	2-Methylphenol	0.41	1.95	108.14	9.00	1.42	3.54	3.70	4.52
11	3-Methylphenol	0.38	1.96	108.14	9.02	1.53	4.14	3.71	10.4
12	4-Methylphenol	0.43	1.94	108.14	8.88	1.34	3.85	3.69	4.16
13	2,4-Dichlorophenol	-0.43	3.06	163.00	9.23	2.16	4.77	4.54	4.82
14	2,4,6-Trichlorophenol	-0.82	3.69	197.45	9.39	1.08	5.14	5.02	2.33
15	Pentachlorophenol	-1.43	5.12	266.34	9.64	1.99	6.29	6.11	2.86
16	2-Nitroaniline	-0.94	1.85	138.13	9.07	8.14	3.93	3.63	7.63
17	3-Nitroaniline	-1.03	1.37	138.13	9.24	8.03	3.35	3.26	2.69
18	4-Nitroaniline	-0.88	1.39	138.13	8.91	12.48	3.48	3.28	5.75
19	2-Nitrophenol	-1.15	1.79	139.11	9.87	6.64	3.58	3.58	0.00
20	3-Nitrophenol	-1.28	2.00	139.11	9.76	5.55	3.90	3.74	4.10
21	4-Nitrophenol	-1.21	1.91	139.11	9.98	7.65	4.34	3.67	15.4
22	2-Chloronitrobenzene	-1.23	2.24	157.56	10.15	7.86	3.79	3.92	3.43
23	3-Chloronitrobenzene	-1.36	2.46	157.56	10.15	5.70	3.80	4.09	7.63
24	4-Chloronitrobenzene	-1.42	2.39	157.56	10.29	0.96	3.79	4.04	6.60
25	3,4-Dichloronitrobenzene	-1.52	3.12	192.00	10.27	6.12	4.48	4.59	2.46
26	2,5-Dichloronitrobenzene	-1.34	3.09	192.00	10.15	5.47	4.54	4.57	0.66
27	3-Bromonitrobenzene	-1.35	2.64	202.01	10.21	6.13	4.18	4.23	1.20
28	2-Iodnitrobenzene	-1.40	2.98	249.01	10.19	7.94	4.21	4.48	6.41
29	Nitrobenzene	-1.22	1.85	123.11	10.30	7.48	3.12	3.63	16.3
30	2-Nitrotoluene	-1.01	2.30	137.14	10.01	7.26	3.37	3.97	17.8
31	4-Nitrotoluene	-1.26	2.37	137.14	10.14	7.99	3.53	4.02	13.9
32	4-Nitrobenzaldehyde	-1.46	1.56	151.12	10.46	6.98	3.60	3.41	5.28

QSARs. Using bivariate correlation analysis of the toxicity and parameters, a linear correlation matrix was obtained (Table 2). The results in Table 2 showed that the remarkable correlation was obtained between toxicity and log*P* or M_W. Moreover, not only E_{HOMO} and E_{LUMO} but also M_W and log*P* were found to be relatively collinear.

TABLE 2
Correlation Matrix Between Toxicity and Parameters

R	log $1/LC_{50}$	log*P*	E_{LUMO}	E_{HOMO}	M_W	μ
log $1/LC_{50}$	1.000	0.900	-0.341	-0.310	0.757	-0.235
log*P*	0.900	1.000	-0.449	-0.432	0.851	-0.183
E_{LUMO}	-0.341	-0.449	1.000	0.823	-0.640	-0.631
E_{HOMO}	-0.310	-0.432	0.823	1.000	-0.516	-0.351
M_W	0.757	0.851	-0.640	-0.516	1.000	0.121
μ	-0.235	-0.183	-0.631	-0.351	0.121	1.000

By the stepwise regression analysis of the toxicity values and parameters for different subclass compounds listed in Table 1, a series of QSAR equations were developed as follows

$\log 1/LC_{50}=0.820 \log P+2.152$

n=15, R^2=0.939, R^2(adj)=0.934, SE=0.232, F=199.96, P=0.000 (1)

$\log 1/LC_{50}=0.730 \log P-0.219 E_{HOMO}+0.397$

n=21, R^2=0.928, R^2(adj)=0.920, SE=0.223, F=116.73, P=0.000 (2)

$\log 1/LC_{50}=0.684 \log P-1.265E_{LUMO}+0.501$

n=11, R^2=0.867, R^2(adj)=0.833, SE=0.185, F=26.01, P=0.000 (3)

In which, n is the number of compounds; R^2 is the square of correlation coefficient; R^2(adj) is the square of adjusted correlation coefficient; SE is the standard error; F is the mean square radio; and P is the probability.

The anilines and phenols not containing nitro group were included in Eq. (1), all the anilines and phenols were included in Eq. (2), and the rest of nitrobenzenes were included in Eq. (3). It should be noted that the compounds containing two nitro groups were not considered in this study.

DISCUSSION

The logarithm of n-octanol/water partition coefficient (log*P*) is hydrophobicity parameter, the higher the log*P* values, the stronger the hydrophobicity, and the easier the compound was bioconcentrated in an organism. Eq. (1) shows that the toxicity of anilines and phenols studied to the carp was controlled mainly by hydrophobicity instead of electronic factor. E_{LUMO} is the energy of the lowest unoccupied molecular orbital and describes how susceptible the molecule is to interactions with a nucleophile and thus is directly related to electron affinity. E_{HOMO} is related to ionization potential. The higher the E_{HOMO} values, the stronger the electron donating ability. Both E_{HOMO} and E_{LUMO} show the tendency of chemicals to undergo orbital-controlled reactions[1]. When six added nitroanilines and nitrop- henols were analyzed in Eq. (2), the toxicity was found to be correlative not only with log*P* but also with E_{HOMO}. The toxicity of these compounds may be related to the oxidizing reaction of the hydroxyl of phenols and amino group influenced by nitro group. The result from Eq. (3) showed that the toxicity of the halogenated nitrobenzens, nitrobenzene and nitrotol- uenes to the fish was probably related not only to their ability to penetrate cells by its cell membrane but also to the reductive reactions of the chemicals with the active sites of action through a variety of electronic processes.

Considering all of the compounds studied, the following equation was obtained:

$\log 1/LC_{50}=0.759 \log P+2.222$,

n=32, R^2=0.824, R^2(adj)=0.818, SE=0.294, F=140.13, P=0.000 (4)

When Eq.(4) was compared with Eq.(1)-(3), the correlation was lower and SE was higher in Eq. (4). However, the correlation of Eq. (4)was still obvious.

There are a large number of studies describing QSARs by using log*P* or log*P* and the descriptors of molecular orbital interaction. Deneer *et al*.[2] developed QSAR equations for the toxicity of 22 nitrobenzenes to *Chlorella pyrenoidosa*, and found that the toxicity of mononitro-substituted benzenes could be modeled by log*P* alone: $\log 1/EC_{50}=0.90 \log P-3.97$, n=15,

R=0.649, SE=0.42. Veith *et al*.[3] established a set of QSARs for aromatic chemicals by respectively using average superdelocalizability (S^{N}_{av}), E_{LUMO} and log$P$ to explain the variation of acute toxicity of substituted benzenes, phenols, and anilines to fish. Schultz *et al*.[4] also established the QSAR model for the toxicity of phenols to *Vibrio fischeri*: -logpT_{30}=0.489 log$P$ + 0.126，n=16, R^2（adj）=0.848, SE=0.124. In which, pT_{30} is 30 min toxic potency. The acute toxicity of 16 organic compounds was thought to elicit their response via the weak acid respiratory uncoupling mechanism. In addition, Lu *et al*.[5] developed QSAR models for the toxicity data of substituted aromatic hydrocarbons to *Vibrio fischeri* with log$P$ and E_{LUMO} or M_W and E_{HOMO}, respectively. The authors thought that the toxicity of aniline, methylphenols and phenol to *V. fischeri* was controlled mainly by hydrophobicity. The toxicity of compounds containing $–NO_2$ was greater, and the enhanced toxicity might be related chiefly to the intracellular reduction of $–NO_2$ obtaining the electron.

Although the chemicals examined in this study all contain the same parent compound (benzene), different mechanisms of toxic action are represented. For example, phenol itself and the most simple alkyl- and/or halogenated substituted phenols are believed to result in polar narcosis, and penta- and selected nitro-substituted phenols might act as weak acid uncouplers[6]. Groups of polar narcotics, including phenols and anilines, have shown hydrophobicity-dependent toxicity[1]. Nitrobenzenes are reactive compounds which may be regarded as pro- electrophiles, yielding the corresponding potentially high toxic C-nitroso compounds[7], and their toxicity is related generally to the energy of the lowest unoccupied molecular orbital.

Eq. (4) was used to predict the toxicity. The predicted values and percentage errors are presented in Table 1. Percentage error (Er%) is defined as the absolute difference between the experimental and predicted values for toxicity divided by the experimental values. The average Er% of Eq. (4) was 6.16%, and the errors for the compounds containing nitro group such as nitrobenzene and nitrotoluenes etc. were more significant. This result was not surprising because of the toxicity of nitrobenzens influenced by hydrophobicity as well as electronic factor. Fold lines comparing the experimental with predicted toxicity are presented in Fig. 1, and the correlation coefficient between the experimental and predicted log1/LC_{50} values was up to 0.91.

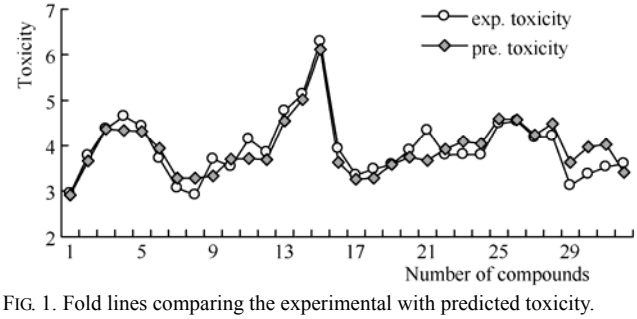

FIG. 1. Fold lines comparing the experimental with predicted toxicity.

CONCLUSION

Fifty percent lethal concentration values (96 h-LC_{50}) of 32 substituted benzenes to the carp were obtained, and the most toxic compound was pentachlorophenol, while the least toxic compound was 4-methylaniline. The parameters log$P$, E_{LUMO}, E_{HOMO}, M_W and μ were selected as structural descriptors to develop the QSARs, and a series of models were obtained. Although the chemicals examined in this study all contain the same parent compound (benzene), different mechanisms of toxic action are represented. Phenols and anilines are polar narcotics, and their toxicity to the carp could be modeled well by log$P$ alone, whereas nitrobenzenes are reactive compounds, their toxicity may be related not only to hydrophobicity but also to electronic interactions.

REFERENCES

1. Seward, J. R., Cronin, M. T. D., and Schultz, T. W. (2001). Structuretoxicity analyses of *Tetrahymena pyriformis* exposed to pyridines─an examination into extension of surface-response domains. *SAR QSAR Environ Res.* **11**, 489-512.
2. Deneer, J. W., van Leeuwen, C. J., and Seinen, W. (1989). QSAR study of the toxicity if nitrobenzene derivatives towards *Daphnia magna*, *Chlorella pyrenoidosa* and *Photobacterium phosphoreum*. *Aquat Toxicol.* **15**, 83-98.
3. Veith, G. D. and Mekenyan, O. C. (1993). A QSAR approach for estimating the aquatic toxicity of soft electrophiles. *Quant Struct-Act Relat* **12**, 349-356.
4. Schultz, T. W. and Cronin, M., T., D. (1997). Quantitative structure-activity relationships for weak acid respiratory uncouplers to *Vibrio fisheri*. *Environ. Toxicol. Chem.* **16**(2), 357-360.
5. Lu, G. H., Yuan, X., and Wang, C. (2003). Quantitative

Structure-Toxicity Relationships for Substituted Aromatic Compounds to *Vibrio fischeri*. *Bull. Environ. Contam. Toxicol.* **70**(4), 832-838.

6. Cronin, M. T. D. and Schultz, T. W. (1996). Structure-toxicity relation-ships for phenols to *Tetrahymena pyriformis*. *Chemosphere* **32**, 1453-1468.

7. Dearden, J. C., Cronin, M. T. D, Schultz, T. W., amd Lin, D. T. (1995). QSAR study of the toxicity of nitrobenzenes to *Tetrehymena pyrifirmis*. *Quant Struct-Act Rela* **14**, 427-432.

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