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Applications of Frontier Molecular Orbital Energies in QSAR Studies

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Quantitative Structure-Activity Relationships (QSAR) are frequently used to study the quantitative relationships between the toxicities and the molecular descriptors of a series of organic compounds (Blum and Speece 1990). Deliberate QSAR equations can be used to estimate the bioactivities of untested compounds, which is valuable for rapid screening toxicants.

Most QSAR studies were based on the Hansch method, and physical-chemical parameters were commonly used to describe the molecular structures (Nevalainen et al 1994). In present study, the quantum descriptors of organic pollutants were introduced into the QSAR method on the basis of "Target Theory" (Hansch et al 1964). The relationships between the molecular orbital energies and the acute toxicities to aquatic organisms of halogenated benzenes, anilines and phenols were studied in this paper.

MATERIALS AND METHODS

The Microtox of 33 organic chemicals were tested in present studies (see Table 1). The purity of all the chemicals tested was analytical grade. The test organism was photobacteria (*Photobacterium phosphoreum*). The concentration values causing 50% inhibition of

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bioluminescence after 15 min exposure (EC50) at 20 °C was tested as toxicity index (displayed in Table 1). Microtox test was performed using DXY-2 toxicity analyzer (Institute of Soil Science, Academia Sinica, Nanjing).

Table 1. The substituted benzenes studied

No.	Chemicals	No.	Chemicals
	Halogenated Benzenes	24*	2,6-dichloroaniline
1*	1,2,4,5tetrachlorobenzene	25*	2,4-dichloroaniline
2*	1,2,4-trichlorobenzene	26*	3,4-dichloroaniline
3*	1,2,3-trichlorobenzene	27*	3-chloro-4-floro-aniline
4*	1,4-dichlorobenzene	28*	4-chloro-aniline
5*	1,3-dichlorobenzene	29*	4-bromo-aniline
6*	1,2-dichlorobenzene	30	2-chloro-4-methyl-aniline
7*	chlorobenzene	31	2-chloro-aniline
8*	1,4-dibromobenzene	32*	aniline
9*	1,3-dibromobenzene		**Phenols**
10*	1-chloro-4-bromobenzene	33*	pentachlorophenol
11*	2,4,5-trichlorotoluene	34*	2,4-dichlorophenol
12*	2,5-dichlorotoluene	35*	4-chlororphenol
13*	4-chlorotoluene	36*	2-chloro-phenol
14*	methylbenzene	37*	2-methylphenol
15*	1,4-dimethylbenzne	38*	1,3-dihydroxybenzne
16*	1,3-dimethylbenzene	39*	phenol
17*	bromobenzene	40	3-methyl-phenol
18	3-chloro-methylbenzene	41	4-methyl-phenol
19	1,2-methylbenzene	42	2,4-methyl-phenol
20	3,4-chloro-methylbenzene	43	2,6-methyl-phenol
21	2,4-chloro-methylbenzene	44	3,4-methyl-phenol
22*	benzene		
	Anilines		
23*	2,4,6-trichloroaniline		

* The compounds used in Microtox test in present study

The 96-hr half-lethal concentration values (LC50) to fathead minnow (*Pimephales promelas*) of 32 chemicals (see Table 1 and Table 2) are from references (Abernethy et al 1988; Hall et al 1989), in which the flow-through protocol was employed in the test.

Table 2. The toxicity to aquatic organisms and molecular descriptors of studied chemicals.

No.	$LogK_{ow}$	E_{homo} (β)	-LogEC50* (mol/L)		-LogLC50** (mol/L)	
			Obs.	Cal.	Obs.	Cal.
1	5.05	0.8596	5.51	5.21	5.85	5.51
2	4.27	0.8872	4.50	4.72	5.00	4.91
3	4.27	0.9233	4.53	4.66	4.89	4.86
4	3.59	0.9043	4.39	4.29	4.62	4.40
5	3.55	0.9271	4.24	4.23	4.30	4.34
6	3.55	0.9232	4.38	4.24	4.40	4.34
7	2.81	0.9500	3.86	3.77	3.77	3.78
8	4.07	0.9015	4.54	4.58		
9	4.07	0.9239	4.99	4.54		
10	3.83	0.9028	4.50	4.43		
11	4.93	0.8300	4.86	5.19		
12	4.04	0.8627	4.38	4.62		
13	3.31	0.8664	3.88	4.19	4.33	4.26
14	2.33	0.9082	3.08	3.56	3.32	3.50
15	3.09	0.8313	3.68	4.12	4.21	4.16
16	3.09	0.8733	3.65	4.05		
17	2.99	0.9464	3.78	3.99	3.89	3.91
18	3.50	0.9180			3.84	4.32
19	3.09	0.9027			3.48	4.05
20	4.21	0.8775			4.74	4.88
21	4.21	0.8766			4.54	4.88
22	2.13	1.0000	3.34	3.30	3.40	3.22
23	3.04	0.5328	4.51	4.55		
24	2.32	0.5138	4.16	4.16		
25	2.32	0.5080	4.09	4.17		
26	2.32	0.5207	4.20	4.15	4.33	4.10
27	1.17	0.5031	3.28	3.50		
28	1.61	0.5228	3.57	3.73		
29	2.05	0.5259	3.92	3.98	3.56	3.90
30	2.27	0.5098			3.60	4.08
31	1.90	0.5280			4.34	3.79
32	0.90	0.5436	3.28	3.28	2.84	3.05
33	5.01	0.7148	5.69	5.41	6.06	5.71
34	2.88	0.7393	4.45	4.14	4.30	4.15

Table 2 (continued)

No.	$LogK_{ow}$	E_{homo} (β)	-LogEC50* (mol/L)		-LogLC50** (mol/L)	
			Obs.	Cal.	Obs.	Cal.
35	2.50	0.7562	4.48	3.89#		
36	2.50	0.7722	4.14	3.86	4.02	3.83
37	2.12	0.7516	3.75	3.68	3.77	3.60
38	0.79	0.7306	3.00	2.93	3.04	2.68
39	1.46	0.7916	3.64	3.23	3.51	3.06
40	2.12	0.7836			3.29	3.54
41	2.12	0.7514			3.58	3.60
42	2.78	0.7331			3.86	4.09
43	2.78	0.7532			3.75	4.06
44	2.78	0.7428			3.90	4.08

* Toxicity to photobacteria
** Toxicity to fathead minnow
# The values of observed and calculated are significantly different statistically ($P<0.05$)

Molecular orbital energy was calculated following Hückel molecular orbital (HMO) methods (Rackstraw 1976). Octanol/water partition coefficients ($LogK_{ow}$) are from Yuanhui Zhao et al. (1993), or calculated according to Hansch and Leo (1979).

The stepwise regression analysis program in Statgraphics software (STSC Inc. Rockville MD. USA 1987) was used in statistical analysis.

RESULTS AND DISCUSSION

"Target Theory" considers that the bioactivities occur as a result of the interactions between toxicants and receptors in organisms.

Two basic assumptions are included in "Target Theory": (1) the interactions between receptors and toxicants were reversible; (2) the biological effect is proportional to

the number of receptors reacted with the toxicants. Based on the above assumptions, theoretical relationship between the acute toxicity of organic chemicals to aquatic organism and their molecular descriptors was obtained (Yuanhui Zhao 1993) :

$$-\text{LogEC50}=\text{LogK} + \text{LogBCF} + \text{C} \qquad (1)$$

Where K is the equilibrium constant of the interaction between chemicals and receptors. BCF is the bioconcentration factor of aquatic organism to chemicals. Equation 1 suggests that the toxicity of organic chemicals are determined by two factors: (1) the bioconcentration abilities of toxicants, which determine the concentration of toxicants around receptors in organisms; (2) the reactivities of toxicants with receptors.

In QSAR studies, the receptor site in an organism is generally assumed specific for the series of chemicals studied; thus, the equilibrium constants of the reactions between toxicants and receptors are anticipated to be mainly affected by the reactivities of toxicant chemicals. Because frontier molecular orbital energies may be used to describe the reactivities of organic chemicals, we may assume that LogK was linearly related to the frontier molecular orbital energies of organic toxicants:

$$\text{LogK}=\text{b}E_{\text{homo}} + \text{c}E_{\text{lumo}} + \text{d} \qquad (2)$$

Where E_{homo} is the highest occupied molecular orbital energy, E_{lumo} is the lowest unoccupied molecular orbital energy, and b, c and d are regression constants.

Earlier studies showed that the bioconcentration factors of organic compounds to aquatic organism can be modeled by octanol/water partition coefficients (Barron 1990) :

$$\text{LogBCF}=\text{a}\,\text{Log}K_{ow}+\text{e} \qquad (3)$$

Where K_{ow} is octanol/water partition coefficient, and a and e are constants. Substituting eq.2 and eq.3 into eq.1, we may obtain:

$$-LogEC50=aLogK_{ow} + bE_{homo} + cE_{lumo} + d \quad (4)$$

Through stepwise multiple regression analysis, the best equations between acute toxicities and molecular descriptors studied were obtained.

For photobacteria:

$$-LogEC50=0.581LogK_{ow}-1.547E_{homo}+3.606 \quad (5)$$
$$n=33,\ R^2=0.83,\ F=74.88$$

For fathead minnow:

$$-LogLC50=0.710LogK_{ow}-1.558E_{homo}+3.261 \quad (6)$$
$$n=32,\ R^2=0.83,\ F=79.97$$

The R^2 values of both eq.5 and eq.6 show the significant regressions, the calculated LC50 and EC50 are also displayed in Table 1. For most compounds, the difference between experimental values and calculated values are insignificant statistically. With regard to the wide range of chemical structures in present study and the uncertainty of toxicity test, the regression models (eq.5 and eq.6) are acceptable, the F values of eq.5 and eq.6 indicate that the significance levels of both equations are less than 0.01.

The coefficient of $LogK_{ow}$ in eq.6 is slightly larger than that in eq.5, which can be explained by the difference of lipid contents in fish and in bacteria. Because the lipid contents in fish is generally more than those in bacteria, the organic chemicals tend to be easier to concentrate in fish than in bacteria.

The coefficients of E_{homo} in eq.5 and eq.6 are approximately equal, which implies that the mechanism of

toxicant-receptor interactions may be similar in fathead minnow and in photobacteria. This inference provides support for the possibility of use of surrogate organisms (Blum and speece 1990).

E_{lumo} is not significant in eq.5 and eq.6, which suggests that the interactions of studied compounds with receptors are mainly affected by E_{homo} of the compounds, while the influence of E_{lumo} is minor.

E_{homo} can be considered as the energy demand of removing an electron from the molecule, which is proportional to the electron-releasing abilities of the compounds as electron-donors. Since the unit of $E_{homo}(\beta)$ is negative, the negative coefficients of E_{homo} in eq.5 and eq.6 indicate that the greater the E_{homo} of the compounds, the stronger their toxicities to fathead minnow and bacteria. The positive correlations between E_{homo} and toxicities suggest that the reactive toxicants among present studied chemicals would react as electron-donors in the toxicant-receptor interactions.

From Table 1, we can see that the E_{homo} values of halogenated benzenes all are approximately 1.00 β, which are the lowest among the studied benzenes, phenols and anilines. The low E_{homo} of halogenated benzenes indicates that they would be inactive in toxicant-receptor interactions, thus they are ascribed nonreactive toxicants, which is consistent with the Verchaar's classification (Verchaar et al. 1993).

Based on "Target Theory", the frontier molecular orbital energies was introduced into the QSAR method in present study. The theoretical model about the acute toxicities to aquatic organisms was established, which was successfully applied to the toxicological studies of some substitutedaromatics, and significant structure-activity correlations are proposed as a result.

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