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A K_{ow}-Based QSAR Model for Predicting Toxicity of Halogenated Benzenes to all Algae Regardless of Species

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Abstract In this study, a total of 40 tests of the toxicity of 8 halogenated benzenes to five algal species were performed. The result demonstrated that the toxicity of halogenated benzenes to five algal species was directly related to the hydrophobicity of the chemicals and the lipid content of the algae. Based on the results, we developed a K_{ow}-based quantitative structure–activity relationship (QSAR) model: $\log(1/EC_{50}) = 1.050\log K_{ow} + 1.429\log(1/lipid) - 3.224$ with $n = 40$, $r^2 = 0.946$, S.E. $= 0.211$, $F = 323.933$ at $p < 0.001$. This model provides evidence that the toxicity of halogenated benzenes to these five algae tested is related to the slower transference into lipid. This model can potentially be generalized to other algal species and toxicants.

Keywords QSAR · Toxicity · Halogenated benzenes · Algal species

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Algae are ubiquitous species that serve as primary producers in aquatic ecosystems. Because of their ecological importance, sensitivity to many toxicants, ready availability, and ease of culture, algae are often used in toxicity testing. Algal toxicity tests have been an important consideration when assessing the hazards of chemicals to aquatic organisms. However, there are millions of organic chemicals in aquatic ecosystems (Grzybowski et al. 2009) and the relative risks of all the chemicals to all the algae are nearly impossible to assess experimentally. Therefore, it is highly desirable to develop and validate rapid, sensitive and cost-effective models to predict the potential risk of these chemicals to algal species.

Quantitative structure–activity relationships (QSARs) have been proved to be important tools for the toxicity prediction of organic chemicals in aquatic toxicology, and the REACH (Registration, Evaluation, and Authorization of Chemical) system has given incentive to develop, validate and apply QSARs for predicting chemical toxicity (Cronin et al. 2003). Many QSAR models have been successfully developed based on the algal toxicity data. For example, Kong et al. (1998) derived two QSAR models based on the toxicity of four halogeno-benzens to green alga *Selenastrum capricornutum*; Netzeva et al. (2004) established a QSAR model by determining the toxicity of 65 aromatic compounds to *Chlorella vulgaris*. However, the universal application of these QSAR models needs to be validated, because each of these models was derived from only a specific alga. In fact, various algal species, even within the same genus, respond differently to even the same chemical. For instance, Sáenz et al. (1997) reported that *Scenedesmus acutus* and *S. quadricauda* had different sensitivity to paraquat. Johnson et al. (2007) found that different algal species had different sensitivity to fluoxetine, fluvoxamine and sertraline. These different algal

sensitivities suggest that each algal species should have its own QSAR model. However, it is not practical to establish the model for every algal species because there are countless algal species. Thus, it is important and urgent to develop a QSAR model for predicting toxicological effect on all algae regardless of their species.

The mechanism of organic chemicals uptake by algae has been studied. A prevailing hypothesis is that the uptake is a rapid surface sorption followed by a slower transference into lipids in the cell matrix (Swackhamer 1991; Tang et al. 1998). Since the rapid sorption may be related to the cell size, Wang et al. (1997a) have found that the bioconcentration factor (log BCF) has linear relationship with the specific surface area (log *S*) of algae cells. And Swackhamer (1991) has pointed out that the large surface area-to-volume ratio (*A/V*) of an alga may promote the uptake of pollutants. The subsequent slower phase includes the transference of chemical molecules into lipids of the cell, so the process may be related to lipid content. For example, Kent and Currie (1995) found that the lipid contents in several species of algae were correlated to the sensitivity to the lipophilic organophosphate insecticide fenitrothion. All these studies have shown that the aforementioned three factors (log *S*, *A/V* and *lipid content*) affect the uptake of organic chemicals by algae. However, since the uptake is unequal to the toxicity (Bradbury and Coats 1985; Call et al. 1987; Daus et al. 2010), it still remains unclear whether these three parameters contribute to the toxicity of organic pollutants to algae, whether there are any other associated factors, and how all these factors work. These were the questions that we attempted to address in this study.

The purposes of this study are (1) to determine the toxicity of 8 halogenated benzenes to five algal species, (2) to find the algal cofactors that may contribute to the toxicity of halogenated benzenes to five algal species, and (3) to develop a K_{ow}-based QSAR model for five algal species using the relationship between the toxicity of halogenated benzenes and the algal cofactors.

Materials and Methods

The following eight halogenated benzene chemicals (reagent grade, purity $\geq$ 98%) were tested in the present study: benzene, chlorobenzene, bromobenzene, 1,4-dichlorobenzene, 1-bromo-4-chlorobenzene, 1,4-dibromobenzene, 1,2,3-trichlorobenzene, 1-bromo-2,3-dichlorobenzene. They werewere purchased from Sigma–Aldrich (St. Louis, MO, USA).

The five algal species in this study were *Nannochloropsis oculata*, *Dunaliella salina var.*, *Platyformas subcordiformis*, *Chlorella marine*, *Skeletonema costatum Gneville*. They were obtained from the South China Sea Institute of Oceanology, Chinese Academy of Sciences. The cultures were maintained in f/2 marine medium (pH 7.8 $\pm$ 0.2) at 23 $\pm$ 1°C, with a 12:12 h light/dark cycle at 2,000–3,000 lux.

For eight chemicals individually studied, six concentrations in a geometric progression ranging from no effect to 100% growth inhibition concentration were planned after preliminary experiments. The alga in the logarithmic growing period was inoculated into 100 mL Erlenmeyer flasks, containing 30 mL culture media, and chemicals. The culture media without test chemicals served as control solution. The initial concentration of the cell inoculum of each species was 2×10^4 cells/mL in both treated and control flasks. All experiments were repeated three times. Data were expressed as effective concentration and its 95% confidence intervals at 50% response. Growth inhibition was determined after 96 h with counting chamber (USEPA 1996) under a microscope. Median effective concentration (EC_{50}) of a chemical to alga was calculated (OECD 2006) based on the decrease in algal biomass.

All statistical analyses were performed using SPSS 16.0 software package (SPSS Inc). The coefficient of determination (r^2), standard error (S.E.), F-ratio, and *p*-value were considered when testing the quality of the regression.

Results and Discussion

The toxicity of eight halogenated benzenes to five algal species was determined and the results are shown in Table 1. The octanol/water partition coefficients (K_{ow}) of these chemicals are shown in Table 2. It is well known that halogenated benzenes affect the organism only by the distribution between water and organic phases (Kőnemann 1981a). Therefore K_{ow} is one of the most effective parameters for QSAR models for nonpolar narcotic chemicals (Kőnemann 1981b). The relationships between $\log(1/EC_{50})$ and $\log K_{ow}$ were derived for individual algae and the results are listed in Table 3.

Equations 1–5 (in Table 3) were characterized by high correlation coefficients and small standard errors. These significance coefficients of the $\log(1/EC_{50})-\log K_{ow}$ relationship once again indicated that the toxicity could be predicted by the partition coefficients (Kőnemann 1981a).

From Eqs. 1–5, since K_{ow} was the effective parameters for QSAR models, a K_{ow}-based QSAR model for all five algal species based on the data listed in Tables 1 and 2 was obtained as follows,

$$\log(1/EC_{50}) = 1.050 \log K_{ow} - 0.343 \quad (6)$$

$n = 40$, $r^2 = 0.810$, S.E. = 0.390, $F = 161.809$, $p < 0.001$.

Table 1 Toxicity of eight chemicals to individual algae

Chemicals		log(1/EC$_{50}$) [mol/L]				
		Nannochlo-ropsis oculata	*Dunaliella salina var.*	*Platyformas subcord-iformis*	*Chlorella marine*	*Skeletonema costatum Gneville*
Benzene	Obs.	1.64 (1.62–1.66)[a]	1.42 (1.40–1.44)[a]	1.49 (1.34–1.64)[a]	1.68 (1.55–1.80)[a]	2.55 (2.53–2.56)[a]
	Pre.[b]	1.61	1.72	1.73	1.89	2.51
	Diff.	0.03	−0.30	−0.24	−0.21	0.04
Chlorobenzene	Obs.	2.63 (2.52–2.73)[a]	2.44 (2.40–2.48)[a]	2.46 (2.41–2.51)[a]	2.66 (2.56–2.76)[a]	3.40 (3.33–3.47)[a]
	Pre.[b]	2.33	2.44	2.45	2.60	3.23
	Diff.	0.30	0	0.01	0.06	0.17
Bromobenzene	Obs.	3.05 (3.03–3.07)[a]	2.81 (2.80–2.83)[a]	2.83 (2.82–2.85)[a]	2.97 (2.92–3.03)[a]	3.69 (3.64–3.74)[a]
	Pre.[b]	2.51	2.63	2.63	2.79	3.42
	Diff.	0.54	0.18	0.20	0.18	0.27
1,4-Dichlorobenzene	Obs.	3.12 (3.10–3.14)[a]	3.11 (3.10–3.13)[a]	3.15 (3.10–3.20)[a]	3.04 (3.03–3.06)[a]	3.69 (3.66–3.73)[a]
	Pre.[b]	3.14	3.26	3.26	3.42	4.06
	Diff.	−0.02	−0.15	−0.11	−0.38	−0.37
1-Bromo-4-chlorobenzene	Obs.	3.41 (3.33–3.48)[a]	3.37 (3.35–3.39)[a]	3.41 (3.40–3.43)[a]	3.37 (3.30–3.44)[a]	4.28 (4.26–4.29)[a]
	Pre.[b]	3.40	3.51	3.52	3.67	4.30
	Diff.	0.01	−0.14	−0.11	−0.30	−0.02
1,4-Dibromobenzene	Obs.	3.77 (3.72–3.82)[a]	3.64 (3.62–3.65)[a]	3.71 (3.63–3.78)[a]	3.78 (3.74–3.82)[a]	4.53 (4.44–4.62)[a]
	Pre.[b]	3.65	3.76	3.77	3.92	4.55
	Diff.	0.12	−0.12	−0.06	−0.14	−0.02
1,2,3-Trichlorobenzene	Obs.	4.09 (3.88–4.30)[a]	3.82 (3.78–3.88)[a]	3.82 (3.80–3.84)[a]	4.17 (3.86–4.49)[a]	4.74 (4.70–4.77)[a]
	Pre.[b]	3.86	3.97	3.98	4.13	4.76
	Diff.	0.23	−0.15	−0.16	0.04	−0.02
1-Bromo-2,3-dichlorobenzene	Obs.	4.38 (4.27–4.49)[a]	4.14 (4.13–4.16)[a]	4.15 (4.13–4.17)[a]	4.15 (4.13–4.17)[a]	5.26 (5.23–5.30)[a]
	Pre.[b]	4.01	4.12	4.13	4.28	4.91
	Diff.	0.37	0.02	0.02	−0.13	0.35

[a] Confidence intervals (95%) of log(1/EC$_{50}$)

[b] The values of Pre. were derived from Eq. 9 in this study. Pre., pre-treatment; Obs., observation; Diff., difference

Table 2 Partition coefficients of eight chemicals

Chemicals	log K_{ow}[a]
Benzene	2.13
Chlorobenzene	2.81
Bromobenzene	2.99
1,4-Dichlorobenzene	3.59
1-Bromo-4-chlorobenzene	3.83
1,4-Dibromobenzene	4.07
1,2,3-Trichlorobenzene	4.27
1-Bromo-2,3-dichlorobenzene	4.41

[a] Values obtained from Cao and Li (1997)

From Eq. 6, the correlation coefficient of r^2 (0.810) was relatively poor when compared with r^2 (>0.935) of Eqs. 1–5. And this relatively poor correlation coefficient indicated that the K_{ow}-based QSAR was not a good model for measuring the toxicity of halogenated benzenes to various algal species, although it was good for single alga. Therefore, it was necessary to improve Eq. 6 by introducing some contribution cofactors.

As pointed out by Swackhamer (1991), the bioaccumulation of HOC (hydrophobic organic chemicals) in algae is related to their surface area-to-volume ratio (*A/V*), and the higher the ratio is, the higher the uptake will be. In other words, the algal size is a determining factor in HOC bioaccumulation. Therefore, based on the data listed in Tables 1, 2 and 4, log (*A/V*) was introduced into Eq. 6 as follows,

$$\log(1/\mathrm{EC}_{50}) = 1.050 \log K_{ow} + 0.721 \log (A/V) - 6.376 \quad (7)$$

$n = 40$, $r^2 = 0.866$, S.E. = 0.333, $F = 119.094$, $p < 0.001$.

However, there was only a little improvement when we compared the value of r^2 (0.866) in Eq. 7 with that ($r^2 = 0.810$) in Eq. 6. This suggested that log(*A/V*) was not a good contribution cofactor for the K_{ow}-based QSAR model in this study.

Table 3 QSARs results for individual algae

Algae	QSAR models		n	r^2	S.E.	F	p
Nannochloropsis oculata	$\log(1/EC_{50}) = 1.061 \log K_{ow} - 0.466$	(1)	8	0.949	0.213	110.737	<0.001
Dunaliella salina var.	$\log(1/EC_{50}) = 1.074 \log K_{ow} - 0.678$	(2)	8	0.972	0.156	211.620	<0.001
Platyformas subcordiformis	$\log(1/EC_{50}) = 1.061 \log K_{ow} - 0.599$	(3)	8	0.976	0.143	245.415	<0.001
Chlorella marine	$\log(1/EC_{50}) = 1.018 \log K_{ow} - 0.347$	(4)	8	0.949	0.203	112.309	<0.001
Skeletonema costatum Gneville	$\log(1/EC_{50}) = 1.036 \log K_{ow} - 0.378$	(5)	8	0.935	0.236	86.156	<0.001

Table 4 The cofactors of five algal species

Algae	log (*A/V*) [1/m]	log (1/*S*) [m^2/g]	log(1/*lipid*) [g/L]
Nannochloropsis oculata	8.39[a]	−3.97[b]	1.82[c]
Dunaliella salina var.	8.04[a]	−4.04[b]	1.90[d]
Platyformas subcordiformis	8.64[a]	−4.30[b]	1.90[c]
Chlorella marine	8.05[a]	−4.00[b]	2.01[c]
Skeletonema costatum Gneville	8.72[a]	−4.07[b]	2.45[e]

[a] Values obtained from Wang et al. (1997b)

[b] Values obtained from Wang et al. (1997c)

[c] Values obtained from Lin and Li (2000)

[d] Values obtained from Renaud et al. (1994)

[e] Values obtained from Yang et al. (2002)

As reported by Wang et al. (1997a), the specific surface area (log S) of algal cells is highly correlated to the bioconcentration factor (log BCF) ($r > 0.95$). The larger the S is, the higher the bioconcentration will be. Therefore, based on the data listed in Tables 1, 2 and 4, log(1/S) was introduced into Eq. 6 in order to improve the quality of the K_{ow}-based QSAR model:

$$\log(1/EC_{50}) = 1.050 \log K_{ow} + 0.333 \log(1/S) + 1.016 \quad (8)$$

$n = 40$, $r^2 = 0.812$, S.E. $= 0.394$, $F = 79.850$, $p < 0.001$.

Comparison of the value of r^2 (0.812) in Eq. 8 with that in Eq. 6 ($r^2 = 0.810$) indicated that there was no improvement with log(1/S), and this proved that log(1/S) was not a contribution cofactor for this K_{ow}-based QSAR.

Lipid content of organisms is important for accumulation of contaminants. For examples, Bruner et al. (1994) found that zebra with higher lipid content had both higher uptake rates and greater accumulations of PCBs; Sijm and Linde (1995) reported that exchange surface and lipid content were the main properties determining bioconcentration kinetics. Since the chemicals used in this study were nonpolar narcotic compounds, it means that these chemicals affect the algae only by interaction with lipids of biomembranes (Kőnemann 1981a). In algae, lipids are important membrane components (Wood 1974) and vary in different algal species. Therefore, it can be assumed that higher lipid content, which has stronger sorption, is possibly related to the higher toxicity. For various algal species, lipid content may be a contribution cofactor for the K_{ow}-based QSAR model. Based on the data listed in Tables 1, 2 and 4, introducing log(1/*lipid*) into Eq. 6, we obtained the improved QSAR model:

$$\log(1/EC_{50}) = 1.050 \log K_{ow} + 1.429 \log(1/lipid) - 3.224 \quad (9)$$

$n = 40$, $r^2 = 0.946$, S.E. $= 0.211$, $F = 323.933$, $p < 0.001$.

The value of r^2 (0.946) in Eq. 9 indicated that introduction of the log(1/*lipid*) greatly improved the quality of the K_{ow}-based QSAR model. Furthermore, as seen from Eq. 9, for various algal species, both logK_{ow} and log(1/*lipid*) were the toxicity contributors. The logK_{ow} describes the hydrophobicity of nonpolar narcotic chemicals, and it means that the higher the logK_{ow} is, the more hydrophobic chemicals are, and the greater toxicity will be (Cleuvers 2004). The log(1/*lipid*) describes the lipid content of various algal species, and it means that the lower the log(1/*lipid*) is, the more lipid content the algae have, and the greater risk for strong lipid-soluble chemicals.

Furthermore, from Eq. 7, since the contribution cofactor *A/V* also had a little improvement of K_{ow}-based QSAR model, log(*A/V*) was introduced into Eq. 9 as follows:

$$\log(1/EC_{50}) = 1.050 \log K_{ow} + 1.290 \log(1/lipid) + 0.226 \log(A/V) - 4.831 \quad (10)$$

$n = 40$, $r^2 = 0.950$, S.E. = 0.205, $F = 228.666$, $p < 0.001$.

The value of r^2 (0.950) in Eq. 10 showed that there was just a little improvement when compared with that ($r^2 = 0.946$) in Eq. 9. This improvement suggested that the parameter log(*A*/*V*) was not the major contribution cofactor, which was proved by Eq. 7 above. Therefore, we conclude that the toxicity of nonpolar narcotic chemicals to the algae tested may be generalized to other algal species, and is directly related to the hydrophobicity of the chemicals and the algal lipid content.

However, it should be pointed out that, before Eq. 9 is assumed as the basic QSAR model for predicting the toxicity of chemicals to all algae regardless of their species, both the wider range of chemicals and algal species have to be tested to validate this approach.

In addition, as pointed out by Bradbury and Coats (1985), Call et al. (1987), and Daus et al. (2010), uptake is unequal to toxicity. The uptake of hydrophobic organic compounds consists of two phases: an initial rapid surface sorption and a subsequent slower transference (Swackhamer 1991; Tang et al. 1998). Because the rapid phase is due to the affinity of organic molecule to algal cell surface, Wang et al. (1997a) have pointed out that log BCF has linear relationship with log *S* of algal cells, and Swackhamer (1991) has pointed out that the large(*A*/*V*) of an alga may accelerate the uptake. However, it seems that the toxicity is related to the second phase of the uptake (the slower transference), because in this study we have found that log(1/EC_{50}) is highly correlated to both the log K_{ow} of organic chemicals and log(1/*lipid*) of the algae, which indicates that the toxicity of nonpolar narcotic chemicals is mainly attributable to lipid solution.

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References

Bradbury SP, Coats JR (1985) Differential toxicity and uptake of two fenvalerate formulations in fathead minnows (*Pimephales promelas*). Environ Toxicol Chem 4:533–541

Bruner KA, Fisher SW, Landrum PF (1994) The role of the zebra mussel, Dreissena polymorpha, in contaminant cycling: I. The effect of body size and lipid content on the bioconcentration of PCBs and PAHs. J Great Lakes Res 20:725–734

Call DJ, Brooke LT, Kent RJ, Knuth ML, Poirier SH, Huot JM, Lima AR (1987) Bromacil and diuron herbicides: toxicity, uptake, and elimination in freshwater fish. Arch Environ Contam Toxicol 16:607–613

Cao CZ, Li Zl (1997) Estimation and prediction of partition coefficient for some substituted benzene. Chin Environ Sci 17(6):501–503

Cleuvers M (2004) Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol Environ Saf 59(3):309–315

Cronin MTD, Jaworska JS, Walker JD, Comber MHI, Watts CD, Worth AP (2003) Use of QSARs in international decision-making frameworks to predict health effects of chemical substances. Environ Health Perspect 111(10):1391–1401

Daus B, Weiss H, Altenburger R (2010) Uptake and toxicity of hexafluoroarsenate in aquatic organisms. Chemosphere 78:307–312

Grzybowski BA, Bishop KJM, Kowalczyk B, Wilmer CE (2009) The 'wired' universe of organic chemistry. Nat Chem 1:31–36

Johnson DJ, Sanderson H, Brain RA, Wilson CJ, Solomon KR (2007) Toxicity and hazard of selective serotonin reuptake inhibitor antidepressants fluoxetine, fluvoxamine, and sertraline to algae. Ecotoxicol Environ Saf 67(1):128–139

Kent RA, Currie D (1995) Predicting algal sensitivity to a pesticide stress. Environ Toxicol Chem 14(6):983–991

Könemann H (1981a) Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and observed results. Toxicology 19:229–238

Könemann H (1981b) Quantitative structure-activity relationships in fish toxicity studies. Part 1: relationship for 50 industrial pollutants. Toxicology 19:209–221

Kong FX, Hu W, Liu Y (1998) Molecular structure and biochemical toxicity of four halogeno-benzens on the unicellular green alga *Slenastrum capricornutum*. Environ Exp Bot 40:105–111

Lin XZ, Li GY (2000) Biomass, total lipids and fatty acid EPA/DHA composition of 11 species of microalgae. Oceanogr Huang Hai Bohai Sea 18(2):36–40

Netzeva TI, Dearden JC, Edwards R, Worgan ADP, Cronin MTD (2004) QSAR analysis of the toxicity of aromatic compounds to *Chlorella vulgaris* in a novel short-term assay. J Chem Inf Comput Sci 44:258–265

Organization for Economic Cooperation and Development (2006) OECD guidelines for the testing of chemicals: freshwater alga and cyanobacteria, growth inhibition test. Paris. p 201

Renaud SM, Parry DL, Thinh LV (1994) Microalgae for use in tropical aquaculture I: gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. J Applied Phycol 6:337–345

Sáenz ME, Alberdi JL, Di Marzio WD, Accorinti J, Tortorelli MC (1997) Paraquat toxicity to different green algae. Bull Environ Contam Toxicol 58:922–928

Sijm DTHM, Linde AVD (1995) Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. Environ Sci Technol 29:2769–2777

Swackhamer DL (1991) Bioaccumulation of toxic hydrophobic organic compounds at the primary trophic level. J Environ Sci (China) 3(3):15–21

Tang JX, Hoagland KD, Siegfried BD (1998) Uptake and bioconcentration of atrazine by selected freshwater algae. Environ Toxicol Chem 17(6):1085–1090

United States Environmental Protection Agency (USEPA) (1996) Ecological Effects Test Guidelines OPPTS 850. 5400 Algal Toxicity, Tiers I and II. Washington:EPA 712-C-96-164

Wang XL, Ma YJ, Yu WJ, Geyer HJ (1997a) Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: quantitative relationship between bioconcentration factor and surface area of marine algae or

octanol/water partition coefficient. Chemosphere 35(8):1781–1797

Wang XL, Ma YJ, Li JL, Yu WJ, Sun HZ (1997b) Determining surface areas of marine phytoplankton cells by acid-base titration method. Oceanologia et limnologia sinica 28(4):356–362

Wang XL, Ma YJ, Su YL (1997c) Determining surface areas of marine alga cells by acid-base titration method. Chemosphere 35(5):1131–1141

Wood BJB (1974) Saponifiable lipids and fatty acids. In: Stewart WDP (ed) Algal physiology and biochemistry. Blackwell, Oxford, p 989

Yang S, Wu RSS, Kong RYC (2002) Physiological and cytological responses of the marine diatom Skeletonema costatum to 2, 4-dichlorophenol. Aquat Toxicol 60:33–41