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Structure–Activity Relationships for Di and Tri Alkyl and/or Halogen Substituted Phenols

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There is increasing evidence that a separate quantitative structure-activity relationship (QSAR) can be developed for each mechanism of toxic action (McKim et al 1987a). Schultz et al (1986), in comparing the toxic response of the fathead minnow and *Tetrahymena* to a select set of phenols, described QSARs for two modes of action. One, polar narcosis or type II narcosis, is slightly more toxic than baseline nonpolar narcosis or narcosis I (Veith et al 1983). The second mechanism modeled by Schultz et al (1986), respiratory uncoupling or the uncoupling of oxidative phosphorylation, is even more toxic and named because two classic uncouplers, 2,4-dinitro phenol and pentachlorophenol model by this QSAR.

Recent studies in our laboratory with mono alkyl and halogen derivatives of phenol (Schultz and Cajina-Quezada 1987), have shown that the predictability of the 1-octanol/water partition coefficient (log K_{ow}) dependent model for polar narcotics can be improved by the addition of an electronic or ionization parameter, such as pK_a, as a second molecular descriptor. These findings are consistent with those of McLeese et al (1979) and Saarikoski and Viluksela (1982).

It was the purpose of the present study to determine the relative toxicity of a series of selected multiple alkyl- and/or halogen-substituted phenols, each representing the polar narcosis mode of action, and examine correlations between the log K_{ow} and pK_a parameters, and toxicity.

MATERIALS AND METHODS

Tetrahymena pyriformis under a static regime was the toxicity test system used (Schultz 1983). This sublethal assay uses population densities of axenic cultures as its end-point. The test compounds selected form a series of di or tri alkyl- and/or halogen-substituted phenols. This series was selected because it provides a representative sample (Table 1) of sufficiently pure (i.e., > 95%), commercially available phenolic derivatives having similar substituents. Additionally, toxicity of all these derivatives was thought to be via a single mode of action, polar narcosis.

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Table 1 Summary of structural types of phenols tested.

subst.	alkyl	halogen	mixed	total
di	6	6	4	16
tri	4	6	4	14
total	10	12	8	30

Each phenol was tested for a minimum of three replicates following range finding experiments. Each replicate was, at minimum, duplicates of a 5-step graded concentration series using freshly prepared stock solutions. Cultures without phenols served as controls. Cultures were reared in 250-mL Erlenmeyer flasks containing 50 mL of proteose-peptone medium. Following 48 hours of incubation, cell population levels were estimated spectrophotometrically as absorbance at 540 nm. Only replicates with control absorbance values of 0.6-0.9, equivalent to late log-growth-phase, were used in the analyses. The IGC_{50}, 50% growth inhibitory concentration, and 95% fiducial limits were determined for each phenol using probit analysis of Statistical Analysis Systems, SAS (SAS Institute Inc. 1985). In these analyses, Y was the absorbance normalized to percent control and X was the concentration of tested phenol in mg/L.

For structure-toxicity analyses, log BR (biological response), i.e., the log of the inverse of the IGC_{50} value in mM/L, was used as the dependent, standard measurement of toxicity and the log K_{ow} and pK_a terms were used as the independent molecular predictors. Log K_{ow} values were computer calculated by the fragment method, with the CLOGP version 3.34 program, or retrieved as measured values from the select list for comparison. The pK_a values were computer calculated (Hunter 1988). The General Linear Model routine for regression analysis from SAS was used to generate the QSARs, with model adequacy being measured as the coefficient of determination (r^2).

Chemical persistence studies were undertaken for each phenol with the aid of HPLC. The analyzed solutions consisted of the appropriate aliquot of stock, added to 50 mL of sterile distilled water in a foam stoppered 250 mL Erlenmeyer flask, to make the final concentration of the phenol approximately equal to that of its IGC_{50} value. For analysis, a Waters Model 840 HPLC (Milford, Massachusetts) with a C-18 reverse phase column, in conjunction with a Waters Model 600 Multisolvent Delivery System and a Model 712 Waters Intelligent Sample Processor, were used. The phenols were eluted using a degassed 65/45 mixture of methanol and 0.5 M ammonium acetate buffer adjusted to a pH of 6.7. The solvent flow rate was set at 1.0 mL/min. The absorbance detector, set at 254 nm, recorded directly onto a Digital Professional 350 microprocessor (Maynard, Massachusetts). At t = 0 hr, a 10-20 μL aliquot was injected into the HPLC and eluted for 4 to 10 min depending on the derivative. Peaks were integrated using Waters Expert Software. The sample-containing flasks were then placed under the same environmental conditions as those for the bioassay. At t = 48 hr, the test solutions were again injected onto the HPLC and analyzed using the same method. Percent loss was measured as the difference between the t = 0 and t =

48 concentration. Each test chemical was analyzed for persistence twice or, in the case of those showing a loss greater than 10%, three times.

RESULTS AND DISCUSSION

Table 2 summarizes the relative toxicity and molecular descriptor data for the tested phenols. Two compounds, 2,4,6-tri(tert)butylphenol and 2,4,6-triphenylphenol, did not elicit an IGC_{50} at saturation. In all other cases, the data fit the probit models extremely well, with $P < \chi^2$ being greater than 0.9, based on $n > 30$.

Table 2 Summary of toxicity and molecular descriptor data.

COMPOUND	CAS[a] Number	Log[b] BR	Log[c] K_{ow}	pK_a[d]
2,6-difluorophenol	6418-38-8	0.396	1.65	7.51
2,3-dimethylphenol	526-75-0	0.122	2.77	10.30
2,5-dimethylphenol	95-87-4	0.009	2.77	10.30
3,4-dimethylphenol	95-65-8	0.122	2.77	10.40
3,5-dimethylphenol	108-68-9	0.113	2.77	10.20
3-chloro-4-fluorophenol	2613-23-2	0.842	2.78	8.96
2-chloro-5-methylphenol	615-74-7	0.640	2.85	8.54
2-bromo-4-methylphenol	6627-55-0	0.789	2.91	8.67
2,5-dichlorophenol	583-78-8	1.128	3.07	7.58
2,3-dichlorophenol	576-24-9	1.271	3.07	7.58
4-chloro-2-methylphenol	1570-64-5	0.700	3.13	9.67
4-chloro-3-methylphenol	59-50-7	0.795	3.13	9.52
3,5-dichlorophenol	591-35-5	1.562	3.35	8.27
2,4-bromophenol	615-58-7	1.403	3.37	7.87
3,4,5-trimethylphenol	527-54-8	0.930	3.42	10.50
2,3,5-trimethylphenol	697-82-5	0.360	3.33	10.48
2,4,6-trichlorophenol	88-06-2	1.695	3.69	7.04
4-chloro-3,5-dimethylphenol	88-04-0	1.203	3.78	9.65
4-bromo-2,6-dichlorophenol	697-86-9	1.779	3.84	6.75
2,4,5-trichlorophenol	95-95-4	2.100	3.85	7.04
4-bromo-6-chloro-2-methylphenol	7530-27-0	1.277	3.87	8.20
4-bromo-2,6-dimethylphenol	2374-05-2	1.278	3.93	10.00
2,4,6-tribromophenol	118-79-6	2.050	4.02	6.31
2-(tert)butyl-4-methylphenol	2409-55-4	1.297	4.10	11.40
4-chloro-2-isopropyl-5-methylphenol	89-68-9	1.862	4.71	10.00
6-(tert)butyl-2,4-dimethylphenol	1879-09-0	1.245	4.75	11.70
2,6-diphenylphenol	2432-11-3	2.113	5.25	9.92
2,6-di(tert)butyl-4-methylphenol	128-37-0	1.788	6.08	12.60
2,4,6-triphenylphenol	3140-01-0	no effect	7.36	12.70
2,4,6-tri(tert)butylphenol	732-26-3	no effect	7.42	12.60

[a] Chemical Abstract Services registry number

[b] log of the inverse of the IGC_{50} value in mM/L

[c] from CLOGP version 3.34

[d] from Hunter (1988)

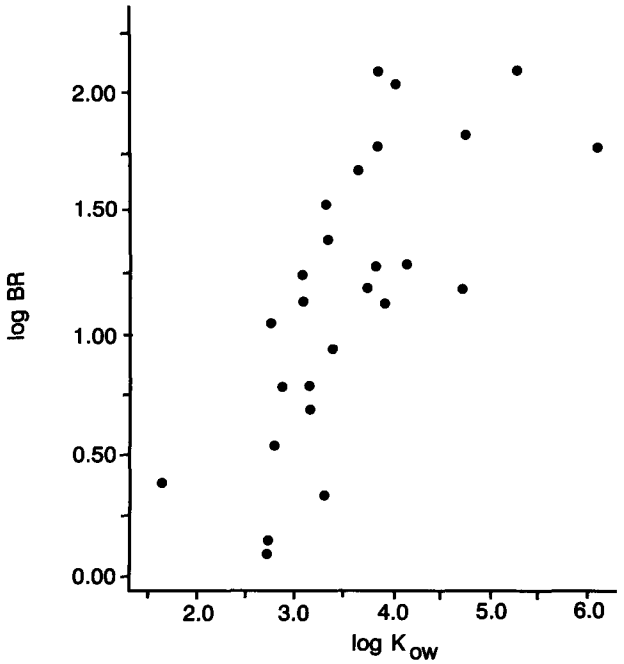

Figure 1 A scatter plot of log BR vs log K_{ow}. Two hidden values.

A scatter plot of log BR vs log K_{ow} for all tested phenols is shown in Figure 1. Regression analysis of these data yielded the QSAR:

$$\log BR = 0.5085 (\log K_{ow}) - 0.6936$$

$$n = 28, r^2 = 0.517, s = 0.448, f = 27.78 \quad [1].$$

In this first QSAR, log K_{ow} was a highly significant predictor of toxicity ($P > f = 0.0001$; *df* 1,26). Analysis of residual values revealed that their distribution was not significantly different from normal.

Regression analysis of log BR vs log K_{ow} and pK_a for all tested phenols gave the QSAR:

$$\log BR = 0.6791 (\log K_{ow}) - 0.2632 (pK_a) + 1.1183$$

$$n = 28, r^2 = 0.903, s = 0.205, f = 116.59 \quad [2].$$

In this second QSAR, both log K_{ow} and pK_a were highly significant descriptors ($P > f = 0.0001$; *df* 2,25).

Table 3 Summary of 48-hr abiotic loss data.

Compound	Percent Loss + std. dev.
2,6-difluorophenol	4.4 ± 3.2
2,3-dimethylphenol	0.8 ± 0.8
2,5-dimethylphenol	-0-
3,4-dimethylphenol	-0-
3,5-dimethylphenol	1.7 ± 0.8
3-chloro-4-fluorophenol	1.4 ± 1.0
2-chloro-5-methylphenol	30.8 ± 5.1
2-bromo-4-methylphenol	21.3 ± 3.6
2,5-dichlorophenol	13.9 ± 3.2
2,3-dichlorophenol	0.8 ± 0.8
4-chloro-2-methylphenol	1.6 ± 1.8
4-chloro-3-methylphenol	4.6 ± 4.6
3,5-dichlorophenol	-0-
2,4-bromophenol	2.6 ± 2.6
3,4,5-trimethylphenol	1.0 ± 1.0
2,3,5-trimethylphenol	3.2 ± 1.3
2,4,6-trichlorophenol	34.2 ± 3.0
4-chloro-3,5-dimethylphenol	2.1 ± 2.1
4-bromo-2,6-dichlorophenol	4.3 ± 4.3
2,4,5-trichlorophenol	-0-
4-bromo-6-chloro-2-methylphenol	33.3 ± 2.6
4-bromo-2,6-dimethylphenol	3.4 ± 0.1
2,4,6-tribromophenol	-0-
2-(tert)butyl-4-methylphenol	1.2 ± 1.2
4-chloro-2-isopropyl-5-methylphenol	2.2 ± 0.5
6-(tert)butyl-2,4-dimethylphenol	-0-
2,6-diphenylphenol	0.6 ± 0.6
2,6-di(tert)butyl-4-methylphenol	-0-
2,4,6-triphenylphenol	NA[a]
2,4,6-tri(tert)butylphenol	NA

[a] NA, Not assayed

Chemical persistence data is shown in Table 3. Typically abiotic loss was less than 10% over 48 h. However, in a few cases, the 2-chloro-5-methyl, 2-chloro-4-methyl, 2,3-dichloro, and 2,5-dichloro derivatives, greater losses were observed. Nevertheless, abiotic loss did not appear to be correlated with Eq. [2] residual values. The relative abiotic loss of the tested phenols is not considered to have affected the toxicity test results and, therefore, the QSAR modeling.

The studies of McLeese et al (1979) and Saarikoski and Viluksela (1982) were among the first to show that the addition of pK_a as an additional molecular descriptor can improve the predictability of the $\log K_{ow}$-dependent QSAR for phenols. Lipnick and co-workers (1986), noted that the predicted toxicity of phenols, which are poorly ionized under test conditions, was not significantly

altered by QSARs not including pK_a as a descriptor. Recent investigations in our laboratory (Schultz and Cajina-Quezada 1987; Schultz 1987), with mono alkyl or halogen substituted phenols and a heterogeneous series of para-substituted phenols, respectively, have demonstrated that the predictability of the log K_{ow} -dependent QSAR can be sharply enhanced by the addition of pK_a as a second independent variable. The result of the present investigation, which included a limited number and type of polysubstituted derivatives, is in agreement with these previously noted studies. In fact, combining the data from each of these three studies and subsequent regression analysis resulted in the QSAR:

$$\log BR = 0.6038 (\log K_{ow}) - 0.2302 (pK_a) + 1.1784$$

$$n = 75,\ r^2 = 0.903,\ s = 0.220,\ F = 329.15 \qquad [3].$$

In Eq. [3] both log K_{ow} and pK_a were highly significant descriptors ($P > f = 0.0001$; df 2,71).

The QSAR presented in Eq. [2] is thought to model the polar narcosis or type II narcosis (Veith and Broderius 1987) mechanism of action. Ferguson (1939) used the term "polar" narcotic to distinguish the more toxic narcotics, which are more water soluble, from the more traditional nonpolar narcosis or type I narcosis. McKim and co-workers have defined mechanisms of toxic action by fish acute toxicity syndromes or FATS (McKim et al 1987a). These FATS were toxic response sets for respiratory-cardiovascular parameters measured in spinally transected rainbow trout. A FATS has been defined for both the nonpolar narcosis and respiratory uncoupling mode of action (McKim et al 1987b). More recently, Bradbury et al (1989) have expanded this work and identified a FATS for polar narcotics including 2,4-dimethylphenol. The development of severe seizures was the most striking response associated with polar narcotics (Bradbury et al 1989). While there are obvious phylogenetic differences between the single cell protozoan, *Tetrahymena*, and the multicellular fish, it is interesting that both systems similarly model several different modes of toxic action. This suggests that the biochemical bases of toxicity, at least for the more common modes of action, may be universal to eucaryotic systems.

In conclusion, the present study showed that relative toxicity (log BR), monitored as 48-hr cell population growth in the *Tetrahymena* test system, of a series of 30 di and tri alkylated and/or halogenated phenols can be accurately modeled by log K_{ow} and pK_a. This is consistent with earlier findings using monosubstituted phenols. Each of the phenols examined in the present study is thought to elicit a toxic response via the polar narcosis mode of action.

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