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Analysis and Prediction of Structure-Reactive Toxicity Relationships of Substituted Aromatic Compounds

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The fundamental differentiation of toxicity is between reactive and nonreactive toxicity. Reactive toxicity is associated with a specific mechanism for the reaction with an enzyme or inhibition of a metabolic pathway, and nonreactive toxicity is related directly to the quantity of toxicant acting upon the cell. The quantitative structure-activity relationships(QSARs) have been successfully used in the nonreactive toxicity, such as prediction of the toxicity of nonreactive compounds based on their solubility in the lipids of organisms (Mackay 1988; Yoshioka et al. 1986). The elements of molecular structure that are most closely related to nonreactive toxicity are those that describe the partitioning of the toxicant into the organism (Blume and Speece 1990), while QSARs for the reactive toxicity are less common in the environmental toxicology literature. With the recent increase in the use of synthetic substituted benzenes as industrial chemicals, the accurate analysis of the effect of reactive toxic chemicals has become recognized with QSAR (Nendza et al. 1988). For this purpose, we selected the fish (Carassias auratus) as the test organism, measured the acute toxicity of 50% lethal concentration (LC_{50}) of the chemicals and the adenosine triphosphate (ATP) content of the liver cells for the organism. These determined the relationships of the acute toxicity of some substituted benzenes with their physicochemical structural parameters. The effects on the ATP content was also compared to predict biological reactivities of the chemicals, so as to find some clues to explain the mode of mechanism of the toxicity.

MATERIALS AND METHODS

The chemicals in our research included chlorobenzenes, bromobenzenes, fluorobenzene, iodobenzene, substituted anilines, and nitrobenzene derivatives (Table 1). Each chemical was purchased from Shanghai Chemical Agent Co., Shanghai, China, and each had a purity of 95% or better. The ATP and albumin bovine were obtained from Sigma Chemical Co.(USA). The conditions of the experimental water were: temperature, 20±l C; dissolved oxygen(DO), 8.2 ±0.5 mg/L; pH, 7.5 ±0.3; hardness(as $CaCO_3$), 110 10 mg/L. The experimental procedures of the acute toxicity test were according to the OECD guidelines for testing chemicals on freshwater fish (OECD 1984).

The organisms (Carassias auratus) were purchased from a commercial source (hatched about 35 d, Nanjing, China) and kept 10 d in the experimental water for acclimation before the bioassays. They were fed a commercial and standard food (dry *Daphnia*) for freshwater fish. Each fish was approximately 3.5 g weight and 4.0 cm length. The 48-hr acute toxicity tests on the chemicals were conducted with a semi-static method (water renewal at each 12 hr), and four organisms in each 6-L glass beaker containing 4 L experimental solutions (16 hr light / 8 hr darkness as photoperiod). Four to six concentrations were tested with C. auratus with two replicates of each concentration

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of the chemicals. Fish were not fed during the exposure to chemical. The acute toxicities of 38 chemicals in terms of LC_{50} values were determined after the probit transformation of the lethal percentage of the fish (Paul 1986; Devillers 1987). The liver cellular ATP content of the fish was measured after the organism was exposured for 24 hr in the test solution, and expressed as the bioluminescent intensity values while the concentration of test chemicals was 1/2-1/4 LC_{50} Value according to the method from literature (Harada et al. 1992; Rudd 1973). Briefly, after 24 hr, took about 10 mg liver of the test organism to homogenize for 8 min with phosphate-buffered saline (PBS, pH 7.4) in a microtissue glass homogenizer (at 1-4 ° C). Then samples were centrifuged at 11000 rpm for 3 min. One mL 0.02 M Tris-HCl reagent (pH 7.7) and 1 mL ATP buffer (pH 7.7) were added to 1 mL the liver cells suspension (about $1x10^5$ cells /mL phosphate-buffered saline), and mixed thoroughly for 3 min to extract ATP at 90 °C. The extract (50 µL) was used for luminometric measuring (Instrument model, Hitachi U-3210; wavelength 260 nm) of ATP content.

QSAR methods indicated that the effects of substituents on the strength of toxic reaction were a combination of the hydrophobic, electronic, steric, and dispersive factors (Hansch et al. 1964; Kamlet 1987). The $LogK_{ow}$ (octanol/water partition coefficient) value was obtained from reference (Verschueren 1983) or calculated according to Hansch and Leo (Lyman et al., 1982). The E_{homo} (energy of the highest occupied molecular orbital) value was computed from reference (Heilbronner and Bock 1976). The Es(steric effect constant of substituents) value was obtained by the Taft-Kutter-Hansch Es parameter (Kutter and Hansch 1969). Two indicator variables, HB_1 and HB_2 were introduced in this QSAR analysis (Fujita 1977). HB_1 represents hydrogen accepting substituents such as $-NO_2$-Br,-F,and -I, HB_2 represents amphiprotic substituents such as $-NH_2$ and -OH. Also a value of one or zero was given to the chemicals with or without these substituents, respectively. The correlation between LC_{50} and ATP content value was analyzed by linear regression with computer system, and $P < 0.05$ was the significant level of the regression cases.

RESULTS AND DISCUSSION

Table 1 showed the LC_{50} and ATP values along with $logK_{ow}$, E_{homo} Es, HB_1 and HB_2 values used for the correlation analysis. For the test chemicals, we obtained that LC_{50} value was approximately related linearly with the ATP value (Equations 1-3). These results indicated that the ATP value decreased as the $log1/LC_{50}$ value increased (the toxicity increased), which may suggest the effect of the cellular oxidative phosphoryl action decreasing. The ATP value varied from 0.202 to 0.427 ug/mL and the $log1/LC_{50}$ value was from 2.67 to 6.87 mol/L (see Table 1). The linear correlation equations between $log1/LC_{50}$ and ATP were given:

Halogenated benzenes:

$$Log1/LC_{50} = 8.31 - 9.90\ ATP \quad (1)$$
$$r^2 = 0.87,\ SE = 0.331,\ F = 59.47,\ n = 18$$

Anilines:

$$Log1/LC_{50} = 7.71 - 12.62\ ATP \quad (2)$$
$$r^2 = 0.94,\ SE = 0.122,\ F = 51.27,\ n = 10$$

Nitrobenzenes:

$$Log1/LC_{50} = 9.70 - 16.65\ ATP \quad (3)$$
$$r^2 = 0.90,\ SE = 0.271,\ F = 44.42,\ n = 10$$

Where r^2 is the correlation coefficient, SE is the standard error, F is the variance ratio, and n is the number of compounds. As can be seen from Table 1, the order of toxicity for the substituted compounds was para- > meta- > ortho- substitutive groups, and $-NO_2$ > $-NH_2$ substituents. For halogenated compounds, I- > F- > Br- > Cl- substituents. For Nitro functional groups, the toxicity was greater than the other substitutive groups. Blume and Speece (1990) reported that the enzymatic reduction of the nitro group to reactive intermediates such as hydroxylamines was a likely mechanism in toxicity for different nitroaromatic isomers. This showed that the nitro functional groups may have a more specialized active toxicity and further studies are required.

Considering the effects of hydrogen accepting substitutents and amphiprotic substituents on

the reactive toxicity, the equations were formulated as follows:

Table 1. Molecular parameters and bioeffects of aromatic compounds

no.	compounds	LgKow	Ehomo (β)	Es	HB1	HB2	Lg1/LC50 (mol/L)	ATP (ug/mL)
1	l-chlorobenzene							
2	1,2-dichlorobenzene	3.38	0.93	0.54	2	0	4.62	0.410
3	1,3-dichlorobenzene	3.38	0.92	0.84	2	0	4.49	0.393
4	1,4-dichlorobenzene	3.39	0.92	1.14	2	0	4.65	0.381
5	1,2,3-trichlorobenzene	4.24	0.91	0.81	3	0	5.16	0.342
6	1,2,4-trichlorobenzene	4.25	0.90	1.11	3	0	5.16	0.322
7	1,2,3,4-tetrachlorobenzene	5.03	0.88	1.08	4	0	5.78	0.267
8	1,2,4,5-tetrachlorobenzene	5.05	0.86	1.38	4	0	5.75	0.285
9	pentachlorobenzene	5.23	0.86	1.39	5	0	5.97	0.252
10	hexachlorobenzene	6. 41	0.85	1.42	6	0	6.87	0.203
11	bromobenzene	2.79	0.95	0.08	1	0	4.19	0.395
12	1-chloro-4-bromobemnzene	3. 72	0.91	0.95	2	0	4.87	0.272
13	1,2-dibromobenzene	3.79	0. 87	0.57	2	0	4.79	0.347
14	1,3-dibromobenzene	3.81	0. 88	0.46	2	0	4.77	0.348
15	1,4-dibromobenzene	4.25	0. 85	0.76	2	0	5.01	0.264
16	fluorobenzene	2.61	0. 92	0.78	1	0	4.48	0.372
17	iodoberuene	3. 32	0. 91	-0.16	1	0	5.29	0.307
18	diphenyl	4.23	0.88	0.24	0	0	5.38	0.299
19	aniline	0. 95	0.87	0.63	0	1	2.67	0.404
20	4-aminoaniline	2.09	0.78	1.86	0	2	3.37	0.344
21	2-chloroaniline	1.83	0.84	0.90	1	1	3.29	0.350
22	3-chloroaniline	1.88	0.83	1.20	1	1	3.36	0.345
23	4-chloroaniline	1.90	0.82	1.50	1	1	3.37	0.337
24	4-bromoaniline	2.21	0.81	1.01	1	1	3.48	0.314
25	3-iodoaniline	2.85	0.76	0.77	1	1	4.37	0.264
26	2,6-dichloroaniline	2.56	0.80	1.17	2	1	4.01	0.305
27	3,4-dichloroaniline	2.74	0.79	1.47	2	1	3.86	0.308
28	2!4-dichloroaniline	2.75	0.77	1.47	2	1	4.01	0.302
29	nitrobenzene	1. 80	0.86	-1.28	1	0	3.33	0.345
30	2--methylnitrobenzene	2. 50	0.82	-1.28	1	1	4.53	0.324
31	3--methylnitrobenzene	2. 52	0.82	1.28	1	1	4.53	0.324
32	4--methylnitrobenzene	2. 52	0.81	-0. 98	1	1	4.55	0.323
33	2-nitroaniline	2. 33	0.58	-0.65	1	1	4.92	0.293
34	3-nitroaniline	2. 37	0.56	-0.65	1	1	5.00	0.286
35	4-nitroaniline	2. 34	0.55	0. 45	1	1	5.07	0.281
36	2-chloronitrobenzene	2. 21	0.52	-1.01	2	0	5.32	0.261
37	3-chloronitrobenzene	2. 22	0.50	-1.01	2	0	5.54	0.244
38	4-chloronitrobenzene	2. 24	0.51	1. 01	2	0	5.55	0.242

Halogenated benzenes:

$$Log1/LC_{50} = 0.731LogK_{ow} - 0.243E_{homo} + 0.211Es + 0.014HB_1 + \quad (4)$$
$$r^2 = 0.92,\ SE = 0.242,\ n = 18$$

$$ATP = -0.047LogK_{ow} + 0.668E_{homo} - 0.003Es + 0.007HB_1 - 0.103 \quad (5)$$
$$r^2 = 0.86,\ SE = 0.033,\ n = 18$$

Anilines:

$$Log1/LC_{50} = 0.399LogK_{ow} - 7.056E_{homo} - 0.298Es + 0.103HB_1$$
$$- 0.060HB_2 + 8.716 \quad (6)$$
$$r^2 = 0.94,\ SE = 0.128,\ n = 10$$

$$ATP = -0.093LogK_{ow} + 0.121E_{homo} - 0.002Es + 0.028HB_1$$
$$+ 0.042HB_2 + 0.552 \quad (7)$$
$$r^2 = 0.95,\ SE = 0.008,\ n = 10$$

Nitrobenzenes:

$$Log1/LC_{50} = 1.903LogK_{ow} - 3.051E_{homo} + 0.014Es + 0.254HB_1$$
$$- 0.298HB_2 + 2.293 \quad (8)$$
$$r^2 = 0.97,\ SE = 0.064,\ n = 10$$

$$ATP = -0.145K_{ow} + 0.242E_{homo} - 0.001Es + 0.051HB_1 + 0.094HB_2 + 0.346 \quad (9)$$
$$r^2=0.97, SE= 0.005, n= 10$$

In these equations, the importance of hydrogen-bonding characteristics of substituents in governing the toxicity and the ATP content was shown by HB_1 and HB_2. To the hydrogen accepting substituents (HB_1), the contribution of HB_1 can increase the values of $Log1/LC_{50}$ and ATP (Equations 4-9). While to the amphiprotic substituents (HB_2), the contribution of HB_2 can increase the values of ATP and decrease that of $Log1/LC_{50}$. These indicated that the hydrogen accepting substituents have a positive effect on the toxic reaction while the amphiprotic substituents have a negative effect (Equations 4,6,8), and both of these hydrogen-bonding patterns of substituents may have an effect on the energy metabolism site of ATP inside the liver cells (Equations 5,7,9). However, further analysis should be done by increasing the number of test chemicals.

For acute toxicity, Equations 4,6,8 indicated that toxicity increased with $LogK_{ow}$, E_{homo}(a negative number), HB_1 values increasing and HB_2 value decreasing. Because the highest energy occupied orbital places the greatest election density between the reactive molecular orbitals, and the b value of E_{homo} is a negative number(Heilbronner and Bock 1976), so the electronic effect of molecular orbital (E_{homo}) is positively correlated with toxicity of the test compounds. The steric parameter Es increased with increasing $Log1/LC_{50}$ values in Equation 4 and Equation 8. But for anilines, Es was negatively related with $Log1/LC_{50}$ in Equation 6. For the oxidative phosphorylation of energy metabolism, the ATP content decreased with increasing $LogK_{ow}$ E_{homo}, Es values and decreasing HB_1, HB_2 values from Equations 5,7,9. These situations indicated that the higher the electron density of molecular orbital of substituent(E_{homo}), the molecular hydrophobicity($LogK_{ow}$), and the steric effect of substituent(Es) at the hydrogen-donating/accepting sites in functional groups are, the greater the inhibition of the process of energy metabolism in the cell, and the larger the effect of the hydrogen bonding between the compound and the target site where the metabolism is at work. The amphiprotic substituents such as $-NH_2$ and -OH were more prone to interact with the target site than the hydrogen acceptor substituents such as $-NO_2$, -Cl or -Br from the slope of the equations 7, 8, and 9. The biological mechanism of toxicity had been interpreted to relate to enzyme leakage, inhibition of respiration, uncoupling of oxidative phosphorylation, resistance of transport process, and impairment of organism growth (Fry and Bridges 1976). In our research, the results indicated that the substituted benzenes cause a linear depletion of ATP content with the toxicity($Log1/LC_{50}$) increasing. The ATP content showed a good correlation with $Log1/LC_{50}$. The LC_{50} and ATP values can be estimated by the hydrophobicity(K_{ow}), molecular orbital energy(E_{homo}), and steric parameters(Es), in addition to the hydrogen-bonding capability (Equations 4-9). In this predicted relationship, the K_{oW} and the E_{homo} are the main factors. This suggested that toxicity of the test compounds was mainly due to the damage to the energy metabolism and reduction of ATP content.

Although a few experimental as well as theoretical problems are still left for the complete elaboration of compounds and descriptive parameters, the present results could be used to predict the reactive toxicity of substituted aromatic compounds as well as other analogs having similar simple substituents. For a more comprehensive understanding of the mechanism of the reactive toxic action and to explore possible relationships between chemical and organism toxicities, future extensive studies are necessary by increasing the number of test compounds.

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