Bull Environ Contam Toxicol (2007) 78:107–111
DOI 10.1007/s00128-007-9070-5

Quantitative Structure–Activity Relationships for Predicting the Joint Toxicity of Substituted Anilines and Phenols to Algae

G. H. Lu · C. Wang · Z. Y. Tang · X. L. Guo

Published online: 6 April 2007

Aniline, phenol, and their derivatives are widely used industrial chemicals that consequently have a high potential for environmental pollution. The toxicity of these chemicals has been investigated extensively using fish, *Tetrahymena pyriformis* and *Vibrio fischei* (Cronin and Schultz, 1996; Könemann and Musch, 1981; Lu et al., 2003). However, in aquatic ecosystems, there often are many coexistent chemicals, and information on the joint toxicity of a mixture of aniline and phenol derivatives is scarce.

Quantitative structure–activity relationships (QSARs) are powerful tools for predicting the toxicologic effect of chemicals. In the past two decades, QSARs for the toxicity of single compounds have been developed. The joint toxicity of organic chemicals can be assessed objectively by QSAR studies, and the toxicologic effect of coexistent pollutants on aquatic organisms can be predicted.

In an early study analyzing the joint toxicity of binary mixtures, Placket and Hewlett (1967) identified four types of actions: simply additive, more than additive (synergism), less than additive (partial addition), and no interaction (independent). These actions have been quantitatively classified using the Toxic Unit (TU), the Additivity Index (AI), the Similarity Parameter (λ), and the Mixture Toxicity Index (MTI).

Recently, joint toxicity prediction based on QSARs has been attempted. Xu and Nirmalakhandan (1998) applied QSAR models derived from single chemical toxicity assays to predict the joint toxicity of mixtures of organic chemicals to microorganisms. Yuan et al. (2002) determined the joint toxicity of binary mixtures of 2,4-dinitrobenzne, 8 nitrobenzenes, and anilines to *Vibrio fischeri* according to an equiconcentration ratio (1:1) and developed a QSAR for joint toxicity using E_{LUMO} as the structural descriptor. Huang et al. (2003) measured the joint toxicity of phenol derivatives to tadpoles (*Rana japonica*) and predicted mixture toxicity using log*P*. This study aimed to determine experimentally the toxicity of substituted anilines and phenols and related binary mixtures to the algae *Scenedesmus obliquus*, and to assess, model, and predict joint toxicity using molecular structural descriptors.

Materials and Methods

Scenedesmus obliquus was supplied by Wuhan Institute of Hydrobiology, Chinese Academy of Science. The toxicity test method was referred to the algae inhibition test (OECD, 1981). The culture was maintained in a liquid medium, and the formula of culture medium was referred to Lu et al. (2001). For each compound studied, seven concentration steps in a logarithmic gradient ranging from no effect to 100% growth inhibition concentration were planned after range-finding experiments. The algae in the logarithmic growing period were inoculated into 250-mL Erlenmeyer flasks, which amounted to 60 mL of the culture media, the compound, and the algae. The culture media without test compounds served as the control solution. The initial algae cell concentration in the test culture was approximately 3×104 cells/mL. The culture was incubated at 20 ± 1°C. A 12-h light and 12-h dark photoperiod was

G. H. Lu · C. Wang (✉) · Z. Y. Tang · X. L. Guo
Key Laboratory for Integrated Regulation and Resources, Development on Shallow Lakes, Ministry of Education, College of Environmental Science and Engineering, Hohai University, Nanjing 210098, People's Republic of China
e-mail: cwang@hhu.edu.cn

programmed, and the average illumination intensity was about 4,000 lex produced by a white fluorescent lamp. All experiments were performed with two replicates of each treatment run simultaneously.

Growth was monitored using the electron microscope (×400). Data were handled according to the following formulas: $\mu = \ln(N_t/N_0)/(t - t_0)$, where μ is the average specific growth rate, N_0 is the initial cell concentration, N_t is the cell concentration after culturing for 48 h, and t-t_0 is the experimental period (48 h in this study); and $I = [\mu(b) - \mu(tox)]/\mu(b) \times 100\%$, where I is the inhibition rate, $\mu(b)$ is the average specific growth rate of the control, and $\mu(tox)$ is the average specific growth rate of added toxic compound. Logarithms of the inverse median effective inhibition concentration after 48 h of exposure, expressed as $\log 1/EC_{50}$ (mol/L), were gained through one variable linear regression analysis of the negative logarithm of compound concentrations and the inhibition rates as the relative toxic potency for each single chemical.

To test the toxicity of the mixture, binary mixtures consisting of 2,4-dichloroaniline and another anilines or phenols were conducted at an equitoxic ratio (1:1) based on observed EC_{50} values. All toxicity testing procedures of mixtures were performed as with the single chemicals. The joint toxicity of mixtures was described as follows based on the work of Preston et al. (2000):

$$EC_{50\text{mix}} = (C_A + C_B)/(C_A/EC_{50A} + C_B/EC_{50B}). \quad (1)$$

In this equation, $EC_{50\text{mix}}$ is the median effective inhibition concentration of binary mixtures; EC_{50A} and EC_{50B} are the median effective inhibition concentrations of components A and B; and C_A and C_B are the concentrations of components A and B in binary mixtures.

The logarithm of the n-octanol/water partition coefficient (log$P$) was obtained from ClogP for Windows software (version 3.55; Biobyte Company, Claremont, CA, USA). The measured values of log$P$ were selected for QSAR study. The energy of the lowest unoccupied molecular orbital (E_{LUMO}) and the energy of the highest occupied molecular orbital (E_{HOMO}) were obtained from the ChemOffice 2004 program using the quantum chemical method MOPAC (http://www.cambridgesoft.com). The parameter values of the chemicals studied are listed in Table 1. The linear regression analyses were carried out using the SPSS statistical package (version 9.0; SPSS Company, Chicago, IL, USA).

Results and Discussion

The observed 48-h $\log 1/EC_{50}$ of 21 substituted anilines and phenols and the $\log 1/EC_{50\text{mix}}$ of 20 binary mixtures are given in Table 1. The compounds exhibited a reasonably wide range of algal toxicity, and $\log 1/EC_{50}$ values ranged from 2.60 for aniline to 4.56 for α-naphthol. For the purpose of comparison, the 5-min bioluminescent bacterium Shk1 EC_{50} values of phenolic compounds (Ren et al., 2003) and the 15-min *Chlorella vulgaris* EC_{50} values of anilines (Netzeva et al., 2004) also are shown. When the toxicity of four anilines on *S. obliquus* was compared with those from *C. vulgaris*, all the chemicals tested exhibited higher toxicity on *S. obliquus* than on *C. vulgaris*. The comparison of *S. obliquus* and Shk1 EC_{50} data shows that chlorophenols and nitrophenol were discovered to be more toxic to Shk1, but for phenol itself and Naphthols, the situation was the converse.

It is well known that the nonspecific toxicity of chemicals can be described by two kinds of action: nonpolar narcosis (type 1 narcosis) and polar narcosis (type 2 narcosis). Nonpolar narcotic chemicals are considered baseline toxicants. Their toxicity is proportional to their concentrations at the site of action and caused solely by membrane perturbation (Schultz et al., 1986). Polar narcotic chemicals, typified by most phenols and anilines, exhibit toxic potency higher than that estimated by their hydrophobicity due to the existence of polar substituents in the molecules (Kamlet et al., 1986). The addition of an electronic parameter can improve the prediction of a log octanol/water partition coefficient (log$P$) dependent model (Schultz et al., 1989).

The relationship of the individual toxicity of 21 substituted anilines and phenols with log$P$ was analyzed, and Equation 2 was obtained. However, the log$P$-dependent model explains only 57.1% of the variance.

$$\log 1/EC_{50} = 0.663(0.132)\log P + 1.913(0.300) \quad (2)$$

$n = 21$, $r^2 = 0.571$, $r^2_{adj} = 0.548$, $SE = 0.471$, $F = 25.25$, $p < 0.001$.

In this equation, n is the number of observations; r^2 is the square of the correlation coefficient; r^2_{adj} is the adjusted r^2 value; SE is the standard error; F is the mean square ratio; and p is the probability greater than the F value.

To improve the prediction for the toxicity of polar narcotic anilines and phenols studied in this report, the frontier orbital energy gap ΔE was introduced and defined as $\Delta E = E_{LUMO} - E_{HOMO}$, where ΔE is a critical parameter determining the molecular admittance. It can be said that the larger the ΔE value, the more stable the molecule, and thus the harder the rearrangement of its electron density under the presence of an external charge or external electric field (Mielczarek, 2005). The following two-descriptor model was obtained from multivariable regression analyses:

Table 1 Toxicity data and structural parameters

Compounds	Log1/EC_{50}			log1/EC_{50mix}		log$P$	logP_{mix}	E_{HOMO}	E_{LUMO}	ΔE	ΔE_{mix}
	Obs.	Pre[a]	Obs.	Obs.	Pre[b]			eV	eV	eV	eV
2,4-Dichloroaniline	3.40	3.63				2.78		–8.96	–0.09	8.87	
2-Chloroaniline	2.81	3.10		3.01	3.15	1.90	2.08	–8.85	0.20	9.05	9.01
3-Chloroaniline	2.90	3.04	2.69[c]	3.05	3.13	1.88	2.09	–8.93	0.19	9.12	9.05
4-Chloroaniline	2.91	3.08		3.09	3.14	1.83	2.06	–8.81	0.22	9.03	9.00
4-Bromoaniline	3.13	3.30	2.67[c]	3.24	3.24	2.26	2.44	–8.87	0.13	9.00	8.95
Diphenylamine	4.49	3.84		3.67	3.50	3.50	2.83	–8.56	0.45	9.01	8.88
Aniline	2.60	2.79	1.66[c]	2.84	2.99	0.90	1.16	–8.28	0.60	8.88	8.88
2,4,6-Trichloroaniline	4.55	4.36	4.11[c]	3.67	3.69	3.52	2.83	–8.71	–0.33	8.38	8.84
α-Naphthylamine	4.29	4.32		3.65	3.60	2.27	2.72	–8.08	–0.36	7.72	8.74
N-Methylaniline	2.79	2.95		3.01	3.09	1.66	1.89	–8.53	0.57	9.10	9.05
2,4-Dichlorophenol	3.42	3.60	3.74[d]	3.41	3.39	3.06	2.92	–9.45	–0.38	9.07	8.97
2-Chlorophenol	2.84	2.98	2.93[d]	3.03	3.13	2.15	2.28	–9.40	–0.07	9.33	9.23
3-Chlorophenol	2.92	3.09		3.10	3.19	2.50	2.57	–9.43	–0.04	9.39	9.26
4-Chlorophenol	3.14	3.19	3.81[d]	3.25	3.21	2.39	2.53	–9.28	–0.08	9.20	9.08
Phenol	2.61	2.51	2.29[d]	2.85	2.92	1.46	1.65	–9.25	0.29	9.54	9.46
α-Naphthol	4.56	4.54	3.43[d]	3.67	3.71	2.85	2.78	–8.31	–0.54	7.77	8.80
β-Naphthol	4.53	4.47	4.08[d]	3.67	3.68	2.70	2.77	–8.31	–0.53	7.78	8.79
3-Nitrophenol	3.15	3.27		3.26	3.22	2.00	2.28	–10.15	–1.26	8.89	8.88
4-Nitrophenol	3.33	3.10	3.51[d]	3.36	3.15	1.91	2.31	–10.30	–1.24	9.06	8.97
Hydroquinone	2.79	2.40		3.00	2.83	0.59	1.02	–9.07	0.12	9.19	9.13
Resorcinol	2.76	2.35	2.76[d]	2.97	2.83	0.80	1.17	–9.24	0.12	9.36	9.28

[a] Predicted log1/EC_{50} calculated by Equation 3

[b] Predicted log1/EC_{50mix} calculated by Equation 6

[c] 15 min log1/EC_{50} for *Chlorella vulgaris* from Netzeva et al. (2004)

[d] 5 min log1/EC_{50} for bioluminescent bacterium Shk1 from Ren et al. (2003)

$$\log 1/EC_{50} = 0.445(080)\log P - 0.801(0.121)\Delta E + 9.501(1.154) \quad (3)$$

$n = 21$, $r^2 = 0.876$, $r^2_{adj} = 0.862$, $SE = 0.261$, $F = 63.33$, $p < 0.001$

Equation 3 was used to predict toxicity, and the predicted values are presented in Table 1. The plot of observed log1/EC_{50} to algae versus calculated values by the model (Equation 3) is shown in Fig. 1.

On the basis of an independence assumption, which implies that partitioning of a mixture is simply the summed partitioning of individual chemicals and ignores the interactions between components, Huang et al. (2003) proposed an equation to predict the n-octanol/water partition coefficient for mixtures (logP_{mix}):

$$\log P_{mix} = (C_A \times \log P_A + C_B \times \log P_B + \cdots)/(C_A + C_B + \cdots). \quad (4)$$

In this equation, logP_{mix} is the n-octanol/water partition coefficient of binary mixtures; logP_A and logP_B are the n-octanol/water partition coefficient of components A and B; and C_A and C_B are the concentration of components A and B in binary mixtures. An obvious correlation between the joint toxicity to tadpoles and logP_{mix} for 23 mixtures of substituted phenols was obtained, and the logP_{mix}-dependent model could explain 88% of variance (Huang et al., 2003).

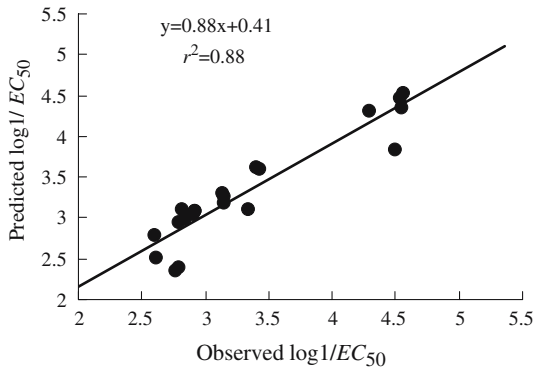

Fig. 1 Calculated log1/EC_{50} from Equation 3 versus observed log1/EC_{50} of single compounds to algae

In our study, the logP_{mix} of 20 binary mixtures were calculated on the basis of Equation 4 (Table 1). In addition, we proposed that the orbital energy of a mixture also is a simple sum of the individual components and calculated the frontier orbital energy gap of mixtures ΔE_{mix}. A regression method similar to single chemical analyses was used to model joint toxicity (log1/EC_{50mix}). The logP_{mix}-dependent model can explain only 66.3% of variance. By adding ΔE_{mix}, a two-descriptor QSAR model was obtained as follows:

$$\log 1/EC_{50mix} = 0.297(0.062)\log P_{mix} - 0.679(0.195)\Delta E_{mix} + 8.699(1.834) \quad (5)$$

$n = 20$, $r^2 = 0.803$, $r^2_{adj} = 0.780$, $SE = 0.137$, $F = 34.7$, $p < 0.001$.

From a predictive standpoint, Equation 5 was not satisfactory. Because the binary mixtures were composed of 2,4-dichloroaniline and other substituted anilines and phenols, respectively, according to an equitoxic ratio (1:1) in this study, there was an obvious relationship between the log$P$ of single compounds and the logP_{mix} of binary mixtures ($r^2 = 0.90$). The toxicity of mixtures then may be related to the structural parameters of single components (Yuan et al., 2002). Through multivariable linear regression analyses, an improved QSAR between log1/EC_{50mix} and log$P$ and ΔE was developed as follows:

$$\log 1/EC_{50mix} = 0.214(0.034)\log P - 0.280(0.051)\Delta E + 5.282(0.489) \quad (6)$$

$n = 20$, $r^2 = 0.873$, $r^2_{adj} = 0.858$, $SE = 0.110$, $F = 58.6$, $p < 0.001$

Equation 6 explains most of the variance (87.3%), with maximum F values (58.6) and the minimum standard error of the estimate (0.110), with neither statistical nor obvious visual outliers observed. This model was used to predict the joint toxicity, and the predicted values are presented in Table 1. The plot of observed log1/EC_{50mix} to algae versus calculated values by Equation 6 is shown in Fig. 2. The quality of model fit and prediction of Equations 3 and 6 were similar to comparable r^2, slope, and intercept in Figs. 1 and 2. It is shown that the model derived from the structural parameters of single components can be used successfully to predict joint toxicity.

The obtained models showed that both individual and joint toxicities of aniline, phenol, and their derivatives to algae are related mainly to their hydrophobicity and electronic properties. Because log$P$ is a hydrophobicity parameter, the higher the log$P$, the stronger the hydrophobicity and the easier the compounds are bioconcentrated in an organism. The substituted anilines and phenols investigated in this study are polar narcotics. Such compounds exhibit effects similar to those of nonpolar narcotics, but at potency levels greater than estimated by their hydrophobicity. The addition of the frontier orbital energy gap ΔE can enormously improve the prediction of log$P$-dependent models. Toxicity increases with greater negative ΔE (i.e., the smaller the value of ΔE, the easier the electron transfers from HOMO orbital to LUMO orbital and the stronger the toxicity). This result is consistent with that reported by Yan et al. (2006).

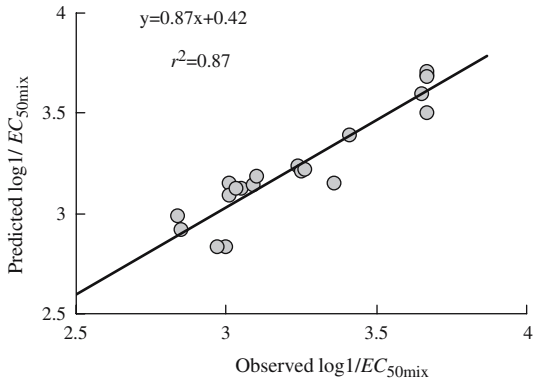

Fig. 2 Calculated log1/EC_{50mix} from Eq.6 versus observed log1/EC_{50mix} of mixtures

In conclusion, the 48-h toxicity of anilines and phenols and their binary mixtures to the algae was determined. Not only for single toxicity, but also for joint toxicity, successful two-descriptor QSAR models, accounting for hydrophobicity and frontier orbital energy gap, were developed. However, this study investigated only the toxicity of binary mixtures containing 2,4-dichloroaniline, and the model derived from the structural parameters of single components can be used to predict joint toxicity. The prediction of the joint toxicity of multiple-component mixtures should be studied continuously.

Acknowledgments This research was funded by the New Century Excellent Talent (2005) of the Ministry of Education of China, the National ''973'' Great Foundation Research Item, and the National Natural Science Foundation of China.

References

Cronin MTD, Schultz TW (1996) Structure–toxicity relationships for phenols to *Tetrahymena pyriformis*. Chemosphere 32:1453–1468

Huang H, Wang X, Shao Y, Chen D, Dai X, Wang L (2003) QSAR for prediction of joint toxicity of substituted phenols to tadpoles (*Rana japonica*). Bull Environ Contam Toxicol 71:1124–1130

Kamlet MJ, Doherty RM, Taft RW, Abraham MH (1986) Solubility properties in polymers and biological media: 7. An analysis of toxicant properties that influent inhibition of bioluminescence in *Photobacterium phosphoreum* (The Microtox Test). Environ Sci Technol 20:690–695

Köneman H, Musch A (1981) Quantitative structure–activity relationships in fish toxicity studies Part 2: The influence of pH on the QSAR of chlorophenols. Toxicology 19:223–228

Lu GH, Yuan X, Wang C (2003) Quantitative structure–toxicity relationships for substituted aromatic compounds to *Vibrio fischeri*. Bull Environ Contam Toxicol 70:832–838

Lu GH, Yuan X, Zhao YH (2001) QSAR study on the toxicity of substituted benzenes on the algae (*Scenedesmus obliquus*) . Chemosphere 44:437–440

Mayer FL, Hamelink JL (1977) Aquatic toxicology and hazard evaluation. Philadelphia: ASTM STP 634 Publication, 99–108

Mielczarek C (2005) Acid–base properties of selected flavonoid glycosides. Eur J Pharmaceut Sci 25:273–279

Netzeva TI, Dearden JC, Edwards R, Worgan ADP, Cronin MTD (2004) Toxicological evaluation and QSAR modeling of aromatic amines to *Chlorella vulgaris*. Bull Environ Contam Toxicol 73:385–391

OECD (1981) Test guideline 201. Paris Decision of the Council C 81:320–323

Placket RL, Hewlett PS (1967) A comparison of two approaches to the construction of models for quantal response to mixtures drugs. Biometrics 23:27–44

Preston S, Coad N, Townend J, Killham K, Paton GI (2000) Biosensing the acute toxicity of metal interactions: Are they additive, synergistic, or antagonistic? Environ Toxicol Chem 19:775–780

Ren S, ASCE M, Frymier PD (2003) Toxicity estimation of phenolic compounds by bioluminescent bacterium. J Environ Eng 129: 328–335

Schultz TW, Holcombe TW, Phipps GL (1986) Relationships of quantitative structure–activity to comparative toxicity of selected phenols in the *Pimephales promelas* and *Tetrahymena pyriformis* test systems. Ectotoxicol Environ Saf 12:146–153

Schultz TW, Wesley SK, Baker LL (1989) Structure–activity relationships for di and tri alkyl and/or halogen substituted phenols. Bull Environ Contam Toxicol 43:192–198

Xu S, Nirmalakhandan N (1998) Use of QSAR models in predicting joint effect in multicomponent mixtures of organic chemicals. Water Research 32:2391–2399

Yan XF, Xiao HM, Gong XD, Ju XH (2006) A comparison of semiempirical and first principle methods for establishing toxicological QSARs of nitroaromatics. J Mol Structure: Theochem 764:141–148

Yuan X, Lu GH, Zhao JS (2002) QSAR study on the joint toxicity of 2,4-dinitrotoluene with aromatic compounds to *Vibrio Fischeri*. J Environ Sci Health A 37:573–578