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Toxicity QSAR of Substituted Benzenes to Yeast *Saccharomyces cerevisiae*

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It is well-known that nonspecific toxicity of chemicals to indicate organisms can be described by two kind of actions: baseline nonpolar narcosis (type I narcosis) and polar narcosis (type II narcosis) (Schultz et al 1986 and Veith et al 1983). Type I narcosis is entirely dependent on the hydrophobicity of chemicals. Type II narcosis is slightly more toxic than type I narcosis. The addition of a electric parameter can improve the predication of a l-octanol/water partition coefficient(LogKow) dependent model(Mcleese 1979 and Schultz 1989).

Recent studies in our laboratory with six series of substitute benzenes (including two narcosis mechanisms) show that narcosis I action to yeast *Saccharomyces cerevisiae* can be modeled by logkow parameter, and narcosis II action by logkow and a electric parameter sigma. It was very interesting to notice that for both narcotic action models, the equation coefficients of LogKow part were almost equal(0.72-0.82). This finding suggested that polar narcosis toxicity can be divided into two parts: the nonpolar narcosis toxicity (baseline toxicity) and polar toxicity(reactive toxicity) It was the purpose of the present study to determine the relative toxicity of six series of substituted benzenes, and then examine the correlation between the Logkow, electric parameter(sigma) and toxicity.

MATERIALS AND METHODS

The test compounds were a series of 6 substituted benzenes, including halogenated benzene, benzoic acids, phenols, mono-nitrobenzens, dinitrobenzene, and anilines. The purity of the chemical was greater that 95 percent.

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The eucaryotic yeast, *Saccharomyces cerevisiae* , was chosen as the test organism because it is easy to handle, and its cellular structure resembles that of higher organisms (Abler et al 1988). DMSO-glycerol (8O:2O w/w) (Liu.D et al 1989) was used as the sample-carrier solvent in determining the minimum inhibition zone concentration of the substituted benzenes on agar plates. A potato-dextrose medium (Gaudy et al 1980) was used as the culture medium. The pH of the medium was adjusted to 5.5 using HCl. For solid medium. 1.5 percent agar was added. The medium was sterilized using high pressure steam at 108°C for 30 minutes. Each agar plate dish (9.0 cm) contained 20 mL of agar medium. The plates were pre-dried for 24 hr at 29°C.

Saccharomyces cerevisiae was grown in 50 mL liquid broth in a 125mL flask on a rotary shaker (100rpm) at 29-30°C. After 24hr, the culture was taken out and allowed to stand at room temperature (in dark) for 48hr before use. The aged yeast culture was dilute 3 times using fresh liquid broth medium to a final concentration of 2 million cells per millimeter. An aliquot of 0.5 mL of cell suspension was evenly spread on the surface of the pre-dried agar plate. After the liquid was completely absorbed (10 minutes or so needed). 5 µL aliquot of test sample (in DMSO-Glycerol solvent) were placed onto the surface of the pre-seeded agar plate using a 5 µL micropipette. A 9cm dish could accommodate 5 concentrations and one control. The test agar plates were incubated at 29°C overnight. The minimum concentration that produced a clear inhibition zone represented the relatively toxicity of the chemicals. In practice when single yeast colonies were distinguished in the inhibition zone, we considered it to be clear. When inhibition in the controls were observed, the results were discarded and the yeast culture renewed. For each test, three parallel plates were employed.

The test included two pre-test steps and one toxicity test. In the final toxicity test the concentration gradient between the two neighbor samples could be no more than 10 percent. Table 1 is an example of determining the minimum inhibition zone concentration Cmiz for 1,4-dichawrobenzene.As shown in the table, the minimum inhibition concentration for 1,4-dichlorobenzene was l.6µg/µL.

Minimum inhibition zone concentration (Cmiz) were analyzed using Statistical Graphics System program version 4.0 (Statistical Graphics Corp.1989). In the analysis, Y was the Log1/Cmiz(in mM/L), X was the LogKow and Hammet sigma parameter.

Table 1 The determination of the minimum inhibition zone concentration Cmiz on agar plate for 1,4-dichlorobenzene

pre-test 1

	control	1	2	3	4	5
μg/μL	0	0.5	1.0	2.0	4.0	8.0
Result	NI	V	V	CIZ	CIZ	CIZ

pre-test 2

	control	1	2	3	4	5
μg/μL	0	1.0	1.2	1.4	1.7	2.0
Result	NI	V	V	V	DSC	CIZ

Toxicity test

	control	1	2	3	4	5
μg/μL	0	1.4	1.5	1.6	1.7	1.8
Result	NI	V	V	DSC	DSC	DSC

In the table:
NI: No Inhibition V: Vague inhibition zone
CIZ: Clear Inhibition Zone
DSC: Distinguish Single Colonies in inhibition zone

According to Leeuween 1992, halogenated benzenes produce Type I narcosis. The work of Rittich(1992) and Cohen(1988) has shown that the toxicity of benzoic aids and substituted phenols to eucaryotic fungi also produce Type I narcosis. Aniline and nitrobenzenes are believed to be chemicals producing polar narcosis (Veith & Broderius, 1990). Our studies were consistent with these results. The toxicities of nonpolar narcosis and polar narcosis chemicals are listed in table 2 and table 3 respectively.

For nonpolar narcotic chemicals, the toxicity depends on its hydrophobicity. Regression of Log1/Cmiz vs LogKow obtained the following results:

Halo-benzenes(toluene):

$$\text{Log1/Cmiz} = 0.82\ \text{LogKow} - 1.03 \quad (1)$$

$n=10 \quad r^2=0.98 \quad s=0.07 \quad F=534.81 \quad P<0.001$

Benzoic acids:

$$\text{Log1/Cmiz} = 0.74\ \text{LogKow} - 0.17 \quad (2)$$

$n=8 \quad r^2=0.99 \quad s=0.06 \quad F=988.89 \quad P<0.001$

Phenols:

$$\text{Log1/Cmiz} = 0.82\ \text{Logkow} - 0.38 \quad (3)$$

$n=7 \quad r^2=0.97 \quad s=0.13 \quad F=223.64 \quad P<0.001$

Table 2 Summary of toxicity and LogKow for nonpolar narcosis chemicals

Compound	Log1/Cmiz	LogKow
Halo-benzenes(toluenes)		
chlorobenzene	1.18	2.81
bromobenzene	1.40	2.99
1,2-dichlorobenzene	1.96	3.55
1,4-dichlorobenzene	1.96	3.59
1,3-dibromobenzene	2.32	4.09
4-bromo-chlorobenzene	2.08	3.82
1,2,3-trichlorobenzene	2.41	4.20
1,2,4-trichlorobenzene	2.54	4.27
2,5-dichlorotoluene	2.33	4.04
2,4,5-trichlorotoluene	2.91	4.93
Substituted benzoic acids		
3-chloro-benzoic acid	1.72	2.68
4-chloro-benzoic acid	1.85	2.65
3-bromo-benzoic acid	1.94	2.87
4-fluro-benzoic acid	1.37	2.07
4-bromo-benzoic acid	1.95	2.86
2-amino-benzoic acid	0.79	1.21
3-amino-benzoic acid	0.32	0.64
4-amino-benzoic acid	0.23	0.64
Substituted phenols		
pentachlorophenol	3.83	5.04
2,4-dichlorophenol	2.43	2.96
2-methyl-phenol	1.38	1.96
2-chlorophenol	1.43	2.18
4-chlorophenol	1.63	2.39
2,6-dimethyl-phenol	1.35	2.36
phenol	0.86	1.46

* the Cmiz of substituted phenols were calculated in un-ioned concentration of the chemicals (Cohen,1988)

For polar narcosis, the QSAR may be modeled by LogKow and an electric parameter(sigma):

Mono-nitrobenzenes:

$$\text{Log1/Cmiz} = 0.76\ \text{LogKow} + 0.17\ \sigma + 0.18 \quad (4)$$

$n=10 \quad r^2=0.99 \quad s=0.02 \quad F=1040.50 \quad P<0.001$

Dinitrobenzenes:

$$\text{Log1/Cmiz} = 0.73\ \text{LogKow} + 0.46\ \sigma + 0.03 \quad (5)$$

$n=7 \quad r^2=0.96 \quad s=0.08 \quad F=108.68 \quad P<0.001$

Aniline:

$$\text{Log1/Cmiz} = 0.72\ \text{LogKow} - 0.25\ \sigma + 0.56 \quad (6)$$

$n=9 \quad r^2=0.98 \quad s=0.06 \quad F=775.10 \quad P<0.001$

In these equations, both LogKow and sigma were highly significant descriptors (significant levels less than 0.005 for each parameter).

Table 3 Summary of toxicity and molecular descriptors data for polar narcosis chemicals

Compound	Log1/Cmiz	LogKow	Sigma*
Substituted mono-nitrobenzenes			
nitrobenzene	1.01	1.86	0
2-chloro-nitrobenzene	1.65	2.58	0.47
3-chloro-nitrobenzene	1.64	2.58	0.37
4-chloro-nitrobenzene	1.65	2.58	0.23
4-bromo-nitrobenzene	2.13	2.73	0.23
2-methyl-nitrobenzene	1.52	2.53	-0.04
3-methyl-nitrobenzene	1.52	2.53	-0.07
4-methyl-nitrobenzene	1.50	2.53	-0.17
3,4-dichloro-nitrobenzene	2.20	3.29	0.60
2,4-dichloro-nitrobenzene	2.24	3.29	0.70
Substituted dinitrobenzene**			
o-dinitrobenzene	1.41	1.84	0****
m-dinitrobenzene	1.45	1.84	0****
p-dinitrobenzene	3.23***	1.84	***
2,4-dinitrobromobenzene	2.47	2.70	0.94
2,4-dinitrochlorobenzene	1.90	2.06	0.94
2,4-dinitrotoluene	2.02	2.28	0.54
2,3-dinitrotoluene	1.97	2.28	0.69
2,6-dinitrotoluene	1.61	2.28	0****
substituted anilines			
3-chloro-aniline	1.80	1.90	0.37
4-chloro-aniline	1.80	1.90	0.23
4-bromo-aniline	1.91	2.05	0.23
2,4-dichloro-aniline	2.40	2.75	0.70
2,4,6-tribromoaniline	3.12	3.97	1.17
2,4,6-trichloroaniline	2.45	3.04	1.17
4-methyl-aniline	0.77	0.33	-0.17
p-phenylenediamine	0.89	0.15	-0.66
2-chloro-4-nitro-aniline	1.42	1.58	1.15

* For ortho-substituted group, sigma data of S.Induct was adopted.(Hansch C 1979)

** For dintrobenzenes, we took one nitro-group as action center, the other as a substituted group.

*** P-dinitrobenzene was not included in the regression since it was obviously a reactive chemical to yeast.

****The sigma values were adopted zero because only a baseline toxicity was observed.

Table 4 is a comparison of regression coefficient of the above equations. In the table, the equation coefficients of LogKow part were found to be almost equal(0.72-0.821, for nonpolar narcosis or for polar narcosis chemicals. This observation implied that polar narcosis action toxicity included a nonpolar narcosis part (baseline toxicity) and a polar toxicity part. For both narcosis action mechanisms, the baseline toxicity part followed the same hydrophobicity dependent pattern. Therefore the slightly more toxic part above the baseline toxicity for polar narcosis mechanisms is

Table 4 Comparison of the regression coefficients for nonpolar and polar narcosis action model

	LogKow	sigma	constant
halo-benzenes(toluene)	0.82		-1.03
benzoic acids	0.74		-0.17
phenols	0.82		-0.38
mono-nitrobenzenes	0.76	0.17	0.18
dinitrobenzenes	0.73	0.46	0.03
anilines	0.72	-0.25	0.56

-- sigma parameter was not selected for nonpolar narcosis chemicals

actually induced by the electric effect of the chemicals. As shown in the equations, the potency of the chemicals to induce the polar toxicity to yeast *Saccharomyces cerevisiae* has the following sequence: dinitrobenzenes>mono-nitrobenzenes>anilines.

The present study showed that the nonpolar narcosis toxicity to yeast can be modeled by logkow, polar narcosis by logkow and electric parameter sigma. And for both narcosis action models, the equation coefficient of the logkow part were almost equal. This observation suggested that polar narcosis can be divided into two parts: nonpolar narcosis part(baselinetoxicity1, and polar narcosis partcreactive toxicity).

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