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Evaluation of Combined Toxicity of Phenols and Lead to *Photobacterium phosphoreum* and Quantitative Structure–Activity Relationships

L. M. Su · Y. H. Zhao · X. Yuan · C. F. Mu · N. Wang · J. C. Yan

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Abstract The combined toxicity of lead (Pb) and nine phenols were measured. The result indicated that the combined toxicity is not only dependent on the Pb concentrations but also on the positions of substituted groups of phenols. Quantitative structure–activity relationship equations were built from the combined toxicity and physico-chemical descriptors of phenols in the different Pb concentrations. The combined toxicity was related to water solubility and the third order molecular connectivity index (^3X) in low Pb concentration, to solute excess molar refractivity (E) and ionization constant (pKa) in medium Pb concentration and to dipolarity/polarizability (S) in high Pb concentration.

Keywords QSAR · *Photobacterium phosphoreum* · Synergism · Antagonism

The toxicity of each chemical is more commonly investigated in the literature (Schultz 1987; Yangjeh et al. 2006). However, in recent years, there is a growing concern that many toxic effects are not only caused by a single substance, but are actually due to the presence of mixtures of a large number of compounds. These chemical mixtures may result in different interactions on ecosystems and organisms rather than that, which would be observed from individual chemical alone (Mochida et al. 2006). In order to evaluate the risk associated with the pollutants in the real environment, it is necessary to develop techniques to understand, analyze and predict the combined effect of toxic chemicals (Xu and Nirmalakhandan 1998).

Phenols have been used widely as materials in the manufacture of plastics, as constituents of industrial disinfectants and as chemical reagents in industrial processes (Yangjeh et al. 2006). They are often been found in industry waste water and very toxic to aqueous organisms. The heavy metal lead (Pb) was found in the environment as a consequence of both natural and anthropogenic processes, with mining and smelting, coal burning, cement manufacturing, and use in gasoline contributing most to Pb contamination of aquatic environments (Grosell et al. 2006). Some studies have been carried out on the combined toxicity among heavy metals (Shaw et al. 2006; Otitoloju 2002) and combined toxicity among organic pollutants (Nirmalakhandan et al. 1997; Chen et al. 2005). Fewer studies have examined the combined toxicity of heavy metals and organic pollutants, especially phenols and Pb to aqueous organism. The quantitative structure–activity relationship (QSAR) of single toxicity of serial organic pollutants including phenols has been investigated (Schultz 1987; Cronin and Schultz 1996; Yangjeh et al. 2006). Xu and Nirmalakhandan (1998) used the QSAR model from single toxicity to predict the combined toxicity of organic chemicals, whereas those predictions are on the basis of mixtures causing toxicity by simple addition. In fact, besides simple addition, some studies indicated that the combined effects of contaminants can be larger or smaller than simple addition (Nirmalakhandan et al. 1997). It means that simple addition, synergism and antagonism may exist among mixture actions. Building QSAR models to predict the combined toxicity of chemical mixtures is currently a major challenge in aquatic ecotoxicology. In

L. M. Su · Y. H. Zhao · X. Yuan (✉) · C. F. Mu · N. Wang · J. C. Yan
College of Urban and Environmental Sciences, Northeast Normal University, 130024 Changchun, China
e-mail: yuanx@nenu.edu.cn

L. M. Su
e-mail: sulm932@nenu.edu.cn

this paper, we used the *Photobacterium phosphoreum* as the tested organism to evaluate the acute toxicity of both single compounds and mixtures of phenols and Pb. Because the combined ratios of mixtures might lead to different toxic properties (Otitoloju 2002), Pb was set in three levels (low, medium and high concentrations). QSAR models were developed in different combination levels to predict the combined toxicity of phenols and Pb.

Materials and Methods

The freeze-dry powder of *P. phosphoreum* was supplied by the Nanjing Institute of Soil Science, Chinese Academy of Science. The tests were performed according to Yuan et al. (2005). The binary combined toxicity of Pb and nine phenols to *P. phosphoreum* were determined on the basis of single toxicity in three concentrations of Pb (0.2 EC_{50}, 0.5 EC_{50}, 0.8 EC_{50}), respectively. All bioassays were carried out in triplicate for statistical purpose. The median effective concentrations (EC_{50}) in units of ($molL^{-1}$) and the corresponding confidence interval (CI) at 95% were determined by method of Probit analysis (Thomulka et al. 1996) with the SPSS statistic package (version 11.5, SPSS Company, Chicago, IL, USA).

The physico-chemical descriptors of phenols were calculated from different computer programs. The logarithm of *n*-octanol/water partition coefficient (log K_{ow}) and water solubility (WS) were calculated with the software WSKowwin (Version 1.41, EPA, USA). The heat of formation ($\triangle H_f$), the energy of the highest occupied molecular orbital (E_{HOMO}), the energy of the lowest unoccupied molecular orbital (E_{LUMO}) and the core–core repulsion (CCR) were calculated by the quantum chemical method PM3 in MOPAC 2000 from the Chemoffice 2002 program (www.cambridgesoft.com). The ionization constant (p*K*a) was calculated with the software of Micro QSAR (version 2.0, EPA, USA). Molecular connectivity indexes (^2X, ^3X, ^4X) and Molecular valence connectivity indexes ($^2X^v$, $^3X^v$, $^4X^v$) were calculated with the software of Molecular Modeling Pro (version 3.01, ChemSW Software Inc., http://www.chemsw.com). The calculation of dipolarity/polarizability parameter(*S*) and solute excess molar refractivity (*E*) is referred to Abraham et al. (2000) and Zissimos et al. (2002).

Results and Discussion

To analyze and quantify the combined effects of Pb and phenols, additive index (AI; Xu and Nirmalakhandan 1998) was used in this paper. The combined effects were derived from AI values as following: AI > 0, synergism; AI < 0, antagonism; and AI = 0, addition. The results are listed in Table 1. In the Pb concentration of 0.2 EC_{50}, the binary combined effects of Pb and phenols were antagonism. In the Pb concentration of 0.5 EC_{50}, the binary combined effects of Pb and phenols were related to the position of substituted groups of phenols. The combined effects of Pb and *ortho*-substituted phenols were mainly antagonistic. The combined effect of Pb and *meta*-substituted phenol (*m*-nitrophenol) was synergistic. Whereas the combined effects of Pb and *para*-substituted phenols were mainly close to additive action. In the Pb concentration of 0.8 EC_{50}, the binary combined effects of Pb and phenols were synergistic. When Pb was combined with *p*-nitrophenol and *p*-aminophenol, the combined toxicity showed so strong synergistic action that experimental values (log1/EC_{50}) can not be obtained. The result indicated that the ecology risk would be different when Pb concentrations

Table 1 Assessment of combined action of binary mixtures of phenols and Pb

Mixture	Pb (EC_{50})	Toxicity of phenols in mixtures and CI at 95% (log1/EC_{50})	AI
Pb + phenol	0.2	2.89 (2.78–2.97)	−0.37
	0.5	3.22 (3.01–3.38)	−0.04
	0.8	4.03 (3.79–4.71)	0.13
Pb + *o*-nitrophenol	0.2	3.40 (3.34–3.47)	−0.28
	0.5	3.32 (3.26–3.41)	−0.82
	0.8	5.01 (4.62–6.08)	0.21
Pb + *m*-nitrophenol	0.2	3.23 (3.07–3.39)	−0.45
	0.5	4.56 (4.40–4.86)	0.79
	0.8	6.86 (6.36–7.20)	0.25
Pb + *p*-nitrophenol	0.2	3.82 (3.69–3.91)	−1.10
	0.5	4.88 (4.63–4.90)	−0.16
	0.8	–	–
Pb + *o*-aminophenol	0.2	3.97 (3.91–4.09)	−1.09
	0.5	4.08 (4.00–4.17)	−0.97
	0.8	5.63 (5.26–6.39)	0.19
Pb + *p*-aminophenol	0.2	4.88 (4.80–4.97)	−0.11
	0.5	5.18 (5.06–5.29)	0.05
	0.8	–	–
Pb + 2,4-dinitrophenol	0.2	4.28 (4.21–4.34)	−0.08
	0.5	4.88 (4.81–4.96)	0.39
	0.8	5.83 (5.69–6.11)	0.21
Pb + *o*-methoxyphenol	0.2	3.31 (3.24–3.37)	−6.16
	0.5	3.28 (3.10–3.39)	−6.88
	0.8	5.35 (4.69–5.95)	0.16
Pb + *p*-benzenediol	0.2	5.68 (5.57–5.85)	−0.01
	0.5	5.98(5.65–6.68)	0.11
	0.8	6.70 (6.28–7.30)	0.41

and the position of substituted groups of phenols were changed.

The toxicology data of phenols combined with Pb in the concentrations of 0.2 EC_{50} Pb, 0.5 EC_{50} Pb and 0.8 EC_{50} Pb were analyzed with 15 physico-chemical descriptors of phenols using the stepwise linear regression method in the statistic package SPSS11.5, respectively. Model quality was characterized by the number of observations (n), the square of correlation coefficient (R^2), standard error of estimate (SE), mean square ratio (F) and a significant level (p). The different linear structure-toxicity models were obtained in different combinations of Pb and phenols.

When Pb was set in the concentration of 0.2 EC_{50}, the QSAR equation built from the toxicology data of phenols in mixtures and descriptors was expressed as Eq. 1:

$$\log 1/EC_{50} = 0.808 + 2.207 \times 10^{-5}\ WS + 0.853^3X \quad (1)$$

$n = 9$, $R^2 = 0.961$, SE $= 0.201$, $F = 74.802$, $p = 0.000$.

Equation 1 explains most of the variance (0.961) with maximum F value (74.802) and minimum standard error of estimate (0.201). There are two descriptors (WS and ^3X) in Eq. 1. The first parameter entering the equations is WS, the water-solubility of a molecule with units of (mg L^{-1}). The second is the third order molecular connectivity index (^3X). It encodes topological information of a molecule and reflects the size of a molecule and the influence of substituted group position. Schultz (1987) had found that the single toxicity of phenols was related to ionization constant (pKa). Lu et al. (2007) have studied the combined toxicity of phenols and anilines to algae and found that the combined toxicity was related mainly to their electronic properties and hydrophobicity. Those results were not consistent with that of in this paper properly. The reason maybe is that the combined systems (combination of organic and inorganic chemicals) and the combined actions (antagonism in low Pb concentration) are different.

When Pb was set in the concentration of 0.5 EC_{50}, the QSAR equation built from the toxicology data of phenols in mixtures and descriptors was expressed as Eq. 2:

$$\log 1/EC_{50} = -12.039 + 12.315E + 0.502\ pKa \quad (2)$$

$n = 9$, $R^2 = 0.884$, SE $= 0.381$, $F = 22.906$, $p = 0.002$.

It was shown from Eq. 2 that the toxicology data of phenols in mixtures were well correlated to E and pKa with square of correlation coefficient (0.884), F value (22.906) and standard error of estimate (0.381). E is the solute excess molar refractivity in units of ($cm^3\ mol^{-1}$)/10 and can be obtained by the summation of fragments or substructure (Zissimos et al. 2002). The ionization constant (pKa) reflects the proton releasing ability of a compound. Lower the pKa value, stronger the proton releasing ability, and stronger the electrophilic ability; while higher the pKa value, weaker the proton releasing ability, and thus stronger the nucleophilic ability (Yuan et al. 2005). In this combined level of phenols and Pb, the combined actions changed with the position of substituted groups of phenols, antagonism, addition and synergism all exist, so the descriptors in Eq. 2 are not common with those in low Pb concentration.

When Pb was set in the concentration of 0.8 EC_{50}, the QSAR equation built from the toxicology data of phenols in mixtures and descriptors was expressed as Eq. 3:

$$\log 1/EC_{50} = 2.221 + 2.882S \quad (3)$$

$n = 7$, $R^2 = 0.634$, SE $= 0.647$, $F = 8.658$, $p = 0.032$.

It was shown from Eq. 3 that the toxicology data of phenols in mixtures were related to the dipolarity/polarizability (S) of a compound that can be obtained from gas liquid chromatographic measures on polar stationary phases, or more generally from water–solvent partition coefficients (Zissimos et al. 2002). Since the toxicities are synergistic in this combined level, the QSAR model varied

Table 2 The descriptors of phenols and the relative errors from QSAR models

Compounds	WS	^3X	E	pKa	S	Relative error values		
						Er. (1)[a]	Er. (2)[a]	Er. (3)[a]
Phenol	26,160	1.89	0.81	9.92	0.89	−3.72	9.44	−18.76
o-Nitrophenol	2,500	3.03	1.02	6.80	1.05	−1.40	−18.55	−4.73
m-Nitrophenol	6,290	2.92	1.05	8.27	1.57	−6.43	−10.60	1.67
p-Nitrophenol	7,507	3.00	1.07	7.15	1.72	7.52	3.13	–
o-Aminophenol	32,360	2.54	1.11	4.84	1.10	7.08	0.48	4.24
p-Aminophenol	101,200	2.30	1.15	5.47	1.20	−2.53	6.00	–
2,4-Dinitrophenol	1,971	4.10	1.20	4.03	1.49	−1.61	2.42	−11.75
o-Methoxyphenol	7,226	2.81	0.84	9.92	0.91	−1.64	−0.17	9.47
p-Benzenediol	129,500	2.30	1.06	9.55	1.27	0.92	2.86	12.22

[a] Er. (1), Er. (2) and Er. (3) are relative errors from Eqs. 1, 2 and 3 which are defined as the difference between the experimental and predicted values divided by the experimental values × 100%

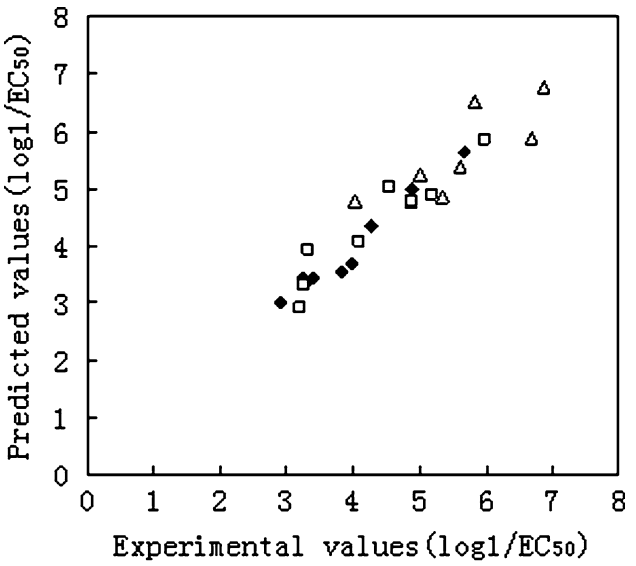

Fig. 1 Plot of experimental and predicted values of phenols in mixtures from Eqs. 1 (◆), 2 (□) and 3 (△)

and the combined toxicity is only related to dipolarity/polarizability parameter (*S*).

The combined actions of Pb and phenols were related to the Pb concentrations in mixtures. It is shown from Eqs. 1, 2 and 3 that different QSAR models were obtained when Pb was combined with phenols in low, medium and high concentration. The different combined actions in three Pb concentrations may be one reason. The descriptors of phenols in QSAR equations and the relative errors (Yuan et al. 2005) from Eqs. 1, 2 and 3 are listed in Table 2.

As shown in Table 2, the 80% relative error values are lower than 10% when predicted by three equations, whilst all the relative error values remain within 20%. The plot of the predicted values from Eqs. 1, 2 and 3 versus the experimental values is shown in Fig. 1. There is a good agreement between the predicted values and experimental values with $R^2 = 0.898$.

In conclusion, when Pb was set in different concentrations, the combined toxicity of phenols and Pb was related to different physico-chemical descriptors. The binary combined effects of Pb and phenols were antagonistic in low Pb concentration and the combined toxicity was related to WS and ^3X. In the medium Pb concentration, the combined effects were changed with the position of substituted groups of phenols and the combined toxicity was related to *E* and p*K*a. In the Pb concentration of 0.8 EC_{50}, the binary combined effects were synergistic and the combined toxicity was related to *S*.

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