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Quantitative Structure-Activity Relationships of Organic Acids and Bases

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Study on the toxicity of organic acids and bases to aquatic organisms has received limited investigation (Blum and Speece 1990). Barron (1990) found that although both ionized and nonionized forms of a weak electrolyte were absorbable, the uptake of the nonionized form was generally faster. The result supports the point that only nonionized fraction contributes significantly to toxicity (Blum and Speece 1991). Good correlations have been achieved between toxic concentrations and pK_a under the assumption that the nonionized fraction of the chemical partitions most significantly into the lipid phase and contributes to toxicity (Saito et al. 1991; Schuurmann 1990). However, even when the toxic concentrations of chemicals with low pK_a values were reduced to account for the ionized fraction of the chemical, the lowest pK_a, chemicals still showed greater toxicity than expected. Blum and Speece (1991) suggested that this was due to reactive toxicity. Schultz (1987) found that pK_a could be used to distinguish between the toxic mechanisms of polar narcosis and the uncoupling of oxidative phosphorylation by phenols for *Tetrahymena pyriformis.* For the 14 chemicals considered, he found that polar narcotics had pK_a values greater than 8.0, while uncoupling agents had pK_a values less than 6.5. The investigation by Blum and Speece (1991) of ionisable compounds was primarily limited to phenols and reported an enhanced or reactive toxicity for compounds with pK_a values equal to or less than 6.0. While they suggest this cutoff value, they noted there was no distinct cutoff but rather a graded response was observed. They attributed this response to a balance between partitioning and the uncoupling mechanism of toxic action.

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In this paper, we will study further quantitative structure-activity relationships (QSARs) of organic acids and bases based on QSARS of hydrophobic organic chemicals and references and attempt to develop QSAR equations to estimate the toxicity of organic acids and bases to aquatic organisms.

MATERIALS AND METHODS

organic acids and bases tested and used in our QSARs study were: chlorobenzoicacids, bromobenzoicacids, aminobenzoic acids, phthalic acids, anilines, phenols. The organic acids and bases were purchased from different commercial sources (analytically pure) and were not repurified before testing. They were diluted with reconstituted hard water for *Daphnia magna* tests. All the test solutions were always regulated at pH=6.0± 0.1, pH=7.8±0.1, pH=9.0±0.1 with HCl or NaOH solution and the pH values were determined at beginning and end of tests with pH meter (make model pHS-25) during the tests.

Daphnia magna was cultured parthenogenetically in an enviromntal chamber at 22±2°c, with a photoperiod of 14 hr daylight/10 hr darkness. *Daphnia magna* were fed with a diet of green alga, and 6-24 -hr old *Daphnia magna* were used for the toxicity tests. Acute toxicity tests of organic acids and bases were conducted with a static method for 24 hr, with 10 *Daphnia magna* in 25 mL of test water. Each test used 60 *Daphnia magna* and numbers of immobilization were determined after 24 hr. The immbilization concentrations for 50% of *Daphnia magna* (IC_{50}, mol/L) at 24 hr were calculated from regression equations between percentages of immobilization and log of concentrations using STATGRAPHICS program. *Daphnia magna were* not fed during experimentations. The results were considered valid if dissolved oxygen measured at the end of the test was at least equal to 60% of saturation and if percentage of immobilization observed for the controls was zero between pH=6-9.

Descriptions of test procedure of concentrations for 50% mortality after 48 *hr* to carp (*Cyprinus carpiol,* LC_{50}, mol/L) and concentrations resulting in 50% inhibition of bioluminescence after 15 min exposure *to Photobacterium phosphoreum* (15 min-EC_{50}, mol/L) have appeared in a previous paper (Zhao and Wang 1993).

Octanol/water partition coefficients were determined by our laboratory (Wang et al. 1989). Molecular connectivity index values of first-order $({}^1\chi^v)$ were calculated according to reference (Kier and Hall 1976). Heteroatom valence delta values are: -Br(0.254), -Cl

(0.690), -F(20), =0(6), -OH(0.600), $-NH_2$(8). pK_a values were obtained from reference (Weast et al. 1988). The relationship between the physical-chemical parameters and toxicity was quantified by multiple linear regression (STATGRAPHICS program). For each regression, the following descriptive information is provided here: number of observations used in analysis (n), adjusted correlation coefficient (r^2), root mean square error (S). F tests were used to ensure that the relationship was significant at the 95% level.

RESULTS AND DISCUSSION

In recent work, Zhao and Wang (1993, 1995) have established a theoretical equation of quantitative structure-activity to estimte the toxicity of hydrophobic organic chemicals to aquatic organisns. The resulting equation:

$$\log\frac{1}{[A]_w} = a^1\chi^v + b\ \log K_{ow} + c \qquad (1)$$

where $[A]_w$ is toxic concentration of a chemical in water causing a specific biological effect, usually expressed by EC_{50}, LC_{50}, or IC_{50}; $^1\chi^v$ is connectivity index of first-order; K_{ow} is octanol/water partition coefficient; a,b,c are constants.

Using Eq.1, good relationships between toxicity data (LC_{50}, EC_{50}, IC_{50}) and parameters were achieved for three aquatic species (*Photobacterium phosphoreum, Daphnia magna* and fathead minnows (*Pimephales promelas*), respectively) with wider range of chemicals:

$$\log\frac{1}{EC_{50}} = 0.853\,^1\chi^v + 0.188\ \log K_{ow} + 0.820 \qquad (2)$$
$$n = 77 \quad r^2 = 0.86 \quad S = 0.37 \quad F = 221$$

$$\log\frac{1}{IC_{50}} = 0.494\,^1\chi^v + 0.312\ \log K_{ow} + 1.836 \qquad (3)$$
$$n = 53 \quad r^2 = 0.82 \quad S = 0.26 \quad F = 114$$

$$\log\frac{1}{LC_{50}} = 0.640\,^1\chi^v + 0.457\ \log K_{ow} + 0.749 \qquad (4)$$
$$n = 38 \quad r^2 = 0.90 \quad S = 0.35 \quad F = 147$$

The chemicals used in Eq.2-4 include 77 compounds such as chlorobenzenes, brombenzenes, nitrobenzenes, anilines, phenols, benzaldehydes, toluenes, and some chlorinated alkanes.

In contrast with hydrophobic organic chemicals, organic acids and bases have two forms in water: ionized and nonionized. Because Kow values of organic acids we observed are the octanol/water partition coefficients of nonionized form (Wang et al. 1989), predicted toxicity data by Eq.l-4 are the toxicity data of nonionized form other than toxicity data of total acids. For convenience sake, we use $[AH]_w$ to express monoacid concentration of nonionized form, $[A^-]_w$ to express monoacid concentration of ionized form, $[AH]_T$ to express total concentration of monoacid. So $[AH]_w+[A^-]_w = [AH]_T$. Barron (1990) found that although both ionized and nonionized forms of a weak electrolyte are absorbable, the uptake of the nonionized form is generally faster in aquatic organisms. If we presume that only the nonionized fraction contributes significantly to toxicity and neglect contribution of ionized fraction to toxicity (Blum and Speece 1991), the toxicity of nonionized fraction can be estimated by Eq.1-4:

$$\log\frac{1}{[AH]_w} = a^1x^v + b\log K_{ow} + c \qquad (5)$$

Generally, the toxic concentrations of organic acids and bases to aquatic organisms we observed are expressed by total concentrations of nonionized and ionized forms other than concentrations of nonionized form. Therefore we should change nonionized form concentration into total concentration in Fq.5. Let us consider the dissociation equilibria of a monoacid AH in water.

$$AH = A^- + H^+ \qquad K_a^A = \frac{[H^+]\,[A^-]_w}{[AH]_w} \qquad (6)$$

$$\frac{[AH]_w}{[AH]_w + [A^-]_w} = \frac{[AH]_w}{[AH]_T} = \frac{1}{1+K_a^A/[H^+]} = \frac{1}{1+10^{pH-pK_a^A}}$$

$$[AH]_w = \frac{[AH]_T}{1+10^{pH-pK_a^A}} \qquad (8)$$

where pK_a^A is dissociation constant of monoacid. Introducing Eq.8 into Eq. 5, we obtain Eq.9:

$$\log\frac{1}{[AH]_T} = a^1x^v + b\log K_{ow} + c - \log(1+10^{pH-pK_a^A}) \qquad (9)$$

Similar to monoacid, we could obtain Eq.10-12 to estimate the toxicity of diacid [AH_2], monobase [BH^+], both acid and base [AH_2^+] to aquatic organism.

Diacid (AH_2)

$$\log\frac{1}{[AH_2]_T} = a\,{}^1x^v + b\log K_{ow} + c - \log(1+10^{pH-pK_{a1}^A}+10^{2pH-pK_{a1}^A-pK_{a2}^A}) \qquad (10)$$

Monobase (BH^+)

$$\log\frac{1}{[BH^+]_T} = a\,{}^1x^v + b\log K_{ow} + c - \log(1+10^{pK_a^B-pH}) \qquad \mathbf{(11)}$$

Both acid and base (AH_2^+)

$$\log\frac{1}{[AH_2^+]_T} = a\,{}^1x^v + b\log K_{ow} + c - \log(1+10^{pK_{a1}^B-pH}+10^{pH-pK_{a2}^A}) \qquad (12)$$

where $[AH]_T$, $[AH_2]_T$, $[BH^+]_T$, $[AH_2^+]_T$ are total concentrations of organic acids and bases in water causing a specific biological effect, usually expressed by EC_{50}, LC_{50} or IC_{50}; pK_a^B is dissociation constant of organic base.

Eq.9-12 are the theoretical equations we established to estimate the toxicity of organic acids and bases to three aguatic species. Introducing Eq.3-4 into Eq.9-12, we obtain Eq.13-14 (Here, only monoacid equations are enumerated):

$$\log\frac{1}{IC_{50}} = 0.494\,{}^1x^v + 0.312\log K_{ow} + 1.836 - \log(1 + 10^{pH-pK_a^A}) \qquad (13)$$

$$\log\frac{1}{LC_{50}} = 0.640\,{}^1x^v + 0.457\log K_{ow} + 0.749 - \log(1 + 10^{pH-pK_a^A}) \qquad (14)$$

The LC_{50}, IC_{50} and EC_{50} values predicted by Eq.2-4,12-14, together with physical-chemical parameters are summarized in Table 1. Anilines and phenols mainly consist of nonionized format pH=6-9 according to the calculation of Eq.9-12. Thus the toxicity of anilines and phenols should be predicted by regression equations of hydrophobic chemicals (Fq.2-4). Table 1 shows that predicted toxicities are close to

Table 1 Toxicity data of *Photobacterium phosphoruem, Daphnia magna,* Carp and physical-chemical parameters of organic acids and bases

					$\log 1/EC_{50}$		$\log 1/IC_{50}$							$\log 1/LC_{50}$[a]		
Organic acids and bases	$^1\chi^v$	$\log K_{ow}$	pK_{a1}	pK_{a2}	Obs	Cal[b]	Obs	Cal[c]	Obs	Cal[c]	Obs	Cal[c]	Cal[d]	Obs[e]	Cal[f]	Cal[g]
					(pH=7)		(pH=6)		(pH=7.8)		(pH=9)			(pH=7)		
Benzoic acid	3.01	1.86	4.21		4.09	3.74	2.74	2.11	2.24	0.31	2.05	−0.89	3.90	<2.39	0.74	3.53
4-Chlorobenzoic acid	3.52	2.65	3.98		4.39	4.32	3.02	2.38	2.41	0.58	2.32	−0.62	4.40	≤2.80	1.19	4.21
3-Chlorobenzoic acid	3.52	2.68	3.82		4.32	4.33	3.11	2.23	2.75	0.44	2.27	−0.76	4.41	≤2.80	1.03	4.23
2-Chlorobenzoic acid	3.54	2.66	2.94		4.17	4.33		1.35		−0.45		−1.65	4.41	≤2.80	0.17	4.23
4-Bromobenzoic acid	3.91	2.86	4.00		4.83	4.69	3.41	2.60	2.55	0.80	2.46	−0.40	4.60	≤2.90	1.56	4.56
3-Bromobenzoic acid	3.91	2.87	3.86		4.66	4.69	3.29	2.52	3.11	0.72	2.61	−0.48	4.66	≤2.90	1.42	4.56
4-Fluorobenzoic acid	3.03	2.07	3.87		3.96	4.79	2.61	1.85	2.15	0.04	1.57	−1.14	3.98	<2.75	0.50	3.63
4-Aminobenzoic acid	3.10	0.64	2.38	4.89	4.00	3.58	3.01	2.46	2.40	0.66	2.12	−0.54	3.57	<2.43	0.92	3.03
3-Aminobenzoic acid	3.10	0.64	3.07	4.74	3.87	3.58	2.88	2.31	2.45	0.51	2.20	−0.69	3.57	<2.43	0.77	3.03
2-Aminobenzoic acid	3.12	1.21	2.05	4.95	4.00	3.71	3.47	2.70	2.88	0.89	2.47	−0.30	3.75	<2.43	1.25	3.30
m-Phthalic acid	4.01	1.66	3.62	4.60	4.23	4.55	1.67	0.56	1.44	−3.05	1.34	−5.45	4.33	<2.52	−0.71	4.07
o-Phthalic acid	4.03	1.61	2.95	5.41	4.40	4.55	1.58	0.69	1.53	−2.87	1.41	−5.27	4.33	<2.52	−1.58	4.06
Aniline	2.09	0.93	4.63		3.28	2.78		3.16	3.58	3.16		3.16	3.16	2.84	2.51	2.51
3,4-Dichloroaniline	3.12	2.55			4.20	3.96			4.26				4.17	4.33		3.91
4-Bromoaniline	2.99	2.05	3.86		3.92	3.76		3.95	4.35	3.95		3.95	3.95	3.56	3.60	3.60
4-Chloroaniline	2.60	1.90	4.15		3.57	3.40		3.71	3.85	3.71		3.71	3.71		3.28	3.28
2,4,6-Trichloroaniline	3.64	3.04			4.51	4.49			4.20				4.58			4.47
3-Chloro-4-fluoroaniline	2.63	2.04			3.28	3.45			3.69				3.77			3.36
Phenol	2.56	1.46	9.89		3.64	3.28		3.56	3.36	3.56		3.51	3.56	3.51	3.05	3.05
2-Chlorophenol	3.08	2.18	8.55		4.14	3.86		4.04	3.95	3.97		3.46	4.04	4.02	3.72	3.72

a)Carp (*Cyrinus carpiol*) toxicity data, experiments show toxicity data of organic acids to carp are close to toxicity data of chemicals to fathead minnows (Abernethy et al. 1988 and Hall et al. 1989). b)Calculated from Eq.2. Calculated from Eq.13 or 12. d)Calculated from Eq.3. e)No concentration with more than 50% inhibition could be tested for organic acids. f)Calculated from Eq.14 or 12. g)Calculated from Eq.4.

observed toxicity for phenols and anilines using Eq.2-4.

Table 1 shows that observed toxicity of organic acids to *Photobacterium phosphoreum* is higher than the toxicity of organic acids to carp and *Daphnia magna.* No concentration with more than 50% inhibition could be tested to carp. For some organic acids, we did not observed biological effect even at concentrations approaching saturation, pH=7. We suggest that the difference can be attributed to different constitution of species studied. *Photobacterium phosphoreun* is an unicellular organism. The obstruction of the cell wall to ionized form is smaller than the obstruction of fish or *Daphnia magna* tissues. Not only nonionized form but also ionized form could pass through the cell membrane to contribute toxicity. Therefore the toxicity of organic acids *to Photobacterium phosphoreum* should be predicted by Eq.2. Results show the observed and predicted toxicity data are in good agreement. In contrast with *Photobacterium phosphoreum,* the permeability of the plasm membrane of fish (or *Daphnia magna*) gill and other tissues (e.g., skin) constitutes a common barrier to adsorption and transfer of chemicals (Barron 1990). The uptake of nonionized form is greatly faster than ionized form in fish or *Daphnia magna* (Blum and Speece 1991). Only the nonionized fraction contributes significantly to toxicity. Therefore the toxicity of organic acids to fish or *Daphnia magna* is lower than toxicity *to Photobacterium phosphoreum.* In order to confirm the viewpoint, the toxicity data are predicted by Eq.3-4 (not considering the effect of ionization; both ionized and nonionized forms contribute to toxicity) and Eq.12-14 (only nonionized form contributes to toxicity). Results show that predicted data of Fq.3-4 are greatly higher than the observed data and predicted data of Eq.12-14 are lower than the observed data (Table 1). The results of pH effect on toxicity to *Daphnia magna* are also sumarized in Table 1. The toxicity significantly decreases as pH increases. The lower pH (the amount of nonionized fraction increasing), the higher the toxicity to *Daphnia magna.* The predicted values of toxicity data are close to observed toxicity data at low pH. Above results support the pointthat nonionized fractioncontributes significantly to toxicity. But ionized form can slowly pass through the fish or *Daphnia magna* tissues to contribute insignificantly to toxicity too.

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