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Acute toxicity of substituted phenols to *Rana japonica* tadpoles and mechanism-based quantitative structure–activity relationship (QSAR) study

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Abstract

Acute 12 h and 24 h lethal toxicity (12 h-LC_{50} and 24 h-LC_{50}) of 31 substituted phenols to *Rana japonica* tadpoles was determined. Results indicate that toxicity of phenols to tadpoles varied only slightly with length of exposure and the 12-h test could serve as surrogate of the 24-h test. A mechanism-based quantitative structure–activity relationship (QSAR) method was employed and 1-octanol/water partition coefficient ($\log K_{ow}$)-dependent models were developed to study different modes of toxic action. Most phenols elicited their response via a polar narcotic mechanism and an excellent $\log K_{ow}$-dependent model was obtained. Soft electrophilicity and pro-electrophilicity were observed for some phenols and a good $\log K_{ow}$-dependent model was also achieved. Additionally, the significant dissociation of carboxyl on benzoic acid derivatives sharply reduced their toxicity. A statistically robust QSAR model was developed for all studied compounds with the combined application of $\log K_{ow}$, energy of lowest unoccupied orbital (E_{lumo}), heat of formation (HOF) and the first-order path molecular connectivity dices ($^1\chi_p$). © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Phenols; Acute toxicity; *Rana japonica* tadpoles; QSAR; Mechanism of action

1. Introduction

In the past several decades, large numbers of frogs were killed due to the release of industrial chemicals. *Rana japonica* are typical amphibious animals bridging the gap between aquatic and terrestrial animals. The larvae of frogs, tadpoles, proved to be more sensitive to some pesticides than adults (Gong, 1994). It is necessary and urgent to investigate the adverse effect of industrial materials on tadpoles and the toxic mechanisms to further protect frogs and aquatic environment.

Quantitative structure–activity relationships (QSARs) have been used widely to predict the hazard of untested chemicals with already tested chemicals by developing statistical relationships between molecular structure descriptors and biological activity. The present development and application of QSARs have been evolving from a chemical class perspective to one that is more consistent with assumptions regarding modes of toxic action (Verhaar et al., 1992; Bradbury, 1994). In the most successful QSARs, toxicants have been divided into many subsets according to their mechanism of toxic action (Schultz et al., 1990; Cronin and Dearden, 1995).

Phenol and its derivatives are used widely in pharmacy, industry and agriculture as intermediates of dyes, the organic synthesis process. They are found in disinfectants and antiseptics, and consequently have a high

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potential for environmental pollution. Much effort has been put into studying the mechanisms of toxic action of phenols with *Pimephales promelas* and *Tetrahymena pyriformis* (Bearden and Schultz, 1997; Cronin and Schultz, 1996). QSAR models were developed and explained. Polar narcosis and/or uncoupling of weak acid respiratory mechanisms were established for most phenols (Schultz et al., 1992, 1986) and selected phenols were determined to be bio-reactive (Bearden and Schultz, 1997) in the above two assay systems.

The objective of this paper is to investigate the acute toxic effect of phenols on the frog tadpoles, to develop statistically robust QSAR models, and to study the mechanisms of acute toxicity of phenols to larvae of amphibious animals.

2. Materials and methods

2.1. Chemicals

Acute toxicity of 31 substituted phenols to *R. japonica* tadpoles was determined. All chemicals tested were provided by Department of Chemistry at Nanjing University. All were of sufficient purity (analytical purity) and further purification was not necessary.

2.2. Tadpoles and acute toxicity test

Naturally fertilized egg masses of *R. japonica* were collected from a permanent pond in Nanjing, where *R. japonica* were exclusively bred. *R. japonica* tadpoles were employed for toxicity test because they are very common in China and green frog tadpoles proved to be more sensitive to hazards than other tadpoles (Berrill et al., 1995). All eggs had been laid no more than 2 days prior to collection. Eggs and the resulting tadpoles were cultured in aerated stream water (Cl^- 3.40 mg/l, pH 7.74, dissolved oxygen concentration 9.6 mg/l, Na^+ 2.35 mg/l, Ca^{2+} 48 mg/l) in the laboratory at room temperature (22–25°C). Tadpoles were fed an excess of rinsed, salt free canned spinach daily and water was renewed every other day. Feeding was halted 24 h prior to testing. The tadpoles were staged according to Gosner (1960). Thirty-day-old (stage 30 to stage 32) healthy individuals of similar size and weight were employed for the test. The average body length and body weight were 2.5 ± 0.1 cm and 0.09 ± 0.01 g, respectively.

All exposures took place at water temperature 22–25°C in a controlled environmental chamber. Aerated chloride-free water was used for preparing the stock solutions of chemicals and test dilution. A six-step concentration series in geometric grade ranging from no effect to 100% lethal concentration were set after range finding experiments. The pH of test solutions was measured by a PHS-3C pH-Meter. Ten tadpoles were employed for each test and placed in a 1000 ml pyrex beaker containing 500 ml test solution or aerated chloride-free water. The aerated chloride-free water without test compounds served as the control. Solutions and the control were renewed every 6 h to achieve semi-static exposure. All experiments were performed with three replicates of each treatment group and unexposed controls. The test was terminated on condition that mortality in controls exceeded 10%. Chemical persistence study was omitted for Schultz et al. (1989) demonstrated that abiotic loss of phenols did not affect the toxicity tests. Photoperiod was 14 h (light): 10 h (dark). Mortality was recorded and concentration needed to obtain half mortality for each chemical was calculated based on nominal concentrations. Logarithms of inverse of median lethal concentrations after 12-h and 24-h exposure, expressed as 12 h-LC_{50} (mmol/l) and 24 h-LC_{50} (mmol/l), were calculated for each compound as toxicity using nonlinear regression in STATISTICA for Windows software (1995).

2.3. Physico-chemical descriptors

Log K_{ow} were computer estimated or retrieved as measured values from SRC-WSKOW for Microsoft Windows (1995). *PKa* were cited from Dean (1985) or calculated according to methods described by Harris and Hayes (1982).

Molecular orbital parameters were calculated from the semi-empirical molecular orbital package MOPAC6.0 (Stewart, 1990). The molecular modeling package ALCHEMY II (Tripos Associates, 1985) was used to construct and view and write all molecular structures. Molecular geometry was optimized with Broyden-Flether-Goldfarb-Shanno (BFGS) method in MOPAC6.0. All quantum descriptors were calculated using AM1 Hamiltonian.

Molecular connectivity indices (MCIs) including zero to sixth order simple and valence corrected path and cluster indices were calculated according to the method outlined by Kier and Hall (1986).

2.4. Statistical analysis

STATISTICA for Windows software (1995) was employed for QSAR analysis. The toxic potency, 24 h-LC_{50}, was employed as the dependent variable, and the calculated molecular structure descriptors as the independent variables. Model quality was characterized by the number of observation (n), the square of the correlation coefficient (r^2), the standard error of estimate (S.E.), the Fisher criterion (F), the significance level (P) and the variance inflation factor (VIF). The VIF is defined as $1/(1-R^2)$, where R is the correlation coefficient of one independent variable against others. Large VIF values imply strong correlation.

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3. Results and discussion

Acute toxicity of phenols to tadpoles after 12-h exposure and 24-h exposure was measured. The calculated 12 h-LC_{50} and 24 h-LC_{50} values are listed in Table 1. The pH of test solutions ranged from 3.0 to 7.10. No mortality occurred in the control except for the testing of chemical 25, 26 and 27. Mortality of tadpoles in the control exceeded 10% for the 12-h exposure of chemical 25, 26 and 27, so the 12 h-LC_{50} values of the three chemicals were absent.

To investigate the effect of exposure time on toxicity of phenols to tadpoles, 12 h-LC_{50} and 24 h-LC_{50} values were compared. The result showed that 12 h-LC_{50} and 24 h-LC_{50} values for each compound were approximately identical. Simple regression analysis on 24 h-LC_{50} against 12 h-LC_{50} was performed and model 1 was obtained.

Model 1

$$\text{24 h-LC}_{50} = 1.02 \times \text{12 h-LC}_{50} - 0.03,$$
$$n = 28, \quad r^2 = 0.997, \quad F = 7325,$$
$$\text{S.E.} = 0.05, \quad P < 0.00000001.$$

A slope of near unity and an intercept of near zero for model 1 was evidence that toxic potency for the phenols measured in the acute toxicity assay of *R. japonica* tadpoles varied only slightly with length of exposure. The 12 h-LC_{50} and the 24 h-LC_{50} values were

Table 1
Compounds, observed toxicity after 12-h and 24-h exposure, and calculated 24-h toxicity of phenols to *R. japonica* tadpoles as well as pH of tested solutions on 24 h-LC_{50}

Chemical no.	Chemical name	CAS number	pH[a]	*pKa*	LC_{50} (mmol/l)		
					12 h	24 h	Calculated[b]
1	2-Nitrophenol	88-75-5	5.78	6.8	3.564	3.686	3.958
2	3-Nitrophenol	554-84-7	6.40	8.4	3.429	3.482	3.903
3	4-Nitrophenol	123-30-8	5.95	7.15	4.015	4.052	3.901
4	4-Chloro-2-nitrophenol	89-64-5	6.02	6.48	4.682	4.880	4.419
5	2-Nitroresorcinol	601-89-8	5.41	6.57	3.370	3.380	3.397
6	2-Chlorophenol	95-57-8	6.33	8.5	3.011	3.024	3.326
7	4-Chlorophenol	106-48-9	6.79	9.38	3.281	3.308	3.292
8	2,4-Dichlorophenol	121-83-2	6.47	7.85	3.873	3.996	3.862
9	4-Bromophenol	106-41-2	6.61	9.45	3.560	3.619	3.580
10	2-Bromo-4-methylphenol	–[c]	6.70	8.67	3.717	3.753	3.935
11	2-hydroxyl-5-chlorobenzoic acid	321-14-2	3.10	2.67	3.011	3.012	3.267
12	4-Fluorophenol	371-41-5	6.47	9.79	2.652	2.670	2.429
13	4-Methoxylphenol	150-76-5	6.63	10.21	2.371	2.371	2.646
14	2-Methoxylphenol	90-5-1	6.37	10.33	2.460	2.500	2.510
15	4-Methylphenol	106-44-5	6.60	10.17	2.846	2.928	3.064
16	2-Methylphenol	95-48-7	6.57	10.23	2.822	2.819	3.098
17	2,6-Dimethylphenol	576-26-1	6.55	10.20	3.324	3.331	3.426
18	Salicylaldehyde	90-02-8	6.42	8.34	3.914	3.968	3.382
19	4-Hydroxylbenzaldehyde	123-08-0	5.78	9.23	2.802	2.804	2.836
20	Salicylic acid	69-72-7	3.11	2.98	2.840	2.841	3.068
21	2-Hydroxyl, methyl benzoate	119-36-8	6.55	8.86	3.315	3.303	3.411
22	4-Hydroxyl, methyl benzoate	99-76-3	6.31	8.89	3.160	3.163	2.962
23	Diphenylol propane	80-5-7	6.61	10.2	4.701	4.710	4.457
24	Diphenylol sulfoxide	–[c]	5.75	8.47	2.790	2.853	3.263
25	1,1-Diphenylol ethane	–[c]	6.40	9.84	–[d]	4.014	4.146
26	Resorcinol	108-46-3	6.98	9.44	–[d]	2.077	2.181
27	3-Aminophenol	591-27-5	7.10	4.21	–[d]	2.064	1.931
28	4-Hydroxylacetophenone	99-93-4	5.87	8.05	2.503	2.509	2.719
29	4-Hydroxyl-benzyl acetic acid	156-38-7	3.48	5.67	2.497	2.505	1.914
30	4-Tert-Butylphenol	98-54-4	6.26	10.43	4.168	4.170	3.888
31	Phenol	108-95-2	6.45	9.99	2.779	2.804	2.749

[a] pH of the solution on 24 h-LC_{50} for each compound.
[b] Calculated from model 9.
[c] CAS registry number was unavailable.
[d] Not determined for mortality of controls exceeded 10%.

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almost identical and interchangeable. The 24 h-LC_{50} data set was chosen for the subsequent analysis.

It is generally accepted that the most successful QSARs have divided organic pollutants into many subsets according to their mechanisms of toxic action and separate QSARs can be developed for each mechanism of toxic action (Schultz et al., 1990; Cronin and Dearden, 1995). Log K_{ow}-dependent QSAR models are often developed to study the different toxic mechanisms of chemicals (Bearden and Schultz, 1997; Schultz et al., 1986). In our investigation, the hydroxyl substituent, with its loosely bound lone pair of electrons can conjugate with electron-withdrawing groups by resonance through the aromatic ring of the molecule (Hansch and Leo, 1979). Furthermore, all substituted phenols possess varied other non-log K_{ow}-dependent substituents on the aromatic moiety besides a hydroxyl group, including alkyl, alkoxyl, halogen, nitro, hydroxyl, carbonyl, amino, etc. The toxic mode of action may, to a great extent, depend on other substituents. In addition, the dissociation of hydroxyl group and other weak acid groups will occur. All these non-log K_{ow}-related factors may be important in determining relative toxicity and may suggest multiple mechanisms for the tested compounds.

The pH of the test solutions on 24 h-LC_{50} for all compounds, as well as the corresponding *pKa*, are also presented in Table 1. The comparison of pH and *pKa* for each compound indicated that the pH of test solution was much lower than corresponding *pKa* and dissociation hardly occurred for most tested compounds except for salicyclic acid, 2-hydroxyl-5-chlorobenzoic acid and 3-aminophenol. The pH values of the two benzoic acid derivatives and 3-aminophenol were greater than their *pKa*, which indicated that dissociation would occur significantly for the three compounds.

Fig. 1 is a scatter plot of the 24 h-LC_{50} of 31 phenols versus log K_{ow}, from which two obvious linear relationships can be observed visually, with a few outliers. This may suggest more than one mechanism exists. To confirm this, A simple linear regression of 24 h-LC_{50} with log K_{ow} was performed. Chemicals which proved to be outliers of the obtained log K_{ow}-dependent model were omitted. Then a new linear regression was performed and a new log K_{ow}-dependent model was obtained. The progress was repeated until a statistically robust log K_{ow}-dependent QSAR model was achieved without either statistical or obvious visual outliers observed. The omitted chemicals, the resulting model from each analysis, and the parameters characterizing the models were summarized in Table 3.

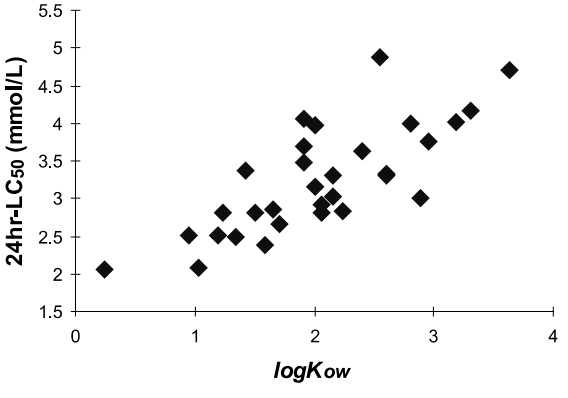


Fig. 1. Relationship of 24 h-LC_{50} (mmol/l) with log K_{ow} of phenols to *R. japonica* tadpoles after 24-h exposure.

The relationship of toxicity of all 31 studied phenols with log K_{ow} was analyzed and model 2 was obtained. Four nitro-containing substituted phenols proved to be outliers and omission of the four chemicals prior to reanalysis led to an improved model 3. Again salicylaldehyde and 4-hydroxylbenzaldehyde proved to be outliers of model 3 and the removal of the two chemicals resulted in a further improved model 4 with two outliers: 2-hydroxyl-5-chlorobenzoic acid and salicylic acid. The two benzoic acid derivatives were omitted and a greatly improved model 5 was obtained. 3-aminophenol proved to be the only outlier to model 5 and an excellent linear log K_{ow}-dependent model 6 was observed after deleting 3-aminophenol. Of all log K_{ow}-dependent models, Model 6 explains most of the variance (91%), with maximum F values (194.5) and minimum standard error of estimate (0.21) and with neither statistical nor obvious visual outliers observed.

Polar narcosis was reported to be the main mechanism for most substituted phenols, including alkyl and alkoxyl substituted, mono- and di-halogen substituted phenols, etc (Schultz et al., 1989). Chemicals which were classified as polar narcotics usually exhibited higher toxicity than baseline toxicity due to their strong electron releasing amino or hydroxyl moieties present on an aromatic ring. This resulted in greater dipolarity and/or hydrogen bond donor acidity than in non-polar narcotics (Kamlet et al., 1986). While the molecular structures of the tested chemicals and the resulting excellent log K_{ow}-dependent relationship were considered, the conclusion was drawn that phenols modeled by model 6 acted as polar narcotics. The relationship of toxicity of 31 phenols with *pKa* was investigated but no correlation was observed. And no significant correlation of toxicity with *pKa* was observed either for the 21 polar narcotic phenols or for the remaining 10 phenols. These demonstrated that *pKa* contributed little to toxicity of phenols to *R. japonica* tadpoles under current test conditions.

To explore the toxic mechanism of the 10 omitted compounds, the log K_{ow}-dependent relationship was investigated. Good correlation was obtained between toxicity and hydrophobicity after removing two benzoic

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acid derivatives from the toxic data set: 2-hydroxyl-5-chlorobenzoic acid and salicylic acid. The removal of the two benzoic acid derivatives was justified by the fact that all the outliers are underestimated by the $\log K_{ow}$-dependent model 2 except the two benzoic acid derivatives. Their toxicity is significantly overestimated. The resulting $\log K_{ow}$-based model 7 is as follows:

Model 7

$$LC_{50} = 0.94 \log K_{ow} + 1.60,$$

$$n = 8, \quad r^2 = 0.88, \quad F = 43.4,$$

$$\text{S.E.} = 0.33, \quad P = 0.00057.$$

Bearden and Schultz (1997) concluded that an increase in bio-reactivity would result in a decrease in slope and predictability of $\log K_{ow}$-dependent QSAR models. A smaller slope and decreased predictability were observed for model 7 (0.94 for slope and 0.88 for r^2) compared to model 6 (0.95 for slope and 0.91 for r^2), which might imply different mechanisms for the two QSARs. To justify this, the relationship between toxicity and E_{lumo}, was investigated. Significant correlation was found for the eight compounds:

Model 8

$$LC_{50} = -0.78\ E_{lumo} + 2.71,$$

$$n = 8, \quad r^2 = 0.54, \quad F = 7.1,$$

$$\text{S.E.} = 0.64, \quad P = 0.031.$$

E_{lumo} is the energy of the lowest unoccupied orbital representing the electrophilicity of a molecule and the ability of a molecule to accept electrons. Strong correlation with $\log K_{ow}$ and obvious correlation with E_{lumo} demonstrated that the toxicity of the eight outliers were both hydrophobic dependent and affected by electric properties. Aromatic-nitro-containing compounds and benzaldehyde derivatives were found to possess much more negative E_{lumo} values. This implies that the toxicity of these chemicals involved their strong in vivo redox reactivity.

The two benzoic acid derivatives, 2-hydroxy-5-chlorobenzoic acid and salicylic acid, were outliers with their toxicity being considerably overestimated by $\log K_{ow}$-dependent models 4 and 7. The reason could be the significant dissociation of the carboxyl group as the pH of the test solutions was greater than their corresponding *pKa*, which resulted in the calculated $\log K_{ow}$ values not being a reflection of the true partitioning of them and significant reduction of the toxicity. While another aromatic acid derivative, 4-hydroxylbenzyl acetic acid was correctly modeled by the $\log K_{ow}$-based model 6. A comparison showed that the pH (3.48) of the test solution on half mortality concentration of 4-hydroxylbenzyl acetic acid was far lower than its *pKa* (5.67) and thus dissociation hardly occurred.

The molecular structure of salicylaldehyde is very similar to that of α,β-unsaturated compounds as its carbonyl group is conjugated with aromatic double bonds. It exhibits a highly bio-reactive toxicity, as it may covalently react with soft macromolecular nucleophiles via a nucleophilic Michael-type addition reaction. Excess toxicity of salicylaldehyde was also observed in the *P. promelas* assay system, and salicylaldehyde was classified to be an electrophiles/ proelectrophiles (Russom et al., 1996).

Amino-substituted phenols may be metabolized in vivo to quninone-like compounds that are known as strong electrophiles (Dupuis and Benezra, 1982). In vitro investigations have identified soft electrophilic arylation and redox cycling oxidation as the most likely mechanisms of quinone toxicity (Brunmark and Cadenas, 1978; O'brien, 1991; Monks et al., 1992). Quinones may covalently react with soft macromolecular nucleophiles via nucleophilic Michael-type addition and may also be reduced enzymatically by either one-electron or two-electron processes to semiquinones or hydroquinone, respectively. Semiquinones themselves may be toxic or act as radical anions. The anion radical can reduce O_2 to a toxic superoxide anion leading to oxidative stress. The regeneration of the parent quinone and the reduction of O_2, is a cyclic process and, thus, is futile (Mason, 1990). Whether a quinone acts as an arylating soft electrophile or redox cycler is dependent on many factors including molecular structure, pH, the presence or absence of O_2, and the quinone to thiol ratio. Thus amino-substituted phenols might act as pro-electrophilic regardless of metabolic pathway. In this study, despite of the significant dissociation of 3-aminophenol due to the pH of the test solution greater than its *pKa*, excess toxicity was still observed.

Nitro-substituted phenols exhibited much higher toxicity than predicted by the $\log K_{ow}$-dependent QSAR model 2. Aromatic-nitro-containing compounds were reported to be uncouplers of oxidative phosphorylation and were regarded as pro-electrophiles, yielding the corresponding potentially highly toxic C-nitroso compounds (Robert, 1987). Excess toxicity of 2-nitrophenol and 4-nitrophenol have been studied and consequently regarded as soft electrophiles (Cronin and Dearden, 1995). 4-nitrophenol was also found to be an outlier in the polar narcosis QSAR model and was classified to be bio-reactive (soft electrophilic) in the *E. coli* system (Russom et al., 1996), while excess toxicity of 3-nitrophenols was not testified in other test systems.

To determine a general and robust QSAR model, all calculated descriptors including $\log K_{ow}$, quantum chemical descriptors, *pKa* and MCIs were applied. Four parameters (Table 2) proved to be significant and entered the obtained QSAR model: $\log K_{ow}$, E_{lumo}, HOF,

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Table 2
Molecular descriptors for developing QSARs to model acute 24 h toxicity to *R. japonica* tadpoles of selected phenols

Chemical no.	Chemical name	CAS number	$\log K_{ow}$	E_{lumo}[a]	$^1\chi_p$[b]	HOF[c] (kcal)
1	2-Nitrophenol	88-75-5	1.91	−1.184	4.722	−20.167
2	3-Nitrophenol	554-84-7	1.91	−1.167	4.206	−17.961
3	4-Nitrophenol	123-30-8	1.91	−1.064	4.698	−19.551
4	4-Chloro-2-Nitrophenol	89-64-5	2.55	−1.227	5.109	−21.209
5	2-Nitroresorcinol	601-89-8	1.43	−1.322	5.126	−64.59
6	2-Chlorophenol	95-57-8	2.15	0.066	3.805	−26.666
7	4-Chlorophenol	106-48-9	2.16	0.098	3.788	−29.298
8	2,4-Dichlorophenol	121-83-2	2.8	−0.244	4.198	−35.142
9	4-Bromophenol	106-41-2	2.4	0.02	3.787	−17.594
10	2-Bromo-4-methylphenol	–[d]	2.95	0.027	4.198	−22.134
11	2-Hydroxyl-5-chlorobenzoic acid	321-14-2	2.89	−0.070	5.109	−114.95
12	4-Fluorophenol	371-41-5	1.71	0.629	3.788	−67.056
13	4-Methoxylphenol	150-76-5	1.59	0.305	4.326	−59.35
14	2-Methoxylphenol	90-5-1	1.34	0.262	4.343	−59.648
15	4-Methylphenol	106-44-5	2.06	0.429	3.788	−29.794
16	2-Methylphenol	95-48-7	2.06	0.369	3.805	−29.449
17	2,6-Dimethylphenol	576-26-1	2.61	0.392	4.215	−36.109
18	Salicylaldehyde	90-02-8	2.01	−0.593	4.343	−55.498
19	4-Hydroxylbenzaldehyde	123-08-0	1.23	−0.45	4.326	−53.857
20	Salicylic acid	69-72-7	2.24	−0.557	4.722	−112.75
21	2-Hydroxyl, methyl benzoate	119-36-8	2.6	−0.449	5.607	−105.87
22	4-Hydroxyl, methyl benzoate	99-76-3	2.0	−0.398	5.236	−106.08
23	Diphenylol propane	80-5-7	3.64	0.3691	7.998	−48.193
24	Diphenylol sulfoxide	–[d]	1.65	−0.642	7.998	−99.957
25	1,1-Diphenylol ethane	–[d]	3.19	0.335	7.665	−48.729
26	Resorcinol	108-46-3	1.03	0.272	3.788	−66.597
27	3-Aminophenol	591-27-5	0.24	0.521	3.805	−24.049
28	4-Hydroxylacetophenone	99-93-4	1.19	−0.249	4.698	−58.659
29	4-Hydroxyl-benzyl acetic acid	156-38-7	0.95	0.148	5.182	−119.79
30	4-Tert-butylphenol	98-54-4	3.31	0.47	4.999	−40.563
31	Phenol	108-95-2	1.51	0.398	3.394	−22.241

[a] E_{lumo} = energy of lowest unoccupied orbital.
[b] $^1\chi_p$ = first path simple molecule connectivity indices.
[c] HOF = heat of formation.
[d] CAS registry number was unavailable.

Table 3
Log K_{ow}-dependent models, parameters characterizing model quality and omitted chemicals

Model	24 h-LC50	n	Omitted chemicals[a]	r^2	F	S.E.	P
2	0.76 $\log K_{ow}$ + 1.75	31	4-Chloro-2-nitrophenol;[b] 3-Nitrophenol;[b] 4-nitrophenol[b] 2-Nitroresorcinol;[b] 2-nitrophenol[b]	0.58	39.6	0.48	<0.000001
3	0.88 $\log K_{ow}$ + 1.63	26	Salicylaldehyde[b] 4-Hydroxylbenzaldehyde[c]	0.77	79.9	0.33	<0.000001
4	0.92 $\log K_{ow}$ + 1.54	24	2-Hydroxyl-5-chlorobenzoic acid[b] Salicylic acid[c]	0.84	114.6	0.28	<0.000001
5	0.95 $\log K_{ow}$ + 150	22	3-Aminophenol[c]	0.90	184.7	0.23	<0.000001
6	0.95 $\log K_{ow}$ + 135	21	No outlier	0.91	194.5	0.21	<0.000001

[a] Chemicals which proved to be outliers of the current log K_{ow}-dependent model and were omitted prior to the construction of next the model.
[b] Statistical outliers.
[c] Visual outliers.

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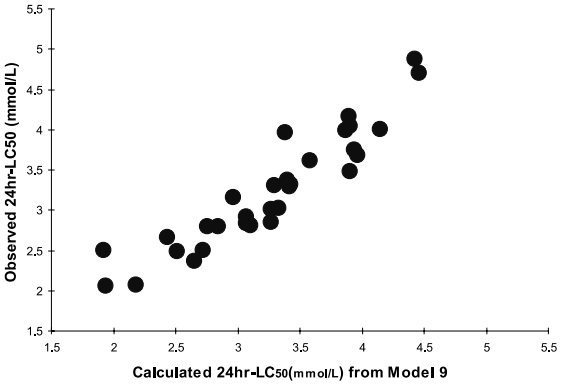


Fig. 2. Calculated 24 h-LC_{50} (mmol/l) from model 9 versus observed 24 h-LC_{50} (mmol/l) of phenols to *R. japonica* tadpoles.

and $^1\chi_p$. The QSAR model containing four variables is shown as model 9. Fig. 2 is the plot of observed 24-h toxicity of phenols to tadpoles versus the calculated 24-h toxicity by model 9. Model fitting results were listed in Table 4.

Model 9

$$\mathrm{LC}_{50} = 0.65\log K_{\mathrm{ow}} - 0.39E_{\mathrm{lumo}} + 0.38\mathrm{HOF} + 0.21{}^1\chi_p + 1.76,$$
$$n = 31, \quad r^2 = 0.85, \quad F = 37.7,$$
$$\mathrm{S.E.} = 0.30, \quad P < 0.000001.$$

Parameters reflecting hydrophobicity, electric properties and steric effect are selected to construct the QSAR model 9. All four variables contain clear physiochemical meaning: $\log K_{\mathrm{ow}}$ represents the hydrophobicity of chemicals and reflects the ability of chemicals to penetrate the bio-membrane and reach the interacting sites. E_{lumo} describes the electric properties of molecules and measures the reactivity of compounds. HOF reflects conformation stability of a molecule. And $^1\chi_p$ contains steric information such as molecular branching, molecular size, molecular volume and molecular surface area

Table 4
Model fitting results of model 9 for toxicity of phenols to *R. japonica* tadpoles

Independent variable	Coefficient	Standard error of estimate	*t*-statistics	*P*-level
Constant	1.76	0.24	7.4	0.000000
$\log K_{\mathrm{ow}}$	0.65	0.09	7.5	0.000000
E_{lumo}[a]	−0.39	0.08	−5.1	0.000026
HOF(kcal)[b]	0.38	0.09	4.4	0.000144
$^1\chi_p$[c]	0.21	0.10	2.2	0.039261

[a] Energy of the lowest unoccupied orbital.
[b] Heat of formation (kcal).
[c] First-order simple molecule connectivity indices.

Table 5
Correlation matrix of selected variables in model 9

	$\log K_{\mathrm{ow}}$	HOF[a]	E_{lumo} [b]	$^1\chi_p$ [c]	VIF[d]
$\log K_{\mathrm{ow}}$	1.00				1.327
HOF[a]	0.10	1.00			1.048
E_{lumo} [b]	0.05	0.05	1.00		1.303
$^1\chi_p$ [c]	0.40	−0.39	−0.16	1.00	1.590

[a] HOF = heat of formation (kcal).
[b] E_{lumo} = energy of the lowest unoccupied orbital.
[c] First-order simple molecule connectivity indices.
[d] VIF = variance inflation factor, a VIF $\geqslant$ 5 implies strong correlation.

(Kier and Hall, 1986). The significance of the multiple-variable-containing model is justified by *F*- and *P*-values of model 9, the majority of variances (85%) being explained and the significance of all variables in the model ($P \leqslant 0.039$). The importance of the descriptors is indicated by the sign and magnitude of the *t*-statistics and *P*-levels in Table 4. The selected variables explain the anticipated importance of hydrophobicity and stereo-electronic parameters. While the correlation matrix (Table 5) was studied, no obvious correlation was found among the four variables (absolute values of the correlation coefficients among variables were not greater than 0.40 and VIF values were far less than the limit 5, which indicates a strong correlation), so no overlapped information was included in QSAR model and the model could be explained mechanistically. The QSAR model 9 explains the majority of the variances, covers all studied chemicals with diverse substituents and different toxicity mechanisms. So it is satisfactory enough to act as a general predictor.

4. Conclusion

Acute toxicity of 31 selected phenol derivatives to *R. japonica* tadpoles was determined post 12-h and 24-h semi-static exposure. 12 h-LC_{50} and 24 h-LC_{50} values were found to be identical and interchangeable, and the 12-h toxicity test could serve as a surrogate of the 24-h test.

A mechanism-based QSAR method was employed to study the mechanisms of phenols and $\log K_{\mathrm{ow}}$-dependent QSAR models were developed to classify tested substances according to their mode of toxic action. Polar narcosis proved to be the main mode of action for most of the substituted phenols, including some mono and/or di halogen-, alkyl- and alkoxyl- substituted phenols. Some hydroxyl-substituted aromatic esters, aromatic alcohols and aromatic ketones, and some bisphenols (chemicals 23, 24, 25) also fell into this category. For polar narcotics, a robust $\log K_{\mathrm{ow}}$-dependent QSAR model was obtained ($\mathrm{LC}_{50} = 0.95\log K_{\mathrm{ow}} + 1.35$, $n = 21$, $r^2 = 0.91$). The significant dissociation of carboxyl

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on benzoic acid derivatives greatly reduced their toxicity. The remaining eight chemicals including salicylaldehyde, 4-hydroxylbenzaldehyde, amino- and nitro-substituted phenols, which exhibited excess toxicity, were classified to be bio-reactive (electrophilic and pro-electrophilic) and a good $\log K_{ow}$-dependent QSAR model was successfully obtained ($LC_{50} = 0.94 \log K_{ow} + 1.60$, $n = 8$, $r^2 = 0.88$). For the eight bio-reactive chemicals, a significant E_{lumo}–dependent relationship was also found ($LC_{50} = -0.78E_{lumo} + 2.71$, $n = 8$, $r^2 = 0.54$). *pKa* was proved to contribute nothing in determining acute toxicity of tested phenols to *R. japonica* tadpoles under current test conditions.

A general and robust QSAR model was achieved for all 31 phenols with the combined application of variables reflecting hydrophobicity, electric property, steric effect and conformation stability of molecule, respectively ($LC_{50} = 0.65 \log K_{ow} - 0.39E_{lumo} + 0.38\ HOF + 0.21\ ^1\chi_p + 1.76$, $n = 31$, $r^2 = 0.85$), and the model can be explained mechanistically.

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