ResearchGate

See discussions, stats, and author profiles for this publication at:
https://www.researchgate.net/publication/11533651

Mechanism-based quantitative structure-activity relationships for the inhibition of substituted phenols on germination rate of Cucumis sativus

ARTICLE *in* CHEMOSPHERE · FEBRUARY 2002

Impact Factor: 3.34 · DOI: 10.1016/S0045-6535(01)00082-0 · Source: PubMed

CITATIONS
30

READS
54

4 AUTHORS, INCLUDING:

yu Wang
University of Florida
26 PUBLICATIONS **531** CITATIONS

SEE PROFILE

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: yu Wang
Retrieved on: 25 January 2016

PERGAMON

Chemosphere 46 (2002) 241–250

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Mechanism-based quantitative structure–activity relationships for the inhibition of substituted phenols on germination rate of *Cucumis sativus*

Xiaodong Wang [a,*], Jianzhen Yu [b], Yu Wang [a], Liansheng Wang [a]

[a] *State Key Laboratory of Pollution Control and Resources Reuse, School of Environment, Nanjing University, Nanjing 210093, People's Republic of China*

[b] *Department of Chemistry, Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong*

Received 18 October 2000; accepted 5 March 2001

Abstract

Comparative inhibition activity (GC_{50}) of 42 structurally diverse substituted phenols on seed germination rate of *Cucumis sativus* was investigated. Quantitative structure–activity relationships (QSARs) were developed by using hydrophobicity (1-octanol/water partition coefficient, $\log K_{ow}$) and electrophilicity (the energy of the lowest unoccupied molecule orbital, E_{lumo}) for the toxicity of phenols according to their modes of toxic action. Most phenols elicited their response via a polar narcotic mechanism and a highly significant log K_{ow}-based model was obtained ($GC_{50} = 0.92 \log K_{ow} + 1.99$, $r^2 = 0.84$, $n = 29$). The inclusion of E_{lumo} greatly improved the predictive power of the polar narcotic QSAR ($GC_{50} = 0.88 \log K_{ow} - 0.30E_{lumo} + 1.99$, $r^2 = 0.93$, $n = 29$). pK_a proved to be an insignificant influencing factor in this study. Poor correlation with hydrophobicity and strong correlation with electrophilicity were observed for the nine bio-reactive chemicals. Their elevated toxicity was considerably underestimated by the polar narcotic $\log K_{ow}$-dependent QSAR. The nine chemicals consist of selected nitro-substituted phenols, hydroquinone, catechol and 2-aminophenol. Their excess toxic potency could be explained by their molecular structure involving in vivo reaction with bio-macromolecules. Strong dissociation of carboxyl group of the four benzoic acid derivatives greatly decreased their observed toxicity. In an effort to model all chemicals including polar narcotics and bio-reactive chemicals, a response-surface analysis with the toxicity, $\log K_{ow}$ and E_{lumo} was performed. This resulted in a highly predictive two-parameter QSAR for most of the chemicals ($GC_{50} = 0.70 \log K_{ow} - 0.66E_{lumo} + 2.17$, $r^2 = 0.89$, $n = 36$). Catechol and 2,4-dinitrophenol proved to be outliers of this model and their much high toxicity was explained. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Modes of action; Phytotoxicity; Response-surface analysis; QSAR

1. Introduction

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. 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) and QSARs were developed for each mechanism of toxic action. However, due to the complexity of the interaction of xenobiotics with target sites of toxic action, it is difficult to identify

* Corresponding author. Tel.: +86-25-3599-705; fax: +86-25-3707-034.

E-mail address: wangxdcn@yahoo.com (X. Wang).

0045-6535/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0045-6535(01)00082-0

the exact mechanisms a priori. Recently, Mekenyan and Veith (1993) proposed a response-surface approach, which was developed by Cronin and Schultz (1996) by constructing two-variable QSARs based on hydrophobicity (quantitated by the logarithm of the 1-octanol/water partitioning coefficient, $\log K_{ow}$), and electrophilicity (the most commonly used is the energy of the lowest unoccupied molecule orbital, E_{lumo}), to model ecotoxicological data regardless of the mechanism of toxic action.

Phenolic compounds, on one hand, have many commercial uses in large scale as intermediates of dyes and organic synthesis processes, also as disinfector and antiseptic, most of which involve biocide activity. Due to the many origins and magnitude of uses, phenolic compounds are widespread in the ecosystem and consequently have a high potential for environmental pollution. On the other hand, it is generally known that phenolic compounds are secondary metabolites in plants regulating growth of plants as typical inhibitors. Aquatic toxicity of phenol and its derivatives has been extensively investigated and QSARs were developed to explore their acute toxicity mechanisms. For example, significant research has been done to explore the different modes of toxic action by Schultz and co-workers (Schultz et al., 1986, 1989, 1992; Cronin and Schultz, 1996; Bearden and Schultz, 1997) with *Tetrahymena pyriformis* and several modes of toxic action were discriminated, including polar narcosis, uncoupler of oxidation phosphyration, electrophilicity and pre-electrophilicity. Polar narcosis was proved to the main mechanism of phenol's toxicity and was modeled well by two parameters: the first being hydrophobicity (1-octanol/water partition coefficient) and the second pK_a, acid dissociation constant (Schultz et al., 1986) or a measure of electrophilicity (the energy of the lowest unoccupied orbital) (Cronin and Schultz, 1996). Veith and Broderius (1987) found that the toxicity of compounds acting by polar narcosis (including many phenols) to the fathead minnow (*Pimephales promelas*) to be modeled well by log *P* alone. In the previous study on the acute lethal toxicity of selected phenols to larvae of amphibians (tadpole *Rana japonica*), polar narcosis proved to be the main mechanism and an excellent hydrophobicity-dependent QSAR was developed (Wang et al., 2000a, 2001a). However, in comparison with the extensive study on the acute toxicity and mechanism, not much effort was put into the investigation of the adverse effect of structurally diverse phenols on higher plants.

Information on phytotoxicity is required for 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 (American Society for Testing and Materials, 1991). As a rapid phytotoxicity test method, germination rate and root elongation possess several advantages over other tests such as its sensitivity, simplicity, low cost and suitability for toxicity studies of unstable compounds or samples with renewal or flow-through methods beside the static method (Wang, 1991). In addition, the test can be activated quickly and seed germination and root elongation tests do not need plant nutrients and adjutants in the water control (Mayer and Poljakoff-Mayber, 1982; Wang, 1991). All the advantages make the tests ideally suitable as a stand-by test method and a rapid and cost-effective tool to evaluate the ecological risk of phenolic compounds. Several recent activities suggested that phytotoxicity tests with seed germination rate and root elongation of higher terrestrial plants were a valuable part of ecotoxicology (Wang, 1991; Reynolds, 1989; Hulzebos et al., 1993; Wang et al., 2000b, 2001c).

In this study, the comparative inhibition toxicity of 42 structurally diverse phenols on germination rate of terrestrial macrophytes *Cucumis sativus* was determined. QSARs were developed to investigate the different mechanisms of toxic effect of environmentally hazardous phenols on higher plants. Response-surface analysis was performed to construct a generalized QSAR to model their toxicity without regard to their exact modes of toxic action.

2. Materials and methods

2.1. Toxicity data

The comparative inhibition activity on the seed germination rate of *C. sativus* of the 42 structurally diverse phenol derivatives was measured in our laboratory following the protocol of Wang et al. (2000a). The seeds of *C. sativus* were used and the 2-d static-renewal 50% germination rate inhibition concentration (GC_{50}) was measured. Briefly, measurements were made as follows: the tests were conducted using 100×15 mm^2 disposable petri dishes and Whatman No. 1 filter paper. All dishes containing 5 ml test solutions or deionized water for control were incubated at dark at 25 ± 1°C. For the test of half inhibition, six concentrations in a geometric series were selected ranging from no effect to 100% inhibition concentration. Four replicates were set for each concentration. Solutions were renewed every 12 h to achieve semi-static exposure. After 48 h incubation, the germination rate in each dish was calculated.

GC_{50}, concentration on which the average germination rate was 50% of those in the corresponding control, was calculated by the log-linear regression in STATISTICA for Windows software (version 5.0) (1995) and was converted to the logarithmic form of the inverse molar concentration for each chemical prior to QSAR analysis.

2.2. Molecular descriptors

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) (1996).

Molecular orbital parameters were calculated from the semi-empirical molecular orbital package MOPAC6.0 (Stewart, 1990). The molecular modeling package ALCHEMY II (1985) was used to construct and view all molecular structures and produce input file as internal coordinate. Molecular geometry was optimized with the MM2 method and then with the Broyden–Flether–Goldfarb–Shanno (BFGS) method in MOPAC6.0.

The negative logarithm of the acidity dissociation constants (pK_a) were cited from Dean (1985) or calculated by the classical Hammett-type relationship (Perrin et al., 1981).

2.3. Statistical analysis

The multiple regression analysis procedure of STATISTICA for Windows software (version 5.0) (1995) was employed for QSAR analysis. For the development of QSAR, the comparative inhibition activity (GC_{50}) was utilized as the independent variable and the calculated parameters characterizing hydrophobicity and electrophilicity as the dependent variables. The quality of the model was characterized by the number of observations (n), the square of correlation coefficient (r^2), the standard error of estimate (SE), the Fisher criterion (F) and the significance level (P).

3. Result and discussion

The suitability of germination rate with *C. sativus* as test species and a method was evaluated in our previous studies (Wang et al., 2001b), which was assured by (1) the stability and reproducibility of germination rate in the control test, (2) regularity of dose–response relation for each chemical, (3) comparably high sensitivity and (4) a short exposure time needed. The average germination rate in the control test ranges from 77% to 88%. It can be inferred that the germination rate in the control test is stable and reproducible and *C. sativus* is a favorite species for the toxicity test. Log-linear dose–response relations were observed for all test compounds in this study (Table 1). 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 1.79 mg/l, which demonstrates the regularity of dose–response relations for all test chemicals.

All the toxicity of 42 substituted phenols to seeds of *C. sativus*, expressed as GC_{50}, together with the structure descriptors for the development of QSARs, was summarized in Table 1. The pH of the test solution on GC_{50} for each chemical, as well as the corresponding pK_a, was also presented (Table 1). A comparison of pH and pK_a for each compound reveals that the pH of the test solution on GC_{50} was much less than the corresponding pK_a for most chemicals, except for four benzoic acid derivatives. The comparison result indicated that dissociation hardly occurs for most tested chemicals and these chemicals are present mainly in neutral molecules under current conditions. While for the four benzoic acids, strong dissociation of carboxyl group will occur and a great fraction of benzoic acid derivatives will be present in anion form.

According to McFarland (1970), chemical toxicity is a combination of penetration of the toxicant into the bio-phase and the interaction of the toxicant with the site of action. Hydrophobicity, quantitated by 1-octanol/water partition coefficient ($\log K_{ow}$) was frequently employed to model the ability of toxicant penetrating through bio-membrane and into biophase. The interaction of toxicant with the site of action can be modeled by many electrostereo parameters, such as pK_a and E_{lumo}. Hydrophobicity had been proved to be one key factor that affected phytoxicity (Hulzebos et al., 1993; Shigeoka et al., 1988). 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 substituents on the aromatic moiety besides a hydroxyl group, including alkyl, alkoxyl, halogen, nitro, hydroxyl, carbonyl, amino and so on, 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 the relative toxicity and may suggest multiple mechanisms for the tested compounds.

Fig. 1 is a scatter plot of the comparative inhibition activity of substituted phenols on germination rate of *C. sativus* versus $\log K_{ow}$, from which no good linear relationship can be observed visually. An initial regression of all 42 compounds with $\log K_{ow}$ resulted in Eq. (1):

$$\begin{aligned} &GC_{50} = 0.55 \log K_{ow} + 2.35, \\ &r^2 = 0.30,\ F = 17.3,\ P > F = 0.00016, \\ &SE = 0.35,\ n = 42. \end{aligned} \tag{1}$$

As noted above, this set of substituted phenols with highly diverse substituents may represent several mechanisms of toxic action, so it is expected that they cannot be modeled well as a single set by hydrophobicity. It is generally accepted that the most successful QSARs have divided organic pollutants into many subsets according

Table 1
Toxicity of substituted phenols to higher plants *C. sativus*, dose–response relations and molecular descriptors for QSAR analysis

No.	Chemical name	CAS Number[a]	pH[b]	pK_a[c]	Dose–response relation[d]				Molecular Descriptors[e]	
					GC_{50} (mg/l)	GC_{50} (log mol/l)	R^2	SE	log K_{ow}	E_{lumo}
1	2-Nitrophenol	88-75-5	6.31	7.22	127.0	3.09	0.925	7.74	1.79	−1.184
2	3-Nitrophenol	554-84-7	6.35	8.36	145.2	2.96	0.983	6.50	2.00	−1.167
3	4-Nitrophenol	123-30-8	6.21	7.15	97.1	3.36	0.990	3.27	1.91	−1.064
4	4-Chloro-2-nitrophenol	51-28-5	6.43	4.09	67.3	4.01	0.985	2.81	1.67	−1.888
5	2,4-Dinitrophenol	89-64-5	6.04	6.48	100.6	3.55	0.956	5.27	2.46	−1.227
6	2-Nitro resorcinol	601-89-8	6.34	8.28	89.1	3.55	0.900	11.79	1.56	−1.322
7	4-Fluoro-2-nitrophenol	–[a]	5.89	7.85	121.7	3.26	0.892	7.06	2.11	−1.447
8	2-Chlorophenol	95-57-8	6.20	8.50	162.6	2.77	0.939	5.43	2.15	0.066
9	4-Chlorophenol	106-48-9	6.31	9.38	124.5	3.03	0.947	5.58	2.39	0.098
10	2,4-Dichlorophenol	121-83-2	6.13	7.85	121.8	3.29	0.925	5.95	3.06	−0.244
11	4-Bromophenol	106-41-2	6.32	9.45	150.7	3.14	0.972	6.52	2.59	0.020
12	2-Bromo-4-methylphenol	–[a]	6.49	8.67	140.0	3.29	0.997	1.45	2.95	0.027
13	2-Hydroxyl-5-chlorobenzoic acid	321-14-2	5.77	4.20	196.7	2.87	0.954	5.34	2.89	−0.703
14	4-Fluorophenol	371-41-5	6.46	9.79	165.7	2.61	0.949	6.60	1.91	0.629
15	4-Methoxylphenol	150-76-5	6.23	10.21	229.3	2.39	0.966	3.37	1.58	0.305
16	2-Methoxylphenol	–[a]	6.21	10.19	186.7	2.59	0.928	8.21	1.32	0.262
17	4-Methylphenol	106-44-5	6.42	10.17	169.5	2.55	0.935	6.27	1.94	0.429
18	2-Methylphenol	95-48-7	6.25	10.23	151.3	2.66	0.999	0.75	1.94	0.369
19	2,6-Dimethylphenol	576-26-1	6.42	10.20	161.8	2.72	0.880	8.50	2.36	0.392
20	Salicylaldehyde	–[a]	6.35	9.09	152.2	2.78	0.997	3.38	1.81	−0.593
21	4-Hydroxylbenzaldehyde	123-08-0	6.52	9.23	226.0	2.39	0.990	2.09	1.35	−0.450
22	Salicylic acid	69-72-7	4.74	2.98	164.3	2.83	0.981	3.13	2.26	−0.557
23	4-Hydroxylbenzoic acid	119-36-8	3.66	4.58	188.8	2.48	0.982	5.19	2.55	−0.449
24	2-Hydroxyl methyl benzoate	99-76-3	6.54	8.65	193.9	2.76	0.978	3.49	1.96	−0.398
25	4-Hydroxyl methyl benzoate	–[a]	6.15	8.65	187.3	3.20	0.955	6.97	1.96	0.369

Table 1 (*continued*)

No.	Chemical name	CAS Number[a]	pH[b]	pK_a[c]	Germination rate inhibition activity[d]				Molecular Descriptors[e]	
					GC_{50} (mg/l)	GC_{50} (log mol/l)	R^2	SE (mg/l)	$\log K_{ow}$	E_{lumo}
26	Bisphenol A	–[a]	6.65	9.44	417.0	2.49	0.928	7.18	1.65	−0.642
27	Bisphenol S	–[a]	6.36	8.47	223.0	2.96	0.971	6.32	3.19	0.335
28	Bisphenol E	108-46-3	6.74	9.44	208.4	2.36	0.953	2.82	0.8	0.272
29	Resorcinol	123-31-9	6.37	9.91	168.2	2.58	0.953	5.56	0.59	0.235
30	Hydroquinone	120-80-9	6.26	9.81	103.9	3.06	0.944	5.11	0.88	0.263
31	Pyrocatechol	108-73-6	6.34	9.36	362.0	1.95	0.994	2.75	0.16	0.247
32	Pyrogallol	591-27-5	6.19	9.03	309.6	1.96	0.923	7.43	0.21	0.521
33	3-Aminophenol	95-55-6	6.21	9.28	97.5	3.11	0.974	5.25	0.62	−1.366
34	2-Aminophenol	99-93-4	6.10	8.05	251.1	2.39	0.945	5.73	1.35	−0.249
35	4-Hydroxylacetophenone	98-54-4	6.36	10.43	143.6	3.05	0.944	6.48	3.31	0.470
36	4-Tert-Butylphenol	831-82-3	6.25	10.30	141.6	3.27	0.979	4.01	3.35	0.117
37	4-Phenoxylphenol	–[a]	6.57	10.67	156.3	2.97	0.894	10.66	2.27	−0.064
38	Phenol	108-95-2	6.31	9.99	170.6	2.41	0.987	2.39	1.46	0.398
39	3-Hydroxyl-benzoic acid	–[a]	3.96	4.08	281.8	2.29	0.956	7.51	1.5	−0.377
40	2,6-dihydroxyl acetophenone	–[a]	6.14	10.26	167.5	2.97	0.936	6.43	2.27	−0.064
41	2-Naphthol	135-19-3	7.03	9.57	128.8	3.11	0.979	4.46	2.7	−0.348
42	1-Naphthol	90-15-3	6.96	9.30	116.7	3.21	0.972	4.48	2.85	−0.388

[a] Chemistry abstract service registry number – CAS NO was not available.

[b] The pH of the test solution on germination rate 50% inhibition concentration for each compound.

[c] The negative logarithm form of the acidity dissociation constants for the test chemicals.

[d] The comparative inhibition activity of substituted phenols on germination rate of *C. sativus*. GC_{50} (mg/l) = half inhibition concentration of germination rate for each chemical in mg/l; GC_{50} (log mol/l) is the negative logarithm of GC_{50} in mol/l; R^2 = the square of linear correlation coefficient of the log-linear dose–response relation; SE = the standard error of estimate in mg/l for the log-linear dose–response relation.

[e] Molecular structure descriptors for QSAR analysis: $\log K_{ow}$ = the logarithm of 1-octanol/water partition coefficient; E_{lumo} = the energy of lowest unoccupied molecule orbital.

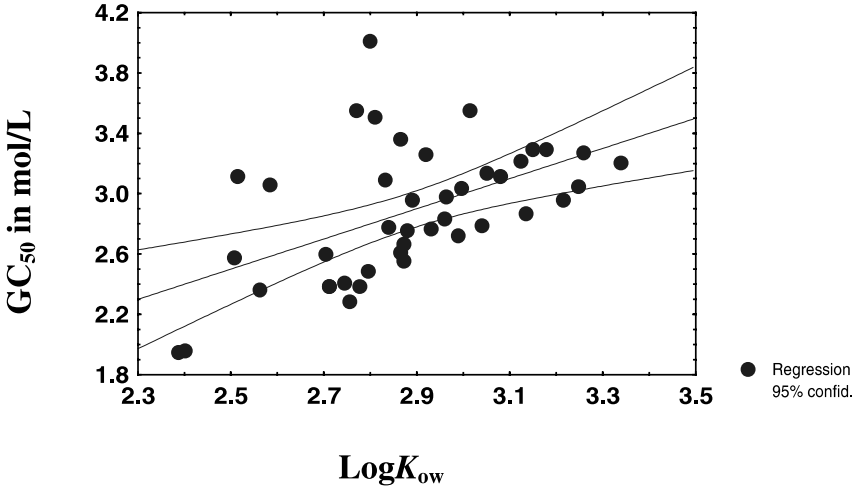

Fig. 1. The scatter plot of the comparative inhibition activity (GC_{50} in negative logarithm of mol/l) versus 1-octanol/water partition coefficient ($\log K_{ow}$) for all 42 substituted phenols.

to their mechanisms of toxic action and a separate QSAR can be developed for each mechanism of toxic action (Schultz et al., 1990; Cronin and Dearden, 1995). Hydrophobicity quantitated by $\log K_{ow}$ was frequently employed as a descriptor to discriminate different modes of toxic action. An examination of the structure of the tested chemicals reveals that all the chemicals fell into three categories: polar narcotic phenols; benzoic acid derivatives; and phenols with bio-reactive substructure. The potential bio-reactive chemicals were identified according to their bio-reactive substructures by making use of the classification of reactive groups by Verhaar et al. (1992) and Hermens (1990). These chemicals include two benzaldehyde derivatives (2-hydroxylbenzaldehyde and 4-hydroxylbenzaldehyde), six nitro-substituted phenols, hydroquinone, catechol and 2-aminophenol. Of all, the molecule structure of the two benzaldehyde derivatives is very similar to the α–β unsaturated aldehyde, thus are capable of undergoing Michael-type addition or Schiff-base formation. 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). Regression analysis of the identified polar narcotic phenols resulted in a highly significant QSAR Eq. (2):

$$\begin{aligned} &GC_{50} = 0.92 \log K_{ow} + 1.97, \\ &r^2 = 0.85,\ F = 143.7,\ P > F = 0.00000, \\ &SE = 0.15,\ n = 27. \end{aligned} \tag{2}$$

An examination of the toxicity of the 11 potential bio-reactive chemicals reveals that except for the two benzaldehyde derivatives, all the nine chemicals with bio-reactive substructure exhibited elevated toxicity and cannot be modeled by the polar narcotic $\log K_{ow}$-dependent QSAR. While the two benzaldehyde derivatives exhibited no elevated toxicity and the inclusion of them in the polar narcotics led to another model with almost identical quality (Eq. (3)), which suggested that the two benzaldehyde derivatives act as also polar narcotics.

$$\begin{aligned} &GC_{50} = 0.92 \log K_{ow} + 1.99, \\ &r^2 = 0.85,\ F = 144.0,\ P > F = 0.00000, \\ &SE = 0.15,\ n = 29. \end{aligned} \tag{3}$$

In an effort to improve the predictive ability, multiple regression using $\log K_{ow}$ and pK_a as independent variables was performed with the aim to account for the influence of dissociation behavior on the observed toxicity of phenols to higher plants *C. sativus*. The regression reveals that pK_a was not a significant variable and cannot enter the step regression procedure. This demonstrated that pK_a contributed little to the toxicity of phenols. This can be explained by the fact that almost all the tested phenols modeled by Eq. (3) are present in the neutral molecule under current test conditions and dissociation hardly occurs. By combining $\log K_{ow}$ and E_{lumo} Cronin and Schultz (1996) successfully modeled the population growth impairment toxicity of phenols to *T. pyriformis*. The introduction of E_{lumo} resulted in a highly predictive two-parameter QSAR model:

$$\begin{aligned} &GC_{50} = 0.88 \log K_{ow} - 0.30 E_{lumo} + 1.99, \\ &r^2 = 0.93,\ F = 173.3,\ P > F = 0.00000, \\ &SE = 0.10,\ n = 29. \end{aligned} \tag{4}$$

As noted earlier, for the four benzoic acid derivatives, significant dissociation of carboxyl group will occur, which makes the $\log K_{ow}$ of the benzoic acid derivatives not a reflection of their true partitioning. Dissociation clearly will significantly reduce their toxicity. All the four benzoic acid derivatives proved to be outliers of the polar narcotic $\log K_{ow}$-dependent Eq. (3) with their toxicity obviously overestimated.

The nine chemicals with bio-reactive substructures, with their toxicity significantly underestimated by the polar narcotic Eq. (3), involved selected nitro-substituted phenols, hydroquinone, catechol and o-aminophenol. Nitro-substituted aromatics are typical bio-reactive chemicals. From the chemicals viewpoint, the nitro group is a strong p-electron acceptor, lowering the electron density of the aromatic ring. Inside the nitro group, excess electronic charges are mainly localized at the oxygen atoms. While the nitrogen atom is typically electron-deficient. The result is that nitro-aromatic compounds often show enhanced reactivity for the attachment of nucleophiles at aromatic ring carbon as well as for reactions with reducing agents (Heike Schmit et al., 2000). Some aromatic-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). The elevated toxicity of the nitro-substituted phenols was also observed in other toxicity test systems (Jaworsky and Schultz, 1994; Schultz and Cronin, 1997; Wang et al., 2000a, 2001a).

Another category of bio-reactive chemicals are catechol, hydroquinone and 2-aminophenol. Hermens (1990) has reviewed the bio-reactive substructures related to aquatic toxicity. Included in these are catechol, hydroquinone and selected amines. Catechol and hydroquinone 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). On one hand, the structure of quinones is similar to α–β unsaturated ketones and thus may covalently react with soft macromolecular nucleophiles via nucleophilic Michael-type addition. On the other hand, they may also be reduced enzymatically by one-electron processes to more electrophilic semiquinones and result in a much more potent toxic mechanism of action, futile redox cycling (O'Brien, 1991). Thus quinone and catechol might act as pro-electrophilic regardless of metabolic pathway. Although most anilines are thought to act by polar narcosis it is impossible that *ortho*-aminophenols may be also metabolized in vivo quinones (Dupuis and Benezra, 1982). The much more potent bio-reactive toxicity of catechol, hydroquinone and amino-substituted phenols was also observed in other test systems with *Rana japonica* tadpole and microorganism *E. coli* (Jaworsky and Schultz, 1994; Wang et al., 2001a).

For bio-reactive chemicals, their toxicity was mainly determined by their in vivo interaction with target sites of action, thus it is expected that their elevated toxicity cannot be modeled by hydrophobicity. This was confirmed by the failure to develop a significant $\log K_{ow}$-dependent QSAR (Eq. (5)):

$$\begin{aligned} &GC_{50} = 0.64 \log K_{ow} + 2.68, \\ &r^2 = 0.41,\ F = 4.9,\ P > F = 0.06, \\ &SE = 0.34,\ n = 9. \end{aligned} \tag{5}$$

The linear regression procedure revealed that $\log K_{ow}$ was not a significant parameter in this relation. For the bio-reactive chemicals, the parameters characterizing eletrophilicity, especially the energy of the lowest unoccupied orbital has been frequently used to model their more potent reactivity (Mekenyan and Veith, 1994). The relationship of the inhibition activity for the nine bio-reactive chemicals was investigated with E_{lumo} and a significant correlation was observed (Eq. (6)):

$$\begin{aligned} &GC_{50} = -0.84 E_{lumo} + 2.81, \\ &r^2 = 0.71,\ F = 17.2,\ P > F = 0.004, \\ &SE = 0.27,\ n = 9. \end{aligned} \tag{6}$$

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. The poor correlation with hydrophobicity and highly significant correlation with E_{lumo} indicated that the toxicity of these chemicals with reactive substructures was mainly determined by their molecule orbital controlled in vivo interaction with bio-macromolecules.

To develop a predictive QSAR for all chemicals including polar narcotics and bio-reactive chemicals, a response-surface analysis was performed with the toxicity, $\log K_{ow}$ characterizing hydrophobicity and E_{lumo} representing electrophilicity. This resulted in a highly significant two-parameter QSAR (Eq. (7)):

$$\begin{aligned} &GC_{50} = 0.56 \log K_{ow} - 0.65 E_{lumo} + 2.26, \\ &r^2 = 0.74,\ F = 52.3,\ P > F = 0.00000, \\ &SE = 0.22,\ n = 42. \end{aligned} \tag{7}$$

The four benzoic acid derivatives proved to be statistical outliers to this relationship with their toxicity significantly overestimated. As earlier noted, due to strong dissociation of carboxyl group, it is impossible to model their toxicity by $\log K_{ow}$-dependent QSARs. The omission of the four benzoic acid derivatives led to another obviously improved QSAR (Eq. (8)):

$$GC_{50} = 0.60 \log K_{ow} - 0.71E_{lumo} + 2.24,$$
$$r^2 = 0.84,\ F = 89.3,\ P > F = 0.00000, \quad (8)$$
$$SE = 0.18,\ n = 38.$$

The examination of residues reveals another two outliers with their toxicity significantly underestimated by Eq. (8). The two chemicals are catechol and 2,4-dinitrophenol. The removal of the two highly toxic chemicals resulted in another highly predictive two-parameter QSAR (Eq. (9)):

$$GC_{50} = 0.70 \log K_{ow} - 0.66E_{lumo} + 2.17,$$
$$r^2 = 0.89,\ F = 133.5,\ P > F = 0.00000, \quad (9)$$
$$SE = 0.14,\ n = 36.$$

Fig. 2 is a plot of the calculated toxicity by Eq. (9) versus the observed toxicity of phenols to higher plants. The Eq. (9) explained the majority of the variability (89%), modeled most of the chemicals without statistical outlier. Catechol and 2,4-dinitrophenol proved to be more toxic than that calculated by this two-parameter Eq. (9). The highly toxic activity of catechol can be explained by its structure. Schultz et al. (1997) noted that catechols are capable of undergoing tautomerization to more electrophilic semiquinones. Semiquinones are known to result in a much more potent toxic mechanism of action, futile redox recycling (O'Brien, 1991). Although most nitro-containing aromatics have been identified to be bio-reactive. The mechanisms of toxic action are complex and far from clear. A number of mechanisms of action have been reported to account for their acute aquatic toxicity. For example, mono-nitrobenzenes may act as pro-eletrophiles. Mono-nitrobenzenes with a halogen substituent are capable of yielding the potentially highly toxic nitroso compounds (Robert, 1987). While di-nitrobenzenes seemed unlikely to act solely as pro-electrophiles since their toxicity was in excess of that of mono-nitrobenzenes (Cronin et al., 1998) and Cronin et al. (1998) concluded that there was no commonality in the bio-reactivity of mono- and di-nitrobenzenes. In this study, 2,4-dinitrophenol is the only dinitrophenol. It is clear that 2,4-dinitrophenol elicited its much high toxicity by much more potent toxic mechanism.

Ideally, toxicological QSARs should be developed based on a clearly defined mechanism of action. The development of mechanism-based QSARs is based on the analysis of chemical structure or identification of bio-reactive substructure a priori to discriminate potential bio-reactive chemicals from narcotics. On one hand, this is a difficult task due to the complexity and diversity of chemical substructure and mechanisms of toxic action. On the other hand, due to the great interspecies difference and great difference among toxicity test protocols. This kind of identification a priori of reactive groups often is not enough to discriminate all bio-reactive chemicals from narcotics. This means that in some test protocols, chemicals with bioreactive structure do not necessarily show elevated bio-reactive toxicity (i.e., the two benzaldehyde derivatives in this study). The response-surface analysis employing hydrophobicity and electrophilicity that was proposed by Mekenyan and Veith (1993) and developed by Cronin and Schultz (1996) successfully model toxicity of most chemicals without reference to their mechanisms and proved to provide a good solution to modeling structurally diverse chemicals. In this study, in spite of the omission of two more toxic chemicals, the developed

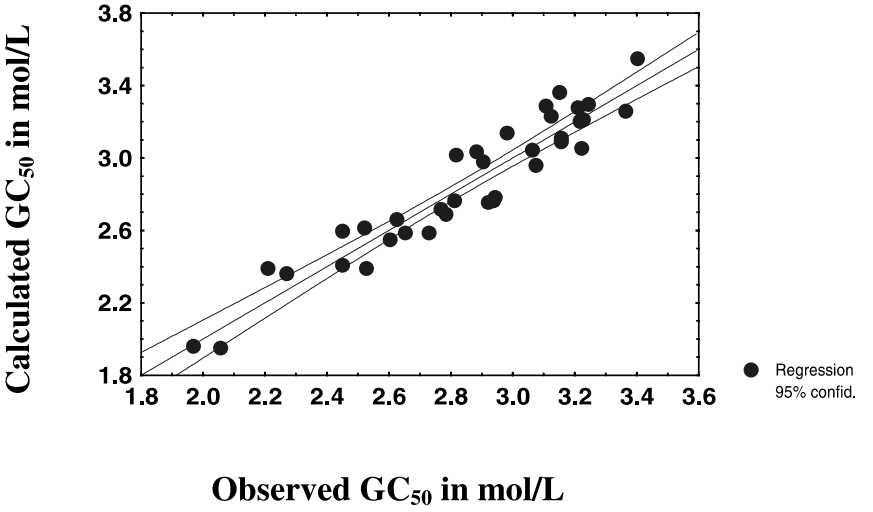

Fig. 2. The calculated toxicity from Eq. (9) versus the observed activity for the comparative inhibition (GC_{50} in negative logarithm of mol/l) of substituted phenols on germination rate of *C. sativus*.

response-surface successfully models the inhibition toxicity of most structurally diverse substituted phenols with different mechanisms of toxic action to higher plants, with high predictive power.

4. Conclusion

In this paper, the comparative inhibition activity of some structurally diverse substituted phenols on seed germination rate of *C. sativus* was investigated. QSARs were developed by using $\log K_{ow}$ and E_{lumo} as descriptors to study the phytotoxicity. The results indicated that most phenols elicited their response via a polar narcotic mechanism. For polar narcosis, a highly significant $\log K_{ow}$-dependent model was obtained and the inclusion of the E_{lumo} greatly improved the predictive power of polar narcotic QSAR. The strong dissociation of carboxyl group of benzoic acid derivatives greatly decreased their observed toxicity. For bio-reactive chemicals, poor correlation with hydrophobicity and significant correlation with electrophilicity were observed, which indicated that their excess toxic potency was mainly determined by their molecular molecule orbital controlled in vivo electrophilic interaction with bio-macromolecules. In an effort to model all chemicals, the response-surface analysis with toxicity, $\log K_{ow}$ and E_{lumo} resulted in a high quality two-parameter QSAR, which successfully modeled the toxicity of most substituted phenols without reference to their exact mechanisms.

Acknowledgements

This paper is financially supported by the National Nature Science Foundation, the Doctoral Program of Higher Education of PR China and the Core University Program, Japan Society for Promotion of Science.

References

ALCHEMY II—Tripos Associates, 1985. A molecular Modeling System for the IBM PC, St. Louis, MO, USA.

American Society for Testing and Materials, 1991. A short-term bio-assay for whole plants toxicity. In: Pfeeger, T., McFarlane, J.C., Sherman, R., Volk, G. (Eds.), Plants for Toxicity Assessment, vol. 2. Philadelphia, PA, USA, pp. 355–364.

Bearden, A.P., Schultz, T.W., 1997. Structure–activity relationships for *pimephales* and *tetrahymena*: a mechanism of action approach. Environ. Toxicol. Chem. 16, 1311–1317.

Brunmark, A., Cadenas, E., 1978. Redox and addition chemistry of quinoid compounds and its biological implications. Free Radical Biol. Med. 7, 435–477.

Cronin, M.T.D., Dearden, J.C., 1995. QSAR in toxicology. 1. Prediction of aquatic toxicity. Quant. Struct. Act. Relat. 14, 1–7.

Cronin, M.T.D., Gregory, B.W., Schultz, T.W., 1998. Quantitative structure–activity analyses of nitrobenzene toxicity to *Tetrahymena pyriformis*. Chem. Res. Toxicol. 11, 902–908.

Cronin, M.T.D., Schultz, T.W., 1996. Structure–toxicity relationships for phenols to *Tetrahymena pyriformis*. Chemosphere 32, 1453–1468.

Dean, J.A., 1985. Lange's Handbook of Chemistry, 13th ed. McGraw-Hill, New York.

Dupuis, G., Benezra, C., 1982. In: Allergic Contact Dermatitis to Simple Chemicals: A Molecular Approach. Marcel Dekker, New York, p. 183.

Hansch, C., Leo, A., 1979. In: Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley, New York, p. 339.

Hermens, J.L.M., 1990. Electrophiles and acute toxicity to fish. Environ. Health Perspect. 87, 219–225.

Hulzebos, E.M., Adema, D.M.M, Dirven-van Breemen, E.M., Henzen, L., Van Dis, W.A., Herbold, H.A., Hoekstra, J.A., Baerselman, R., Van Gestel, C.A.M., 1993. Phytotoxicity studies with *Lactuca sativa* in soil and nutrient solution. Environ. Toxicol. Chem. 12, 1079–1094.

Jaworsky, J.S., Schultz, T.W., 1994. Mechanism-based comparisons of acute toxicities elicited by industrial organic chemicals in procaryotic and ecaryotic systems. Ecotoxicol. Environ. Safety 29, 200–213.

Kamlet, M.J., Doherty, R.M., Veith, G.D., Taft, R.W., Abraham, M.H., 1986. Solubility properties in polymers and biological media, 7. An analysis of toxicant properties that influence inhibition of bioluminescence in *Photobacterium phosphoreum* (the Microtox Test). Environ. Sci. Technol. 20, 690–695.

Mayer, A.M., Poljakoff-Mayber, A., 1982. The Germination of Seeds, third ed. Pergamon Press, Oxford, UK.

McFarland, J.W., 1970. On the parabolic relationship between drug potency and hydrophobicity. J. Med. Chem. 13, 1092–1196.

Mekenyan, O.G., Veith, G.D., 1993. Relationships between descriptors for hydrophobicity and soft electrophilicity in predicting toxicity. SAR QSAR Environ. Res. 2, 335–344.

Mekenyan, O.G., Veith, G.D., 1994. The electronic factor in QSAR: MO-parameters, competing interactions, reactivity, and toxicity. SAR QSAR Environ. Res. 2, 129–143.

Monks, T.J., Hanzlik, R.P., Cohen, G.M., Ross, D., Graham, D.G., 1992. Quinone chemistry and toxicity. Toxicol. Appl. Pharmacol. 112, 2–16.

O'Brien, P.J., 1991. Molecular mechanisms of quinone cytotoxicity. Chem.-Biol. Interaction 80, 1–41.

Perrin, D.D., Dempsey, B., Serjeant, E.P., 1981. pK_a Prediction for Organic Acids and Bases. Chapman & Hall, London, UK.

Reynolds, T., 1989. Comparative effects of heterocyclic compounds on inhibition of lettuce fruit germination. J. Exp. Bot. 40, 391–404.

Robert, D.W., 1987. In: Kaiser, K.L.E. (Eds.), QSAR in Environmental Toxicology-II. D. Reidel, Dordrecht, pp. 295–308.

Schmit, H., Altenburger, R., Jastorff, B., Schuurmann, G., 2000. Quantitative structure–activity analyses of algae toxicity of nitroaromatic compounds. Chem. Res. Toxicol. 13, 441–450.

Schultz, T.W., Wesley, Sonji, K., Baker, Leslie, L., 1989. Structure–activity relationships for di and tri allyl and/or halogen substituted phenols. Bull. Environ. Contam. Toxicol. 13, 192–198.

Schultz, T.W., Cronin, M.T.D., 1997. Quantitative structure–activity relationships for weak acid respiratory uncouplers to *vibrio fisheri*. Environ. Toxicol. Chem. 10, 357–360.

Schultz, T.W., Holcombe, G.W., Phipps, G.L., 1986. Relationships of quantitative structure–activity to comparative toxicity of selected phenols in the *Pimephales promelas* and *Tetrahymena pyriformis* test system. Ecotoxicol. Environ. Safe 12, 146–153.

Schultz, T.W., Lin, D.T., Wesley, S.K., 1992. Quantitative structure–activity relationships for monosubstituted phenols and the polar narcosis mechanism of action. Qual. Assurance: Good Practice, Regulation Law 1, 132–143.

Schultz, T.W., Lin, D.T., Wilke, T.S., Arnold, L.M., 1990. Quantitative structure–activity relationships for the *Tetrahymena pyriformis* population growth endpoint: A mechanism of toxic action approach. In: Karcher, W., Deviller, J. (Eds.), Practical Applications of Quantitative Structure–Activity Relationships (QSAR) in Environmental Chemistry and Toxicology. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp. 241–262.

Schultz, T.W., Sinks, G.D., Cronin, M.T.D., 1997. Identification of mechanism of toxic action of phenols to *Tetrahymena pyriformis* from molecular structure. In: Fredenslund, F.C., Schuurmann, G. (Eds.), Quantitative Structure–Activity Relationships in Environmental Sciences, vol. VII. SETAC Press, Pensacola, FL, pp. 329–342.

Shigeoka, T., Sato, Y., Takeda, Y., Yoshida, D., Yamauchi, F., 1988. Acute toxicity of chlorophenols to green algae, *Selenastrum capricornutum* and *Chlorella vulgaris*, and quantitative structure–activity relationships. Environ. Toxicol. Chem. 7, 847–854.

SRC-WSKOW for Microsoft Windows (version 1. 26, copyright © William Meylan, 1994–1996.

STATISTICA for Windows software: version 5.0, Copyright 1984–1995, StatSoft, Inc., 2325 East 13th Street, Tulsa, OK, 74104, Phone: (918) 583-4149, Fax: (918) 583-4376.

Stewart, J.J.P., 1990. MOPAC 6.0, Frank J. Seiler research Laboratory, US Air force Academy, Corporation 84840, USA.

Veith, G.D., Broderius, S.J., 1987. Structure–toxicity relationships for industrial chemicals causing type (II) narcosis syndrome. In: Kaiser, K.L.E. (Ed.), QSAR environmental Toxicology – II. D. Reidel, Dordrecht, pp. 385–393.

Verhaar, H.J.M., van Leeuwen, C.J., Hermens, J.L.M., 1992. Classifying environmental pollutants. I: Structure–activity relationships for prediction of aquatic toxicity. Chemosphere 25, 471–491.

Wang, W., 1991. Literature review on higher plants for toxicity testing. Water, Air, Soil Pollut. 59, 381–400.

Wang, X.D., Dong, Y.Y., Xu, S.F., Wang, L.S., Han, S.K., 2000a. Quantitative structure–activity relationship for the toxicity to the tadpole *Rana japonica* of selected phenols. Bull. Environ. Contam. Toxicol. 64, 859–865.

Wang, X.D., Dong, Y.Y., Wang, L.S., Han, S.K., 2000b. Structure–phytotoxicity relationship: comparative inhibition of selected nitrogen-containing aromatics to root elongation of *Cucumis sativus*. Bull. Environ. Contam. Toxicol. 65, 435–442.

Wang, X.D., Dong, Y.Y., Wang, L.S., Han, S.K., 2001a. Acute toxicity of substituted phenols to *Rana japonica* tadpoles and mechanism-based quantitative structure–activity relationship (QSAR) study. Chemosphere 44 (3), 447–455.

Wang, X.D., Sun, C., Gao, S.X., Wang, L.S., 2001b. Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chemosphere 44 (8), 1711–1721.

Wang, X.D., Sun, C., Han, S.K., Wang, L.S., 2001c. QSAR: comparative inhibition of nitrogen-containing aromatics on germination rate of *Cucumis sativus*. Environ. Toxicol. Chem. 20 (4), 913–916.

Xiaodong Wang, holds a B.S. and an M.S. degree and is pursuing his Ph.D in Department of Environmental Science & Engineering of Nanjing University. He has worked for three years as a design and project engineer of wastewater treatment and recycling. His research interests are in decontamination technologies, QSAR applications in Environmental Toxicology and chemistry and Ecological Risk Assessment.