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Relative Toxicity of Para-Substituted Phenols: Log K_{ow} and pKa-Dependent Structure-Activity Relationships

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The toxic response of the majority of industrial chemicals which are non-reactive and non -ionic can be quantitatively modeled by the 1-octanol/water partition coefficient (K_{ow}) in a linear fashion following a log-log transformation of the data (Konemann 1981; Veith et al. 1983; Schultz and Moulton 1984). The toxicity of phenols, however, as shown by Schultz et al. (1986) does not model by these same quantitative structure-activity relationships (QSAR).

Earlier studies including those of McLeese et al. (1979), and Saarikoski and Viluksela (1982) have shown that the addition of pKa as a second molecular descriptor improves the predictive capability of log K_{ow} QSAR. Lipnick and co-workers (1986) expanded upon this theme in their examination of fish toxicity screening data for 110 phenols. They note that predicted toxicity of phenol derivatives, which ionize poorly under the test conditions, is not significantly altered by using models in which pKa is not included. (Lipnick et al. 1986).

In an effort to systematically explore the extent to which such log K_{ow} and, log K_{ow} and pKa-dependent QSAR can be used to predict the biological activity of phenols, the first in a series of investigations was conducted using the rapid and inexpensive Tetrahymena population growth impairment assay to determine relative toxicity of 30 para-substituted derivatives.

MATERIALS AND METHODS

Tetrahymena pyriformis under static conditions was the test system of choice. This chronic assay, which uses population densities of axenic cultures as its end-point, has been described in detail (Schultz 1983). Each phenol was tested in duplicate for a minimum of three replicates following range finding experiments. Each replicate was, at minimum, a five-step graded concentration series using freshly prepared stock solutions. Cultures without phenols served as controls. Cell population levels were estimated spectrophotometrically as absorbance at 540 nm. Only replicates with control absorbance values of 0.6 to 0.9, equivalent to late log-growth-phase, were used in the analyses. The IGC_{50}, 50% growth inhibitory concentration was determined for each phenol using probit analysis of the Statistical Analysis System (SAS) software and an IBM 3081 computer. In these analyses, Y was the absorbance normalized to percent control, X was the concentration of tested phenol in mg/L, n was ≥ 30 and P<

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χ^2 greater than 0.9.

The phenols selected for testing are a heterogenous series of 30 para-substituted derivatives. Highly ionizable derivatives (e.g., -COOH) and strong electron-releasing derivatives (e.g., $-NH_2$ and -OH) were excluded from the test set. The test phenols were purchased from commercial sources (Aldrich Chemical Co., Milwaukee, WI, U.S.A. or Pfaltz and Bauer Inc., Waterbury, CT, U.S.A.) and not repurified. All were of purity of 95% or better. Stock solutions of the individual phenols were prepared in dimethylsulfoxide (DMSO) at a concentration of 10, 25, or 50 g/L. The volume of stock solution added to the culture medium was limited so that DMSO did not exceed a final concentration of 0.75%, a level which has no effect on Tetrahymena growth (Schultz and Cajina-Quezada 1982).

For structure-activity analyses, log BR (i.e., the log of the inverse of the IGC_{50} value in mmol/L) was used as the standard measurement of toxicity (Y) and log K_{ow} and pKa terms were selected as molecular descriptors (X). Log K_{ow} values were computer calculated by the fragment method, with the CLOG P-3.2 program (*Leo personal communication*), or retrieved as measured values from the select list for comparison (Hansch and Leo 1979). The pKa values were taken from (Dean 1985) or calculated by the Perrin method (Perrin et al. 1981) using Hammet sigma constants from Hansch and Leo (1979) and the equation: pKa = 9.99 - 2.23 (sigma). The General Linear Model routine for regression analysis from SAS (Ray 1982) was used to generate the QSAR with outliers being identified from a plot of residual values and model adequacy being measured by the r^2 value.

RESULTS AND DISCUSSION

Table 1 is a listing of the molecular descriptor and toxicity data used in the analyses. The log K_{ow} values are distributed rather uniformly over the range of 0.00 to 4.03. However, the distribution of pKa values is skewed toward the upper end of the 10.70 to 7.15 range. The log K_{ow} and pKa values for the phenols used in the investigation are not correlated ($r^2 = 0.074$).

A scatter plot of log BR versus log K_{ow} for all tested phenols is shown in Figure 1. Regression analysis of these data results in Eq. (1).

$$\log BR = 0.5744 (\log K_{ow}) - 0.8652 \qquad (1)$$
$$n = 30;\ r^2 = 0.756;\ s = 0.362$$

For Eq. (1) log K_{ow} is a highly significant descriptor ($P > F = 0.0001$; df 1,28). The toxic response of one compound, the nitro- derivative, resides outside the upper 95% confidence limit of the model.

The addition of pKa as a second molecular descriptor and subsequent regression analysis yields Eq. (2).

$$\log BR = 0.6577 (\log K_{ow}) - 0.3171 (pKa) + 1.9860 \qquad (2)$$
$$n = 30;\ r^2 = 0.912;\ s = 0.221$$

For Eq. (2) both log K_{ow} and pKa are highly significant descriptors ($P > F = 0.0001$; df 2,27). The observed toxicity of all 30 tested phenols lies within the 95% confidence interval for Eq. (2).

Table 1. Toxicity to Tetrahymena pyriformis and molecular descriptor data for para-substituted derivatives of phenols

No.	Derivative of Phenol	Log K_{ow}	pKa	Log[c] BR
1.	$CONH_2$	0.00	9.23	-0.7802
2.	$NHCOCH_3$	0.32	9.99[b]	-0.8198
3.	CH_2CH_2OH	0.72	10.12[b]	-0.8275
4.	CH_2CN	0.90	9.97[b]	-0.3840
5.	OCH_3	1.34	10.20	-0.1425
6.	CHO	1.35	7.62	0.2661
7.	$COCH_3$	1.35	8.05	-0.0932
8.	H	1.49	9.99	-0.4310
9.	COC_2H_5	1.55	8.85	0.0557
10.	CN	1.60	7.95	0.5161
11.	F	1.77	9.89	0.0169
12.	OC_2H_5	1.87[a]	10.52[b]	0.0130
13.	NO_2	1.91	7.15	1.4257
14.	CH_3	1.94	10.26	-0.1920
15.	CL	2.39	9.43	0.5447
16.	C_2H_5	2.58	10.00	0.2058
17.	Br	2.59	9.34	0.6806
18.	I	2.91	9.20	0.8544
19.	OC_4H_9	3.04[a]	10.70[b]	0.7016
20.	$CH(CH_3)_2$	3.05	10.32[b]	0.4732
21.	COC_6H_5	3.07	8.89	1.0237
22.	C_3H_7	3.18	10.28[b]	0.6350
23.	$N{=}NC_6H_5$	3.18	8.56[b]	1.6547
24.	C_6H_5	3.20	9.55	1.3828

TABLE 1 (Continued)

No.	Derivative of Phenol	Log K_{ow}	pKa	Log BR
25.	$C(CH_3)_3$	3.31	10.23	0.9126
26.	OC_6H_5	3.56	10.70[b]	1.3550
27.	$CH_2CH(CH_3)_2$	3.60[a]	10.30	0.9797
28.	cyclopentyl	3.63[a]	9.92	1.2916
29.	$CH_2C_6H_5$	3.69	10.19[b]	1.1946
30.	$CH_2C(CH_3)_3$	4.03[a]	10.50	1.2326

[a] estimated by CLOG P-3.2 program
[b] calculated by the Perrin method
[c] the log of the inverse of the IGC_{50} value in mmol/L

Most industrial organics exhibit narcosis as their mechanism of toxic action. Narcosis represents baseline toxicity and is simply the reversible retardation of cytoplasmic activity due to the partitioning of the chemical into the biophase. Schultz and Moulton (1984) developed the linear narcosis model, log BR = 0.7230 (log K_{ow}) - 1.3459, for Tetrahymena growth impairment by the use of nitrogen aromatic molecules. Eq. (1) differs in both slope and intercept from this narcosis model.

Recently, Veith et al (1985) developed an acute lethal fish toxicity QSAR for industrial esters, log LC_{50} = -0.535 (log K_{ow}) - 2.75; n = 29 r^2 = 0.828, which differed in both slope and intercept from the original fish narcosis model. It is interesting that the slope of Eq. (1) in this study and the slope in Veith's ester model do not differ in absolute value, although they do differ in sign. The sign difference is due to the reciprocal transformation of the Tetrahymena toxicity data. Similarly the r^2 value of these two equations do not differ statistically. Veith and co-workers note that the predictability of their ester or polar-narcosis model may be improved by the addition of an orthogonal electronic parameter (Veith et al. 1985). The present investigation with phenols shows pKa to be such a parameter.

In conclusion the present study shows that the predictability of the polar-narcosis model of phenol toxicity can be improved with the addition of pKa as a second independent variable to a log K_{ow}-dependent equation.

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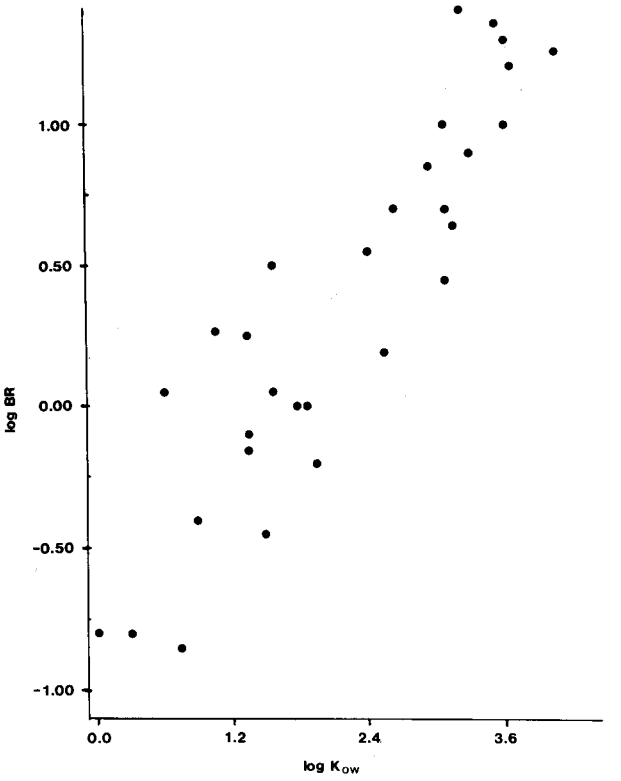

Figure 1. A scatter plot of log BR for Tetrahymena pyriformis versus log K_{ow} for para-substituted derivatives of phenols.

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