Bull. Environ. Contam. Toxicol. (2002) 69:278–285

DOI: 10.1007/s00128-002-0058-x

Bulletin of Environmental Contamination and Toxicology

Response-Surface Analysis for the Inhibition Toxicity of Benzene Derivatives to Yeast *Saccharomyces cerevisiae*

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Received: 15 May 2001/Accepted: 5 March 2002

Increasing concern over the flooded release of newly produced chemicals promoted the assessment of their toxicological effect. The toxicity of chemical compounds can be assessed with vertebrate bioassays, *in vitro* bioassays or bioassays with invertebrates, bacteria or algae. Yeasts, as eucharyotics, are potentially good model systems because they are widely spread in nature playing important roles in many ecosystems. From a practical point of view they also present advantages, since they are easy to maintain and cultivate under controlled conditions, avoiding the problems of variability found with more complex organisms (Soares and Calow 1993). Thus yeast has been already proposed as alternative organism for testing acute toxicity of drugs and environmental chemicals (Koch et al 1993).

Toxicity based Quantitative Structure-Activity Relationships (QSARs) provide useful tools of predicting toxicological effect of chemicals (Bradbury 1994). According to McFarland (1970), the toxicity of chemicals is result of a two-step process: the penetration of chemicals into biophase and the interaction of chemicals with the target site of action. Based on this hypothesis, Mekenyan and Veith (1993) proposed a response-surface analysis approach by developing QSAR models based on hydrophobicity representing penetration term and molecular orbital parameters modeling the interaction term. Cronin and co-workers (Cronin et al 1998; Cronin and Schultz 1996) applied this approach to modeling the toxicity of nitrobenzenes and phenols to *Tetrahemena pyriformis*. This provides a solution to modeling the toxicity of chemicals without regard to the exact mechanisms of toxic action.

In this paper, the comparative inhibition activity toward yeast *Saccharomyces cerevisiae* of 78 heterogeneous benzene derivatives was assessed with response-surface analysis method. The aim of this investigation is to develop a robust QSAR to model the toxicity of chemicals encompassing multiple mechanisms, without regard to their exact modes of toxic action.

MATERIALS AND METHODS

A heterogeneous set of 78 benzene derivatives potentially encompassing several mechanisms of toxic action were assessed. The chemicals include alkyl and halo

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substituted benzenes, phenols, anilines, benzonitriles and benzaldehydes, nitrobenzenes and benzoic acid derivatives. Their toxicity data to yeast *S. cerevisiae* was cited from the literature (Liao et al 1997). The minimum concentration that produced a clear inhibition zone to the culture of yeast *Saccharomyces cerevisiae* after 12hr incubation was regarded as the relative toxic potency of each chemical and was transformed to the negative logarithmic form (C_{miz} in mol/L) for the QSAR analysis. The pH of the test medium is 5.5.

Logarithms of the 1-octanol/water coefficient ($\log K_{ow}$) for all chemicals were estimated or retrieved as measured values from *SRC-WSKOW* for Microsoft Windows (version 1. 26, copyright © William Meylan 1994 –1996). For benzoic acid derivatives, considering the change of the hydrophobicity parameters due to the possible protonization at low pH, partition coefficient of non-ionized fraction in logarithmic form, $\log D_{ow}$, was used instead of $\log K_{ow}$ ($D_{ow} = K_{ow} / (1+ 10^{pH-pKa})$, pH is 5.5, the pH value of the test medium; *pKa* is the negative logarithms of acid dissociation constant). *pKa* values were calculated by the classical Hammett-type relationship (Perrin et al. 1981). Molecular orbital parameters were calculated from the semi-empirical molecular orbital package MOPAC6.0 (Stewart 1990) using PM3 Hamiltonian. Molecular geometry was optimized with MM2 method and then with Broyden-Flether-Goldfarb-Shanno (BFGS) method in MOPAC6.0.

The Multiple Regression Analysis in *STATISTICA* software (version 5.0, Copyright 1984-1995, StatSoft, Inc.) was employed for f response-surface analysis. 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).

RESULTS AND DISCUSSION

A summary of the toxicity data and the calculated molecular descriptors was shown in Table 1. Figure 1 is scatter plot of all toxicity data versus log K_{ow}. An initial regression of all toxicity data with hydrophobicity resulted in Equation 1:

$$C_{miz} = 0.489 \log K_{ow} + 0.445 \qquad 1$$
$$r^2 = 0.569 \quad F=100.3 \quad P< 0.000001 \quad SE = 0.43 \quad n = 78$$

The resultant hydrophobicity dependent QSAR proved to be statistically significant but not predictive. This implied the multiple mechanisms of toxic action for all chemicals and hydrophobicity can't be used alone to model the toxicity towards yeast of all chemicals. The significant correlation of toxicity with hydrophobicity implied the important role of penetration ability of chemicals into bio-phase in determining toxicity of all chemicals. Based on previous studies of aquatic toxicology, halogen and alkyl substituted benzenes are typical nonpolar narcotic compounds (Nendza and Russom 1991). Nonpolar narcosis is considered the baseline toxicity and is mainly the result of irritation of chemicals onto the membrane, which result in non-covalent interaction such as the disruption of Van

Table 1. Toxicological data toward yeast *Saccharomyces cerevisiae* and the calculated molecular structure descriptors for QSAR analysis.

	CHEMICAL	CAS Number	C_{miz} [a]	Log K_{ow} [b]	E_{lumo} [c]
1	chlorobenzene	108-90-7	1.18	2.81	-0.151
2	Bromobenzene	108-86-1	1.40	2.99	-0.398
3	1,2-dichlorobenzene	95-50-1	1.96	3.55	-0.346
4	1,3-dichlorobenzene	541-73-1	1.87	3.38	-0.336
5	1,4-dichlorobenzene	106-46-7	1.96	3.59	-0.398
6	1,3-dibromobenzene	108-36-1	2.32	4.09	-0.653
7	1,4-dibromobenzene	106-37-6	2.37	4.07	-0.545
8	4-chlorobromobenzene	106-39-8	2.08	3.82	-0.454
9	1,2,3-trichlorobenzene	87-61-6	2.41	4.2	-0.478
10	1,2,4-trichlorobenzene	120-82-1	2.54	4.27	-0.565
11	1,3,5-trichlorobenzene	108-70-3	2.41	4.28	-0.478
12	2,5-dichlorotoluene	19398-61-9	2.33	4.04	-0.402
13	2,6-dichlorotoluene	118-69-4	2.47	4.27	-0.265
14	2,4,5-trichlorobenzene	6639-30-1	2.91	4.93	-0.576
15	2-chlorotoluene	95-49-8	1.85	3.28	-0.156
16	4-chlorotoluene	106-43-4	1.8	3.3	-0.184
17	1,4-dimethylbenzene	106-42-3	1.74	3.3	0.039
18	13-dimethylbenzene	108-38-3	1.7	3.3	0.062
19	toluene	108-88-3	1.1	2.65	0.068
20	3,4-dichloronitrobenzene	99-54-7	2.2	3.29	-1.442
21	4-nitrochlorobenzene	100-00-5	1.65	2.58	-1.352
22	3-nitrochlorobenzene	121-73-3	1.65	2.58	-1.305
23	2-nitrotoluene	88-72-2	1.64	2.58	-1.18
24	4-nitrobromobenzene	586-78-7	2.13	2.58	-1.382
25	2,4-dinitrobromobenzene	584-48-5	2.47	2.7	-1.759
26	24-dinitrotoluene	121-14-2	2.06	2.18	-1.713
27	nitrobenzene	98-95-3	1.01	1.86	-1.212
28	2,4-dinitrochlorobenzene	97-00-7	2.02	1.98	-1.55
29	2,6-dinitrotoluene	606-20-3	1.61	2.28	-1.532
30	4-nitrotoluene	99-99-0	1.5	2.53	-1.233
31	3-nitrotoluene	99-08-1	1.52	2.53	-1.222
32	2-nitrotoluene	88-72-2	1.29	2.53	-1.18
33	3-chloro-4-fluoronitrobenzene	350-30-1	1.56	2.71	-1.4
34	1,4-dinitrobenzene	100-25-4	3.23	1.84	-4.237
35	1,2-dinitrobenzene	528-29-0	1.41	1.84	-3.17
36	1,3-dinitrobenzene	99-65-0	1.45	1.84	-1.547
37	4-chlorobenzaldehyde	104-88-1	1.45	2.16	-0.849
38	2-chlorobenzaldehyde	89-98-5	1.67	2.16	-0.832
39	benzaldehyde	100-52-7	0.75	1.43	-0.669
40	pentachlorophenol	87-86-5	2.98	4.24	-0.926
41	2,4-dichlorophenol	120-83-2	2.43	2.90	-0.364
42	2,4-dinitrophenol	51-28-5	2.19	1.47	-1.462

Table 1. (continued).

	CHEMICAL	CAS Number	C_{miz} [a]	Log K_{ow} [b]	E_{lumo} [c]
43	4-nitrophenol	100-02-7	1.24	1.92	-1.155
44	2-methylphenol	95-48-7	1.38	1.37	-0.012
45	2-chlorophenol	95-57-8	1.43	2.18	-0.218
46	phenol	108-95-2	0.86	1.46	0.027
47	4-chlorophenol	106-48-9	1.63	2.39	-0.114
48	2,6-dimethylphenol	576-26-1	1.35	2.26	-0.012
49	3,4-dichloroaniline	96-76-1	1.67	2.55	-0.221
50	4-bromoaniline	106-40-1	1.91	2.05	-0.267
51	4-chloroaniline	106-47-8	1.44	1.9	0.015
52	2,4-dichloroaniline	554-00-7	2.4	2.75	-0.201
53	2,4,6-tribromoaniline	147-82-0	3.12	3.97	-0.814
54	2,4,6-trichloroaniline	634-93-5	1.91	3.04	-0.398
55	4-methylaniline	106-49-0	0.77	0.33	0.147
56	1,4-diaminobenzene	106-50-3	0.89	0.15	0.104
57	4-no2-2-fluroaniline	121-87-5	1.42	1.58	-1.303
58	4-nitroaniline	100-01-6	0.96	1.39	-1.202
59	3-nitroaniline	99-09-2	0.88	1.37	-1.22
60	2-nitroaniline	88-74-4	1.08	1.37	-1.068
61	4-biphenylamine	92-67-1	2.49	3.92	-0.382
62	2,6-dichloroaniline	608-31-1	1.62	2.85	-0.265
63	3-cl-4-fluorobenzonitrile	117482-84-5	0.83	2.04	-0.262
64	3,4-dichlorobenzonitrile	6575-00-4	2.16	2.98	-0.979
65	2-chlorobenzonitrile	873-32-5	1.56	1.97	-0.841
66	4-chlorobenzonitrile	623-03-0	1.44	1.97	-0.858
67	3-chlorobenzonitrile	766-84-7	1.31	1.97	-0.79
68	4-chlorobenzonitrile	623-00-7	1.33	2.24	-0.184
69	benzonitrile	100-47-0	0.92	1.26	-0.667
70	4-chlorobenzoic acid	74-11-3	1.85	1.14	-0.879
71	3-bromobenzoic acid	585-76-2	1.94	1.18	-0.818
72	4-fluorobenzoic acid	456-22-4	1.37	0.71	-0.823
73	2-aminobenzoic acid	118-92-3	0.79	-0.62	-0.568
74	3-nitrobenzoic acid	121-92-6	1.52	-0.21	-1.422
75	4- bromobenzoic acid	586-76-5	1.95	1.35	-0.908
76	3- chlorobenzoic acid	535-80-8	1.72	1.11	-0.811
77	4-aminobenzoic acid	105-13-0	0.32	-1.19	-0.46
78	3-aminobenzoic acid	99-05-8	0.23	-1.33	-0.663

[a] C_{miz} = the negative logarithm of the 12hr minimum inhibition zone concentration (in mol/L) toward yeast *Saccharomyces cerevisiae.*

[b] logK_{ow} = the logarithmic form of the 1-octanol/water partition coefficient, obtained from SRC–WSKOW (version 1.26, copyright © William Meylan 1994 – 1996). For benzoic acids, logK_{ow} = logD_{ow}, partition coefficient of un-ionized fractions in logarithmic form.

[c] E_{lumo} = the energy of the lowest unoccupied orbital, computed from MOPAC6 (Stewart, 1990)

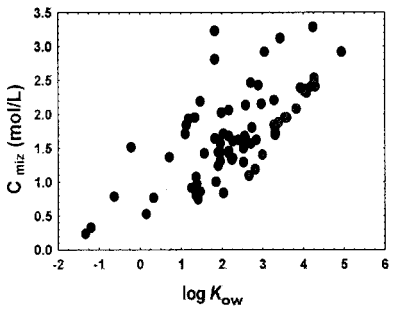

Figure 1. Scatter plot of toxicity toward yeast *Saccharomyces cerevisiae* (C_{miz} in mol/L) vs hydrophobicity (log K_{ow}) for all chemicals

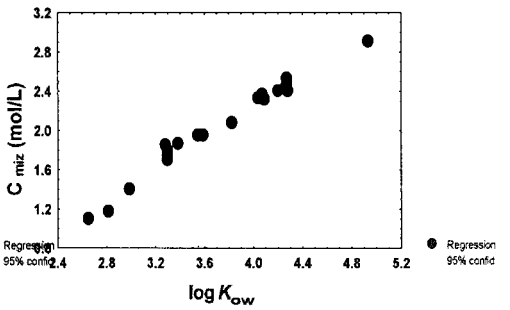

Figure 2. Scatter plot of toxicity toward yeast *Saccharomyces cerevisiae* (C_{miz} in mol/L) vs log K_{ow} for nonpolar narcotic halogen and alkyl substituted benzenes.

der Waal's interactions between lipid and /or protein compounds within the membrane (Franks and Lieb 1990). For narcosis, the penetration ability of chemicals into bio-phase is the overriding factor affecting toxicity, thus a highly significant hydrophobicity dependent QSAR can be developed. Regression analysis of the toxicities of halogen and alkyl substituted benzenes revealed an excellent logK_{ow} -dependent QSAR (Equation 2) and this model was considered as the baseline or nonpolar narcotic QSAR for this endpoint, 12hr minimum inhibition zone concentration of yeast *Saccharomyces cerevisiae.*

$$C_{miz} = 0.793 \log K_{ow} - 0.907 \qquad 2$$
$$r^2 = 0.975 \quad F = 661.0 \quad P < 0.000001 \quad SE = 0.08 \quad n = 19$$

Other chemicals in this study include phenols, anilines, benzonitriles, benzaldehydes, nitrobenzenes, and benzoic acids. According to the toxicophore based on other aquatic toxicological investigations (Bearden and Schultz 1997; Nendza and Russom 1991; Wang et al 2000 and 2001), the majority of phenols and anilines are polar narcotics. For these chemicals, the presence of strong electron-releasing amino or hydroxy moieties on the aromatic ring results in greater dipolarity and /or hydrogen bond donor acidity than in nonpolar narcosis (Kamlet et al 1986). Polar narcosis could be well modeled by logK_{ow} alone or the combination of logK_{ow} and E_{lumo}, the lowest unoccupied molecular orbital energy (Bearden and Schultz 1997). Some derivatives of phenol and aniline with multiple nitro or/ and halogen groups are identified to be weak acid respiratory uncouplers. They are general bulky and electronegative and can cause the inner mitochondrial membrane to become permeable to hydrogen ions, thereby disrupting the hydrogen ion gradient and synthesis of ATP (Terada 1990). According to the review of Hermens (1990), benzaldehydes, nitrobenzenes, and benzonitriles are typical electrophiles, with a similar molecular structure with α-β unsaturated chemicals. The elevated toxicity of electrophiles was mainly determined by their specific electrophilic interaction with bio-macromolecules of target sites and can't be modeled well by a single hydrophobicity based QSAR. Whilst the frontier orbital parameters have been employed for the development of robust QSARs

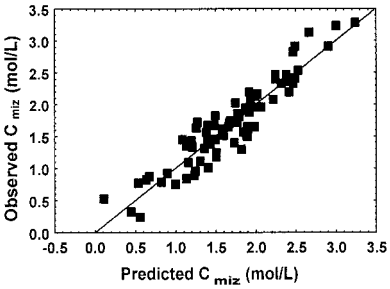

Figure 3. Calculated toxicity from the response surface (Equation 4) versus the observed toxicity to yeast *S. cerevisiae* of benzenes.

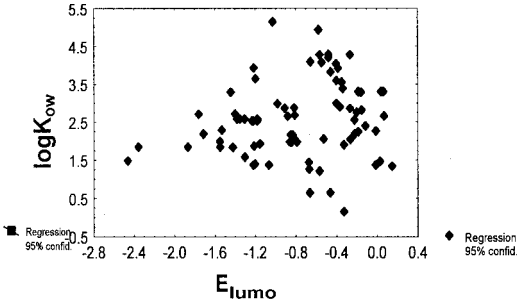

Figure 4. The correlation plot of $\log K_{ow}$ versus E_{lumo} for the response- surface (Equation 4) of the toxicity to yeast *S. cerevisiae* of benzenes.

recently (Mekenyan and Veith 1993). The toxicity mechanism of benzoic acids was far from clear. Based on molecular substructure, benzoic acids were identified to be bioreactive toxicants where an unsaturated carboxyl moiety substitutes directly to an aromatic ring system. However, previous study indicated that toxicity of benzoic acids to *Daphnia* was elicited via a narcosis mode of action (Zhao et al 1998). Nendza and Russom (1991) also considered aromatic carboxylic acids to be polar aromatic toxicants based on the hydrogen bonding capability of the carboxyl moiety. The diversity of the potential mechanisms of toxic action made it difficult to model all chemicals as a single set with hydrophobicity alone. To examine the role of electrophilic reactivity in determining toxicity of chemicals, a regression analysis was performed with toxicity data and E_{lumo} after nonpolar narcotics were excluded from toxicity data set. And the result indicated that E_{lumo} was a statistically significant parameter in this QSAR (Equation 3), although it was poorly predictive.

$$C_{miz} = -0.558\ E_{lumo} + 1.1096 \qquad 3$$
$$r^2 = 0.311 \quad F = 27.8 \quad P < 0.000001 \quad SE = 0.556 \quad n = 59$$

According to Mcfarland (1970), the toxicity of a chemicals is the combination of penetration into bio-phase through bio-membrane and their interaction with the target site of action. Log K_{ow}, was frequently used to model the penetration term. The interaction term can be modeled by many electronic and / or steric parameters according to their specific interactions. Based on Mcfarland's principle, a response surface approach was initially proposed by Mekenyan and Veith (1993). Cronin and co-workers (Cronin and Schultz 1996; Cronin et al 1998) extended its application by developing a two-variable QSAR or a response surface based on hydrophobicity ($\log K_{ow}$) and electrophilic reactivity (E_{lumo}), to model chemicals encompassing a variety of mechanisms of toxic action. The application of response-surface analysis to this investigation resulted in a highly predictive two-variable QSAR or response surface (Equation 4). An examination of residuals revealed no outliers to this relationship and the calculated toxicity from this response surface was very close to the observed toxicity (Figure 3). Correlation

matrix reveals that there was no significant correlation between $\log K_{ow}$ and E_{lumo} (Figure 4).

$$C_{miz} = 0.555 \log K_{ow} - 0.496E_{lumo} - 0.127 \qquad 4$$
$$r^2 = 0.874 \quad F=261.2 \quad P< 0.000001 \quad SE = 0.23 \quad n = 78$$

Recent development of QSARs included the mechanism-based QSAR (Bearden and Schultz 1997), which relies on the *a priori* assignment of chemicals to a mechanism of toxic action. However, this *a priori* identification of mechanism is based strictly on the molecular structure of chemicals. Due to the complexity of the interaction of chemicals with target site of action and the diversity of chemical structure, it is difficult to always identify a correct mechanism. Other reasons such as the interspecies difference and different test protocols also may cause failure to identify the correct mechanism. Thus there has been much interest recently in the modeling the toxicity of structurally diverse chemicals without regard to the mechanism of toxic action. Several applications of response surface analysis to modeling toxicity suggest that the response surface approach developed from multiple regression analysis provides an ideal solution.

In summary, toxicity data to yeast *Saccharomyces cerevisiae* of a set of 78 heterogeneous benzene derivatives was assessed and a robust two-variable QSAR or a response surface based on parameters characterizing hydrophobicity and electrophilicity was developed to model the toxicity of all chemicals. The response surface developed herein allows to model toxicity of substituted benzenes across chemicals and molecular mechanisms.

Acknowledgments. This study was funded by the National Natural Science Foundation (NNSF), the Doctoral Program of Higher Education of P. R. China and the Core University Program, Japan Society for Promotion of Science.

REFERENCES

Bearden AP, Schultz TW (1997) Structure-activity relationships for *Pimephales* and *Tetrahymena*: A mechanism of action approach. Environ Toxicol Chem 16: 1311-1317.

Bradbury SP (1994) Predicting modes of toxic action from chemical structure: An overview. SAR QSAR in Environ Res 2: 89-104.

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

Cronin MTD, Gregory BW, and Schultz TW (1998) Quantitative Structure-Activity Analyses of Nitrobenzene Toxicity to *Tetrahymena pyriformis*. Chem Res Toxicol 11: 902-908.

Franks HB, and Lien WR (1990) Mechanisms of general anesthesia. Environ Health Prospect 87: 199-205.

Hermens JLM (1990) Electrophiles and acute toxicity to fish. Environ Health Prospect 87:219-225

Kamlet MJ, Doherty RM, Veith GD, Taft RW, Abraham MH (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.

Koch HP, Hofeneder M, Bohne B (1993) The yeast tests: An Alternative method for the testing of acute toxicity of drug substances and environmental chemicals. Method Find Clin Pharmacol 15:141-152.

Liao YY, Zhou D, Liu ZT, Han SK, Wang LS (1997) QSARs of the toxicity to Yeast of substituted aromatic compounds (in Chinese). In *Progress in Organic Pollutants Chemistry* (Wang LS and Han SK Eds) pp181-186. Nanjing University Press, Nanjing.

McFarland JW (1970) On the parabolic relationship between drug potency and hydrophobicity. J Med Chem 13: 1092-1196.

Mekenyan OG, and Veith GD (1993) Relationships between descriptors for hydrophobicity and soft electrophilicity in predicting toxicity. SAR QSAR Environ Res 2: 335-344.

Nendza M, and Russom CL (1991) QSAR modeling of the ERL-D fathead minnow acute toxicity database. Xenobiotica 21: 147-170.

Perrin DD, Dempsey B, and Serjeant EP (1981) p*Ka* Prediction for Organic Acids and Bases. Chapman and Hall, London, UK.

Soares AMVM Calow P (1993) Seeking standardization in ecotoxicology. In: Soares, AMVM, Calow P (Eds.) Progress in standardization of aquatic toxicity tests. Chapter 1. Lewis, London.

Stewart JJP (1990) MOPAC 6, Frank J Seiler research Laboratory, U.S. Air force Academy, Corporation 84840, U.S.A.

Terada H (1990) Uncouplers of oxidative phosphorylation. Environ Health Prospect 97: 213-218.

Wang XD, Dong YY, Xu SF, Wang LS, Han SK (2000). Quantitative Structure-Activity Relationship for the Toxicity to the Tadpole *Rana japonica* of Selected Phenols. Bull Environ Contam Toxicol 64: 859-865.

Wang XD, Dong YY, Wang LS, Han SK (2001). Acute Toxicity of Substituted Phenols to *Rana Japonica* Tadpoles and Mechanism-Based Quantitative Structure-Activity Relationship (QSAR) Study. Chemosphere 44: 447-455.

Zhao YH, Ji GD, Cronin MTD, Dearden JC (1998) QSAR study of the toxicity of benzoic acids to *Vibrio fisheri*, *Daphnia magna* and carp. Sci Total Environ 216: 205-215.