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Comparative Inhibition and Quantitative Structure–Activity Relationships (QSARs) of Substituted Phenols to Germination Rate of *Cucumis sativus*

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Quantitative Structure-Activity Relationships (QSARs) that correlate molecular structure to biological activity have been used widely to predict the hazard of untested chemicals and have proved to be a useful tool for use in hazard assessment of organic chemicals (Bradbury, 1994).

Phenol and its derivatives are widely used in pharmacy, industry and agriculture as intermediates of dyes, the organic synthesis process. Due to the many origins and magnitude of uses, they are widespread in ecosystem and consequently have a high potential for environmental pollution. Much effort has been put into studying the toxicity of phenols to aquatic organisms, including *Pimephales promelas*, *Tetrahymena pyriformis* and *Rana Japonica* tadpole (Schultz et al., 1986; 1989; 1992; Bearden and Schultz, 1997; Cronin and Schultz, 1996; Wang et al.2000a and 2001a). However, in comparison with the extensive study of toxicity of phenolic compounds in aquatic organisms, not much attention was paid to adverse effect of phenols on higher terrestrial plants.

Terrestrial macrophytes are primary producers of O_2 and useful energy and organic substances. The effect on plants can directly affect structure and function of an ecosystem, thus inevitably affect other life forms including mammals and human beings (Wang, 1991). So information on phytotoxicity is required for the ecological risk assessment of pollutants. The method of germination rate and root elongation provides valuable information about inhibition, enzyme activation, cell expansion, respiration, and other parameters and possesses several advantages over those toxicity tests using animals and algae, such as sensitivity, simplicity, low cost and suitability for unstable chemicals or samples with renewal or flow-through methods (Wang, 1991; Mayer and Poljakoff-mayber, 1982; Wang, 2000b and 2001b). In this paper the comparative inhibition effect of some structurally diverse substituted phenols on the germination rate of *cucumis sativus* was determined. QSARs were developed to 1) investigate the structural features that determined the phytotoxicity of substituted phenols; 2) establish highly predictive QSAR models to predictive phytotoxicity of chemicals from their molecule structures.

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MATERIALS AND METHODS

All tested chemicals were provided by Department of Chemistry, Nanjing University (Table 1). All were of sufficient purity (analytical purity) and further purification was not necessary. The seeds of *Cucumis sativus* (sprout containing ≥ 95%, purity ≥ 95%) were employed for tests and were purchased commercially. The seeds were sterilized with 0.1% NaClO solution for 20 min, soaked for 10 minutes and washed three times with deionized water before use.

Phytotoxicity was conducted according to OECD guidelines (OECD, 1984) following the protocol of Wang et al. (2000b). Stock solutions of test chemicals were prepared with deionized water for all chemicals after range finding experiments. The tests were conducted using 100×15 mm disposable petri dishes and Whatman No.1 filter paper. Each dish was filled with 5 mL test solution or deionized water in control. Six concentrations in geometric series were set, ranging from no effect to 100% inhibition concentration and four replicates were set for each concentration. Fifteen pre-treated, undamaged and plump seeds with almost identical size were placed evenly on the filter paper in each dish. Solutions were renewed every 12 hr to achieve semi-static exposure. Deionized water without test compounds served as control. After 48hr of incubation in the dark at 25±1°C, the germination rate in each dish was counted and the average germination rate for each concentration was computed. Inverse logarithm of the concentration (mol/L) for each chemical on which the average germination rate was 50% in the control, expressed as GC_{50}, was calculated for each chemical as its toxic potency. A chemical persistence experiment was not undertaken because Schultz et al. (1989) demonstrated that abiotic loss of test compounds had no significant effect on QSAR analysis.

Logarithms of the 1-octanol/water coefficient (log K_{ow}) were computer estimated or retrieved as measured values from *SRC-WSKOW* for Microsoft Windows (version 1.26). Molecular orbital parameters and atomic charges were calculated from the semi-empirical molecular orbital package MOPAC6.0. The negative logarithm of the acid dissociation constants (*pKa*) were cited from Dean (1985) or calculated by the classical Hammett-type relationship (Perrin et al. 1981). Multiple linear regression in *STATISTICA* for Windows software (version 5.0) was employed for QSAR analysis. The quality of QSAR models was characterized by the number of observation (n), the square of correlation coefficient (r^2), the standard error of estimate (SE), the Fisher criterion (F) and the significance level (P).

RESULTS AND DISCUSSION

The suitability of *Cucumis sativus* seeds as test species was assured by the stability and reproducibility of the germination rate in the control test, regularity of dose-response relations for all test compounds, and comparably high species

Table 1. Toxicity of phenols on germination of *C. sativus* and QSAR descriptors.

Chemical Name	CAS NO [a]	pH [b]	*pKa* [c]	GC_{50} [d]	Molecule Descriptors [e]		
					$\log K_{ow}$	Q_{max}	E_{lumo}
2-Nitrophenol	88-75-5	6.31	7.22	3.09	1.79	0.580	-1.18
3-Nitrophenol	554-84-7	6.35	8.36	2.96	2.00	0.566	-1.17
4-Chloro-2-Nitrophenol	51-28-5	6.43	4.09	4.01	1.67	0.580	-1.88
4-Fluoro-2-nitrophenol	-	5.89	7.85	3.26	2.11	0.580	-1.45
2-Chlorophenol	95-57-8	6.20	8.50	2.77	2.15	0.221	0.07
4-Chlorophenol	106-48-9	6.31	9.38	3.03	2.39	0.220	0.11
2,4-Dichlorophenol	121-83-2	6.13	7.85	3.29	3.06	0.231	-0.24
4-Bromophenol	106-41-2	6.32	9.45	3.14	2.59	0.221	0.02
2-Bromo-4-methylphenol	-	6.49	8.67	3.29	2.95	0.221	0.03
4-Fluorophenol	371-41-5	6.46	9.79	2.61	1.91	0.220	0.63
4-Methoxylphenol	150-76-5	6.23	10.21	2.39	1.58	0.226	0.31
2-Methoxylphenol	-	6.21	10.19	2.59	1.32	0.235	0.26
4-Methylphenol	106-44-5	6.42	10.17	2.55	1.94	0.217	0.43
2-Methylphenol	95-48-7	6.25	10.23	2.66	1.94	0.219	0.37
2,6-Dimethylphenol	576-26-1	6.42	10.20	2.72	2.36	0.218	0.39
2-Hydroxymethyl	99-76-3	6.54	8.65	2.76	1.96	0.354	-0.45
4-Hydroxymethyl	-	6.15	8.65	3.20	3.64	0.350	-0.40
Resorcinol	123-31-9	6.37	9.91	2.58	0.59	0.218	0.24
Pyrogallol	591-27-5	6.19	9.03	1.96	0.21	0.223	0.52
3-Aminophenol	95-55-6	6.21	9.28	3.11	0.62	0.217	-1.37
2-Aminophenol	99-93-4	6.10	8.05	2.39	1.35	0.214	-0.25
4-Hydroacetophenone	98-54-4	6.36	10.43	3.05	3.31	0.271	0.47
4-Tert-Butylphenol	831-82-3	6.25	10.30	3.27	3.35	0.217	0.12
4-Phenoxylphenol	-	6.57	10.67	2.97	2.27	0.219	-0.06
Phenol	108-95-2	6.31	9.99	2.41	1.46	0.217	0.40
2-Naphthol	135-19-3	7.03	9.57	3.11	2.70	0.219	-0.35
1-Naphthol	90-15-3	6.96	9.30	3.21	2.85	0.218	-0.39
2,6-dihydroacetophenone	-	6.14	10.26	2.97	2.27	0.301	-0.06
Salicylaldehyde	-	6.35	9.09	2.78	1.81	0.258	-0.59

[a] Chemistry Abstract Service Registry Number. – CAS number was not available.
[b] the pH of the test solution on GC_{50} for each compound.
[c] the negative logarithm form of the acidity dissociation constant.
[d] GC_{50} is the negative logarithm of germination rate 50% inhibition concentration in mol/L.
[e] Molecular descriptors for QSAR analysis: log K_{ow} = the logarithm of 1-octanol / water partition coefficient; E_{lumo} = the energy of lowest unoccupied orbital; Q_{max} is the most positive atomic charge on hydrogen atom.

sensitivity as well as much less exposure time needed than other higher plant test systems, detailed in our previous study (Wang et al., 2001b). The germination rate test proved to be suitable for phytotoxicity test. Regularity of dose-response relation was observed for each test compound in this investigation. Square of correlation coefficients (r^2) and standard error of estimates (*SE*) were used to characterize the linear relation between concentration and toxicity effect of each chemical on germination rate. For dose-response relations of germination rate, r^2 values range from 0.880 to 0.999 and *SE* values range from 0.75 to 11.79 mg/L, which demonstrated the regularity of dose-response relations for all test chemicals.

The comparative inhibition activities of all phenolic compounds on germination rate of *Cucumis sativus*, expressed as GC_{50} in mol/L, together with the physio-chemical descriptors were given (see Table 1). The pH values of the test solutions on GC_{50} for all compounds, as well as the corresponding *pKa*, were also presented (Table 1). A comparison of pH and *pKa* for each compound showed that the pH value of test solution was much less than the corresponding *pKa* for most test chemicals. The comparison result indicates that dissociation hardly occurs for most tested compounds and these compounds exist mainly in neutral molecules.

According to McFarland (1970), chemical toxicity is a combination of the penetration of the toxicant into bio-phase and the interaction between toxicants and target sites. Hydrophobicity, quantitated by 1-octanol/water partition coefficient (log K_{ow}) was frequently employed to model the penetration ability of xenobiotics through bio-membrane and reaching the target site of action. The interaction of xenobiotics with bio-macromolecule can be affected by many molecule structural features. 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). For toxicity of phenols, besides hydrophobicity, eletrophilicity was another most significant toxicity influencing factor. The dissociation of OH group and other weak acid groups may occur. In addition, due to the presence of nitrogen and other heterogeneous atom, hydrogen bonding interaction may occur. All these non-hydrophobicity-related factors may be important in determining relative toxicity and may suggest complexity of mechanisms for phenol derivatives. While these toxicity influencing factors are considered, four parameters were selected to model phytotoxicity: logK_{ow} was used to characterize hydrophobicity of chemicals and model the ability of chemicals penetrating through bio-membrane and reaching action sites, energy of lowest unoccupied molecular orbital (E_{lumo}) to model electrophilicity, *pKa* to model the effect of dissociation on toxicity and Q_{max}, the most positive net atomic charge on hydrogen atom, to model effect of hydrogen bonding interaction of test chemicals with action sites on phytotoxicity of phenols.

Hydrophobicity had been proved to be one key factor that affected phytoxicity (Shigeoka et al., 1988; Hulzebos et al., 1993). A simple regression with the toxicity and hydrophobicity was performed and this resulted in a significant log K_{ow} based Equation 1 (Table 2), which indicated that hydrophobicity was a key

Table 2. Single-and two-variable based QSARs for the inhibition activity of phenols to germination rate of *Cucumis sativus*

	GC_{50}[a]=	r^2	F	SE	$P>F=$	n
1	0.744 logK_{ow} [b]+2.13	0.553	33.5	0.274	0.000004	29
2	- 0.48 pKa [c]+ 4.46	0.226	7.9	0.361	0.009	29
3	- 0.61 E_{lumo} [d] + 2.79	0.372	16.0	0.325	0.0004	29
4	0.435 Q_{max} [e]+ 2.48	0.189	6.3	0.369	0.018	29
5	0.765 logK_{ow} – 0.51 pKa + 3.81	0.811	55.8	0.180	0.000000	29
6	0.722logK_{ow} – 0.58 E_{lumo} + 2.08	0.892	107.3	0.140	0.000000	29
7	0.729logK_{ow} + 0.408Q_{max} + 1.79	0.719	33.3	0.220	0.000000	29

[a] the average germination rate 50% inhibition concentration
[b] the logarithm of the 1-octanol / water partition coefficient
[c] negative logarithm of the acid dissociation constant
[d] energy of the lowest unoccupied molecular orbital
[e] maximum positive atomic charge in molecule of chemical

toxicity influencing factor. However, from a predictive point of view, Equation 1 was not satisfactory and it only explained 55.3% of the variances, which indicated the significance of other non-hydrophobicity factor in determining phytoxicity of phenols. A simple regression of phytotoxicity of phenols was performed with *pKa*, E_{lumo} and Q_{max}, respectively and the resultant QSARs were listed in Table 2 (Equation 2-4). It can be inferred from Table 2 that all of variables including *pKa*, E_{lumo} and Q_{max} were significant variables, which indicated that all non-hydrophobicity factors considered in this investigation proved to play significant role in determining phytotoxicity of phenols.

In our previous study on the acute toxicity of phenols to amphibian larvae *Rana japonica* tadpoles (Wang et al., 2000a), the inclusion of another parameter, *pKa* characterizing dissociation behaviour of weak acid group, in a hydrophobicity based QSAR greatly improved the predictive power. In the investigation of toxicity of large scale data set of phenols to *Tetrahymena pyriformis* by Cronin and Schultz (1996), a two-parameter QSAR combining logK_{ow} and energy of lowest unoccupied orbital was developed to successfully model all non-reactive phenols. Multiple regression was performed combining hydrophobicity with *pKa*, E_{lumo} and Q_{max}, respectively and the resultant two-parameter QSARs were also listed in Table 2 (Equation 5-7). It can be inferred that the inclusion of the three parameters all significantly improved the quality of log K_{ow} based QSAR, which confirmed the importance of dissociation, eletrophilicity and hydrogen bond interaction in determining toxicity. Of all the two-parameter QSARs, Equation 6 consisted of logK_{ow} and E_{lumo} explained most variances and showed strongest predictive power.

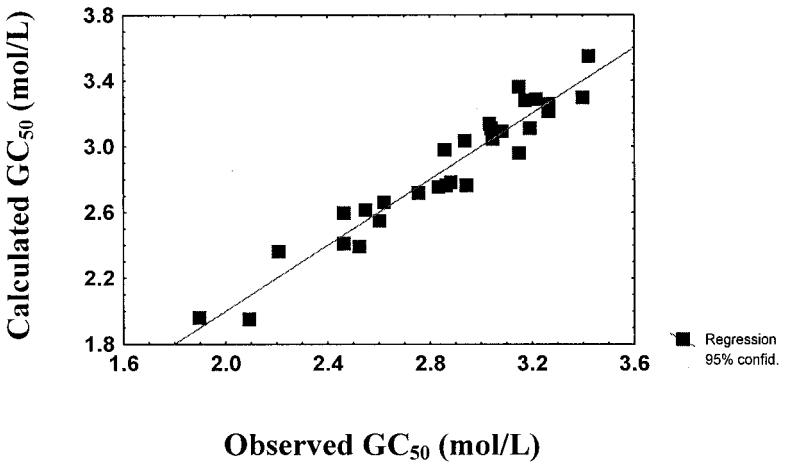

Figure 1. The plot of calculated toxicity by Equation 8 versus the observed toxicity of phenols to the germination rate of *Cucumis saticus*

Table 3. Correlation matrix of variables in Equation 8 for the inhibition toxicity of phenols to germination rate of *Cucumis sativus*.

	Log K_{ow}	E_{lumo}	Q_{max}
Log K_{ow}[a]	1.00		
E_{lumo}[b]	0.04	1.00	
Q_{max}[c]	-0.06	-0.26	1.00

[a] the logarithm of the 1-octanol / water partition coefficient
[b] energy of the lowest unoccupied molecular orbital
[c] maximum positive atomic charge in molecule of chemical

In an effort to develop a multiple-parameter QSAR consisting of all phytotoxicity influencing factors, stepwise variable regression was performed with all four variables. This resulted in a multiple-variable QSAR (Equation 8):

$$GC_{50} = 0.682 \log K_{ow} - 0.710 E_{lumo} + 0.212 Q_{max} + 2.551 \quad (8)$$
$$r^2 = 0.919 \quad F = 94.2 \quad SE = 0.120 \quad P>F = 0.000000 \quad n = 29$$

This QSAR can be explained mechanistically and the selected variables explain the anticipated importance of hydrophobicity and stereoelectronic parameters. As mentioned earlier, $\log K_{ow}$ represents the hydrophobicity of chemicals and reflects the ability of chemicals to penetrate the bio-membrane and reach the interaction

sites. E_{lumo} is a global molecular property that describes the electrophilicity of a compound in general terms, and it measures the ability of a molecule as electron acceptor. E_{lumo} is frequently employed in QSAR and is considered descriptors of reactivity (Mekenyan and Veith, 1994). Q_{max}, the most positive atomic on hydrogen atom, was a parameter employed to describe the contribution of hydrogen bonding interaction (Ramos et al., 1998). The combination of log K_{ow}, E_{lumo} and Q_{max} demonstrated the anticipated importance of hydrophobicity, electrophilicity and hydrogen bond interaction in determining toxicity of phenols. An examination of the residuals indicated that there was neither statistical nor visual outliers to this relation. The variables correlation matrix (Table 3) revealed that there was no significant correlation among the three variables that constructed the model. Equation 8 explained the majority of the variances (91.9%), gave accurate prediction of the phytotoxicity of all investigated phenols (Figure 1) and was satisfactory from both a mechanistical interpretation standpoint and an predictive point of view. It is noted that *pKa* was proved to be an insignificant variable and can't enter the stepwise variable procedure. This may be due to the fact that almost all chemicals in this investigation were present in neutral molecule forms. As we noted earlier, because the pH of the solution on GC_{50} for each chemical was far lower than its corresponding *pKa*, dissociation hardly occurs for most tested phenols.

In summary, the comparative inhibition activity of selected phenol derivatives to germination rate of *Cucumis sativus* was determined. QSARs were developed and the result indicated that hydrophobicity, electrophilicity and hydrogen bond interaction were three most significant toxicity influencing factors. The combination of the three parameters constructed a highly predictive multiple-variable QSAR model, which gave accurate prediction of all tested phenols.

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