Bull Environ Contam Toxicol (2008) 81:525–530
DOI 10.1007/s00128-008-9570-y

QSAR for Predicting Joint Toxicity of Halogenated Benzenes to *Dicrateria zhanjiangensis*

Ming Zeng · ZhiFen Lin · DaQiang Yin · KeDong Yin

Received: 18 December 2007 / Accepted: 24 September 2008 / Published online: 15 October 2008

Abstract In this study, the toxicity of 49 mixed halogenated benzenes to *Dicrateria zhanjiangensis* was determined and the partition coefficient of these mixtures was described by using the C_{18}-Empore^{TM} disks/water partition coefficient (K_{mix}). According to these data, a simple K_{mix}-based QSAR model was successfully used to correlate the toxicity of the mixed halogenated benzenes to *D. zhanjiangensis*.

Keywords QSAR models · Mixture toxicity · Halogenated benzenes · *Dicrateria zhanjiangensis*

In recent decades, researchers have usually focused on the effects of joint toxicity. The major aims of these studies were to explore ways to predict and to identify hazardous combinations of chemicals relevant to the environment.

M. Zeng (✉) · K. Yin
Key Laboratory of Tropical Marine Environmental Dynamics, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West XinGang Road, Guangzhou 510301, China
e-mail: zmrainbow@yahoo.com.cn

Z. Lin (✉) · D. Yin
College of Environmental Science and Engineering, Key Laboratory of Yangtze River Water Environment, Ministry of Education, Tongji University, Shanghai 200092, China
e-mail: lzhifen@yahoo.com

Z. Lin
College of Environmental Science, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China

M. Zeng
Graduate University of Chinese Academy of Sciences, Beijing 100049, China

The octanol–water partition coefficient (K_{ow}) is one of the most effective parameters of QSAR in the field of single chemical (Kőnemann 1981). If the partition coefficient of mixtures can be used to predict the toxicity of mixtures, it would be a good way to develop models for mixtures. Therefore, Verhaar et al. (1995) first extended K_{ow} to the field of mixture in 1995 and he described the partition coefficient of a mixture as follows:

$$K_{mix} = \frac{W}{V} \times \frac{\sum_{i=1}^{n} \frac{Q^0_{water,i}}{1+\frac{W}{VK_{Di}}}}{\sum_{i=1}^{n} Q^o_{water,i} - \sum_{i=1}^{n} \frac{Q^0_{water,i}}{1+\frac{W}{VK_{Di}}}} \quad (1)$$

where K_{mix} is the C_{18}-Empore^{TM} disk/water partition coefficient for a mixture, K_{Di} is the partition coefficient of individual chemical i, W is the volume of solution, V is the volume of hydrophobic phase, Q^0_{water} is the initial amount of chemical i in water and n is the total number of individual chemicals in the mixture. The value of W/V is suggested as 6.8×10^5.

Based on the methods described by Verhaar et al. (1995); Lin et al. (2002) obtained the K_{mix} of 74 mixed halogenated benzenes, determined the relationship between the K_{mix} and their corresponding toxicity to *Photobacterium phosphoreum*, and therefore successfully developed a simple QSAR-based approach to predict the toxicity of mixtures.

$$\log 1/EC_{50M} = 0.928 \log K_{mix} + 0.224 \quad (2)$$

$n = 74$, $r^2 = 0.953$, $SE = 0.130$, $F = 1{,}461.932$, $p < 0.0001$.

However, it is uncertain whether the method described by Verhaar et al. (1995) can be applied to other organism, such as algae. Algae are sensitive indicators of environmental change due to their key position as primary

producers in aquatic ecosystem. They are widely used in the assessment of risk. The purpose of this study therefore are, first to determine the toxicity of 49 mixed halogenated benzenes to *Dicrateria zhanjiangensis*; second to develop a simple QSAR-based approach to predict the toxicity of mixtures by using the relationship between the toxicity of these mixtures and their partition coefficients (K_{mix}); finally to compare the model of this study with the one of Lin et al. (2002).

Materials and Methods

Seven halogenated benzene chemicals (Table 1) were used without repurifying (purity $\geq$ 99%). They were purchased from Sinopharm Chemical Reagent Co., Ltd. (ShangHai, China).

D. zhanjiangensis, marine, single-celled, golden algae, was supplied by South China Sea Institute of Oceanology. It was employed for toxicity testing because it is very common in China and never used in mixture toxicity testing. According to the OECD guideline 201 (Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Draft Revised Guideline 201. OECD, Paris 2002), the culture was maintained in f/2 marine medium. A six-step concentration series in geometric grade ranging from no effect to 100% growth inhibition concentration were planned after range-finding experiments. The chemicals found at elevated concentrations were Chlorobenzene (1.81–2.62 mmol/L), Bromobenzene (0.84–1.03 mmol/L), 1,2-Dichlorobenzene (0.49–0.75 mmol/L), 1-Bromo–2,3-dichlorobenzene (0.04–0.06 mmol/L), 1-Bromo-4-chlorobenzene (0.31–0.56 mmol/L), 1,2,4-Trichlorobenzen (0.11–0.14 mmol/L), 1,4-Dibromobenzene (0.19–0.4 mmol/L). The alga in the logarithmic growing period was inoculated into 100-mL Erlenmeyer flasks, which amounted to 30 mL of the culture media, the compound, and the alga. And pH was at 7.8 $\pm$ 0.2. The culture media without test compounds served as the control solution. The culture was incubated at 27 $\pm$ 1°C (Sun et al. 2005), with a 12:12 h light/dark cycle at 2,000–3,000 lux. All experiments were performed with three replicates of each treatment run simultaneously and expressed as effective concentration and its 95% confidence intervals at 50% response.

Growth inhibition was determined after 96 h (Delorenzo and Serrano 2003; Gatidou and Thomaidis 2007) by microscope with counting chamber. Based on the decrease in algal biomass, median effective concentration (EC_{50}) of the toxicity of a single chemical to *D. zhanjiangensis* was calculated according to the OECD guideline 201 (OECD 2002). The mixture toxicity tests were conducted in a similar manner as the single chemical tests. All the mixtures were composed of the seven single halogenated benzenes randomly and each mixture was almost tested at three ratios 1:2, 1:1, and 2:1 (identical fraction of EC_{50}) based on the observed EC_{50} values. Assumed that the initial concentration of the mixture was 100%, the decrease in algal biomass was measured at six different concentrations, 10%, 18%, 32%, 56%, 80%, and 100%, and therefore the median effective concentration was calculated in the unit of percentage (%). The mixture toxicity was quantitatively described by Eq. 3 (Preston et al. 2000) and the results are given (Table 2).

$$EC_{50M} = \frac{C_M}{\sum_{n}^{i=1} C_i/EC_{50i}} \quad (3)$$

where EC_{50} is the effective concentration required to bring about a 50% decrease in growth rate, C is the concentration of a substance, and the subscripts M and i are the mixtures and the ith individual chemical in the mixtures, respectively. Because of the concentration addition, the concentration of the individual chemical (C_i) could be calculated according to the median effective concentration in the unit of percentage (%), that is C_i = percentage% $\times$ the initial concentration of individual chemical.

According to Verhaar et al. (1995), the correlation between log K_{ow} and log K_D (C_{18}-Empore^{TM} disk/water partition coefficient of single chemicals) was described by Eq. 4. In this study, the K_{mix} and K_D were calculated by

Table 1 Results of the individual toxicity experiment

No	Single chemicals	log *K*ow[a]	log K_D	log 1/EC_{50}[mol/L]		
				Obs.	Pre.	Diff.
1#	Chlorobenzene	2.81	3.50	2.66 (2.61–2.71)[b]	2.69	−0.03
2#	Bromobenzene	2.99	3.68	3.03 (2.96–3.10)[b]	2.84	0.19
3#	1,2-Dichlorobenzene	3.55	4.23	3.23 (3.19–3.27)[b]	3.31	−0.08
4#	1-Bromo–2,3-dichlorobenzene	4.41	5.09	4.28 (4.26–4.30)[b]	4.02	0.26
5#	1-Bromo-4-chlorobenzene	3.83	4.51	3.38 (3.36–3.40)[b]	3.54	−0.16
6#	1,2,4-Trichlorobenzene	4.27	4.95	3.92 (3.89–3.96)[b]	3.91	0.02
7#	1,4-Dibromobenzene	4.07	4.75	3.54 (3.52–3.56)[b]	3.74	−0.20

[a] Cao and Li (1997)

[b] Confidence intervals (95%) of log 1/EC_{50}

Table 2 Results of the mixture toxicity experiment

No	Individual chemicals in the mixture	xEC_{50}:yEC_{50}[a]	log K_{mix}	log $1/EC_{50M}$[mol/L]		
				Obs.	Pre.	Diff.
1	1#:2#	1:1	3.56	2.79 (2.77–2.80)[b]	2.66	0.13
2	1#:2#	1:2	3.59	2.86 (2.81–2.91)[b]	2.69	0.17
3	1#:2#	2:1	3.53	2.62 (2.60–2.64)[b]	2.64	−0.02
4	1#:3#	1:1	3.78	2.83 (2.79–2.87)[b]	2.85	−0.02
5	1#:3#	2:1	3.68	2.76 (2.71–2.81)[b]	2.76	0.00
6	1#:4#	1:1	3.74	2.81 (2.79–2.83)[b]	2.81	0.00
7	1#:4#	1:2	3.89	3.01 (2.78–3.24)[b]	2.94	0.07
8	1#:4#	2:1	3.63	2.70 (2.68–2.72)[b]	2.73	−0.03
9	1#:5#	1:2	4.04	2.84 (2.81–2.87)[b]	3.07	−0.22
10	1#:6#	1:1	3.85	2.84 (2.81–2.87)[b]	2.91	−0.06
11	1#:6#	1:2	4.03	3.10 (2.93–3.27)[b]	3.05	0.04
12	1#:6#	2:1	3.71	2.76 (2.74–2.78)[b]	2.79	−0.03
13	1#:7#	1:1	3.95	2.92 (2.90–2.94)[b]	2.99	−0.06
14	2#:3#	1:1	3.98	3.11 (3.09–3.13)[b]	3.01	0.10
15	2#:3#	1:2	4.06	3.08 (2.95–3.21)[b]	3.08	0.00
16	2#:3#	2:1	3.88	3.13 (3.06–3.20)[b]	2.93	0.20
17	2#:4#	1:1	4.00	3.30 (3.25–3.35)[b]	3.04	0.27
18	2#:4#	1:2	4.18	3.34 (3.30–3.38)[b]	3.18	0.16
19	2#:4#	2:1	3.87	3.11 (3.04–3.18)[b]	2.93	0.18
20	2#:5#	1:1	4.12	3.13 (3.04–3.22)[b]	3.13	0.00
21	2#:5#	1:2	4.25	3.09 (3.07–3.11)[b]	3.24	−0.15
22	2#:6#	1:1	4.13	3.23 (3.19–3.27)[b]	3.14	0.09
23	2#:6#	1:2	4.31	3.31 (3.24–3.38)[b]	3.29	0.02
24	2#:6#	2:1	3.97	3.06 (3.01–3.11)[b]	3.00	0.05
25	2#:7#	1:2	4.37	3.13 (3.09–3.17)[b]	3.34	−0.21
26	3#:4#	1:1	4.39	3.48 (3.41–3.55)[b]	3.36	0.12
27	3#:4#	1:2	4.49	3.50 (3.43–3.57)[b]	3.44	0.06
28	3#:5#	1:1	4.37	3.23 (3.16–3.30)[b]	3.34	−0.10
29	3#:5#	1:2	4.41	3.21 (3.19–3.22)[b]	3.38	−0.17
30	3#:6#	1:1	4.45	3.45 (3.43–3.46)[b]	3.41	0.04
31	3#:6#	1:2	4.56	3.47 (3.40–3.54)[b]	3.50	−0.03
32	3#:6#	2:1	4.36	3.32 (3.25–3.39)[b]	3.33	−0.01
33	3#:7#	1:1	4.47	3.31 (3.24–3.38)[b]	3.42	−0.11
34	3#:7#	1:2	4.55	3.26 (3.24–3.28)[b]	3.49	−0.23
35	4#:6#	1:1	4.99	4.07 (4.06–4.09)[b]	3.86	0.20
36	4#:6#	1:2	4.98	3.99 (3.97–4.01)[b]	3.85	0.14
37	5#:6#	1:1	4.64	3.53 (3.49–3.57)[b]	3.57	−0.04
38	5#:6#	2:1	4.59	3.65 (3.58–3.72)[b]	3.52	0.13
39	6#:7#	1:1	4.81	3.72 (3.68–3.76)[b]	3.71	0.00
40	6#:7#	1:2	4.79	3.70 (3.66–3.74)[b]	3.69	0.01
41	5#:7#	1:2	4.66	3.46 (3.37–3.55)[b]	3.58	−0.13
42	4#:5#	1:1	4.62	3.60 (3.58–3.61)[b]	3.55	0.06
43	4#:5#	1:2	4.57	3.46 (3.44–3.48)[b]	3.51	−0.04
44	4#:7#	1:2	4.79	3.68 (3.64–3.72)[b]	3.69	−0.01
45	1#:5#	2:1	3.75	2.58 (2.56–2.50)[b]	2.82	−0.24
46	2#:5#	2:1	3.97	2.91 (2.87–2.95)[b]	3.02	−0.11
47	1#:7#	2:1	3.79	2.66 (2.57–2.75)[b]	2.86	−0.19

Table 2 continued

No	Individual chemicals in the mixture	xEC_{50}:yEC_{50}[a]	log K_{mix}	log $1/EC_{50M}$[mol/L]		
				Obs.	Pre.	Diff.
48	2#:7#	2:1	4.05	3.03 (2.99–3.07)[b]	3.07	−0.04
49	3#:7#	2:1	4.39	3.36 (3.34–3.38)[b]	3.35	0.01

[a] Binary mixtures were conducted at three ratios 1:2, 1:1, and 2:1 (identical fraction of EC_{50}) based on observed EC_{50} values

[b] Confidence intervals (95%) of log $1/EC_{50M}$

Eqs. 1 and 4 (Verhaar et al. 1995), respectively. The results were given in Tables 1 and 2.

$$\log K_D = 0.995 \log K_{ow} + 0.70 \quad (4)$$

n = 18, $r^2 = 0.93$, SE = 0.24.

Statistical analyses were performed with the SPSS 11.50 software (SPSS Inc.) and Sigmaplot 2001 (SYSTAT Inc.). The square of the correlation coefficient (r^2), standard error (SE), the mean square ratio (F), and p-value were taken into consideration in testing the quality of the regression.

Results and Discussion

The toxicity of seven individual halogenated benzenes to *D. zhanjiangensis* is determined and the results are given in Table 1. The relationship between log $1/EC_{50}$ and log K_D is determined by using Eq. 5.

$$\log 1/EC_{50} = 0.838 \log K_D - 0.242 \quad (5)$$

n = 7, $r^2 = 0.899$, SE = 0.189, $F = 44.640$, $p = 0.001$.

Equation 5 explains most of the variance (89.9%), with maximum F values (44.640) and minimum standard error of the estimate (0.189). This significance of the log $1/EC_{50}$ − log K_D relationship indicates that the toxicity of these chemicals is dependent on the distribution between water and organic phases (Lin et al. 2002). And this dependence shows that the chemicals are nonpolar narcotic chemicals (Verhaar et al. 1992; Lin et al. 2004; Hsieh et al. 2006). Furthermore, the Eq. 5 shows that individual chemicals act in the same way, by the same mechanisms, and differ only in their potencies.

According to the Eq. 5 and Lin et al. (2004), the joint action of these chemicals is a non-interactive process. It is therefore assumed that, similarly to the single chemicals, the mixtures may affect the organism only by interaction with lipids of biomembranes, and this interaction is essentially the partitioning behaviors. In other words, the toxicity of the mixtures may be related to the partition coefficient of the mixtures. Then, based on the data listed in Tables 1 and 2, K_{mix} is calculated by Eq. 1 and the hydrophobicity-based QSAR model is determined for mixtures by using Eq. 6.

$$\log 1/EC_{50M} = 0.836 \log K_{mix} - 0.311 \quad (6)$$

n = 49, $r^2 = 0.879$, SE = 0.124, $F = 341.239$, $p < 0.001$.

The model (Eq. 6) was characterized by a high correlation coefficient ($r^2 = 0.879$) and small standard error (SE = 0.124). Thus it appears that the log $1/EC_{50M}$ is well correlated with log K_{mix}. When the toxicity of mixture was tested in various ratios (2:1, 1:1, 1:2, identical fraction of EC_{50}), the quality of the model was not affected. Obviously, the correlation in this study is similar to that in Lin et al. (2002).

However, the model in this study (Eq. 6) is different from that in Lin's et al. (Eq. 2, 2002), and this difference can be seen from Fig. 1. In Fig. 1, line A and line B are almost parallel, and this parallelism may be due to the toxicity character of chemicals. Both QSAR models are derived from the halogenated benzenes, which are considered to be nonpolar narcotic chemicals (Verhaar et al. 1992; Lin et al. 2004). And the nonpolar narcotic chemicals affect the organism only by interaction with lipids of biomembranes. Therefore, the mixture toxicity is related to the partition coefficient of the mixtures. In other words, both QSAR models belong to hydrophobicity-based ones. This is why the two lines are almost parallel.

Furthermore, it can also be seen from Fig. 1 that line A is above line B. In other words, the log $1/EC_{50M}$ obtained

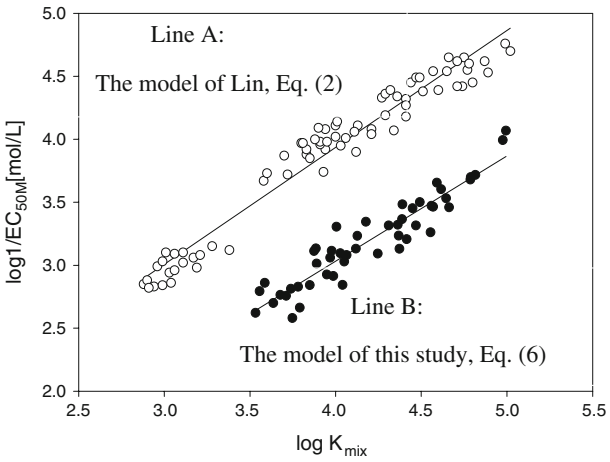

Fig. 1 Comparison of the QSAR of Lin et al. (2002) with the QSAR in this study

Table 3 Results of the log 1/EC_{50M} for 10 other related mixtures

No	Individual chemicals in the mixture	xEC_{50}:yEC_{50}	log K_{mix}	log 1/$EC_{50\ M}$[mol/L]		
				Obs.	Pre.	Diff.
1	1#:3#	1:2	3.90	2.91 (2.89–2.93)[a]	2.95	−0.04
2	2#:7#	1:1	4.21	3.25 (3.20–3.30)[a]	3.21	0.04
3	5#:6#	1:2	4.71	3.63 (3.59–3.67)[a]	3.63	0.00
4	1#:3#:5#	1:1:1	3.97	2.89 (2.84–2.95)[a]	3.01	−0.12
5	1#:4#:7#	1:1:1	4.04	3.01 (2.97–3.05)[a]	3.06	−0.05
6	3#:4#:5#	1:1:1	4.44	3.42 (3.40–3.44)[a]	3.40	0.02
7	2#:5#:6#	1:1:1	4.27	3.31 (3.27–3.35)[a]	3.26	0.05
8	2#:3#:5#:6#	1:1:1:1	4.26	3.27 (3.22–3.32)[a]	3.25	0.02
9	3#:4#:5#:7#	1:1:1:1	4.53	3.46 (3.41–3.51)[a]	3.47	−0.01
10	4#:5#:6#:7#	1:1:1:1	4.71	3.59 (3.57–3.61)[a]	3.63	−0.04

[a] Confidence intervals (95%) of log 1/EC_{50M}

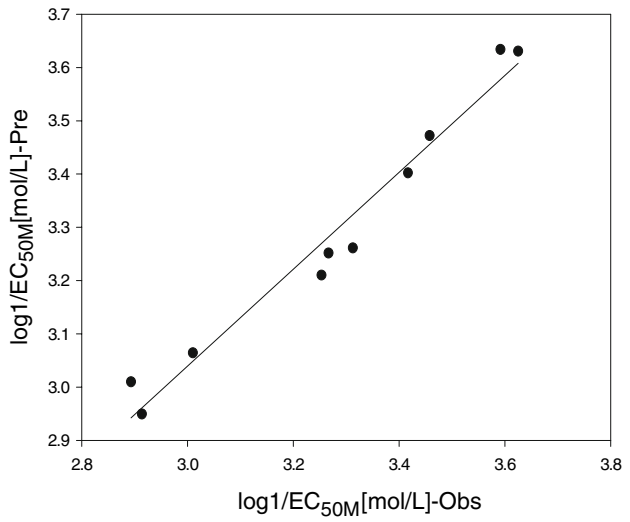

Fig. 2 Observed versus predicted log 1/EC_{50M} of 10 other related mixtures

from Eq. 2 will be greater than that from Eq. 6, provided that K_{mix} is the same. And this difference may be due to the structure of the test organism. *P. phosphoreum*, which belongs to Gram-negative bacteria, has an outer membrane outside of plasma membrane. And this outer membrane is a hydrophobic one because it is embedded by the hydrophobic lipid of an abundant small liporotein (Nicklin et al. 1999). However, for *D. zhanjiangensis*, there is no outer membrane and its membrane shows a marked tendency to became silicified (Morris 1977). Therefore, it can be said that *P. phosphoreum* is more sensitivity to hydrophobic pollutants than *D. zhanjiangensi*, because the former has a hydrophobic membrane while the latter only has the silicified one. In other words, it is the outer membrane of *P. phosphoreum* that lead to the results that line A is above line B.

The predictive capability of the regression equation (Eq. 6) and the statistical validity of the modeling are confirmed by application of the 10 other related mixtures to the model (Table 3). The predicted log 1/EC_{50M} are plotted against the observed ones in Fig. 2, which shows a good consistency between them, with $r^2 = 0.967$, SE = 0.04683 and $F = 235.575$ at a level of significance $p < 0.001$. Since the 10 mixtures are randomly composed of the seven chemicals and the agreement between the observed log 1/EC_{50M} and that predicted by the model is generally satisfactory, the model therefore can be used to predict toxicity of the mixtures.

Acknowledgments This work is sponsored by the National Natural Science Foundation of P.R.C.#: 20677063, the National Natural Science Graveness Foundation of P.R.C.#: 40490264, the Science and Technology Foundation of Guangzhou City, P.R.C.#: 2007J1-C0241, the opening Foundation of Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, P.R.C.#: MEL0504, and the opening Foundation of Key Laboratory of Yangtze River Water Environment, Tongji University, Shanghai, P.R.C.#: YRWEF07004.

References

Cao CZ, Li ZL (1997) Estimation and prediction of partition coefficient for some substituted benzene. China Environ Sci 17(6):501–503. doi:10.3321/j.issn:1000-6923.1997.06.006

Delorenzo ME, Serrano L (2003) Individual and mixture toxicity of three pesticides: atrazine, chlorpyrifos, and chlorothalonil to the marine phytoplankton species *Dunaliella tertiolecta*. J Environ Sci Health B38:529–538. doi:10.1081/PFC-120023511

Gatidou G, Thomaidis NS (2007) Evaluation of single and joint toxic effects of two antifouling biocides, their main metabolites and copper using phytoplankton bioassays. Aquat Toxicol 85:184–191. doi:10.1016/j.aquatox.2007.09.002

Hsieh SH, Tsai KP, Chen CY (2006) The combined toxic effects of nonpolar narcotic chemicals to *Pseudokirchneriella subcapitata*. Water Res 40:1957–1964. doi:10.1016/j.watres.2006.03.026

Kőnemann H (1981) Quantitative structure-activity relationships in fish toxicity studies. Part 1: relationship for 50 industrial

pollutants. Toxicology 19:209–221. doi:10.1016/0300-483X(81)90130-X

Lin ZF, Yu HX, Wei DB, Wang GH, Feng JF, Wang LS (2002) Prediction of mixture toxicity with its total hydrophobicity. Chemosphere 46:305–310. doi:10.1016/S0045-6535(01)00083-2

Lin ZF, Du JW, Yin KD, Wang LS, Yu HX (2004) Mechanism of concentration addition toxicity: they are different for nonpolar narcotic chemicals, polar narcotic chemicals and reactive chemicals. Chemosphere 54:1691–1701. doi:10.1016/j.chemosphere.2003.09.031

Morris L (1977) An introduction to the algae. Hutchinson & Co Ltd, London

Nicklin J, Graeme-Cook K, Paget T, Killington R (1999) Instant notes in microbiology. Bios Scientific Publishers Limited, Beijing, China

Organization for Economic Cooperation and Development (2002) OECD guideline for testing of chemicals: freshwater alga and cyanobacteria, growth inhibition test. Draft Revised Guideline 201. Paris: OECD

Preston S, Coad N, Townend J, Killham K, Paton GI (2000) Biosensing the acute toxicity or metal interactions: are they additive, synergistic, or antagonistic? Environ Toxicol Chem 19:775–780. doi:10.1897/1551-5028(2000)019<0775:BTATOM>2.3.CO;2

Sun CB, Wang XH, Bi HQ (2005) Commercial culture of marine one-celled algae. Fish Sci 24:39–40. doi:cnki:ISSN:1003-1111.0.2005-08-013

Verhaar HJM, Leeuwen CJV, Hermens JLM (1992) Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25(4):471–491. doi:10.1016/0045-6535(92)90280-5

Verhaar HJM, Busser FJM, Hermens JLM (1995) A surrogate parameter for the baseline toxicity content of contaminated water. Environ Sci Technol 29(3):726–734. doi:10.1021/es00003a021