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Comparative Toxicity and Structure-Activity in *Chlorella* and *Tetrahymena:* Monosubstituted Phenols

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Previous comparative toxicity and structure-activity studies of phenols have been limited, for the most part, to chloro-derivatives (Moulton and Schultz 1986) or have used fish mortality and ciliate growth impairment data (Schultz et al. 1986). While the former allowed for a comparison between a variety of test systems, the latter allowed for a comparison of a larger and more diverse data set. Quantitative structure-activity studies have repeatedly shown that toxicity is related to 1-octanol/water partition coefficient (log K_{OW}) and correlation is maintained within the same mode of toxic action. For chemicals which are polar and aromatic yet nonionic and nonreactive in nature, the mode of action is polar narcosis (Schultz et al. 1986; Veith and Broderius 1987). Polar narcosis has been characterized as a fish acute toxicity syndrome (FATS), a series of measured cardiovascular-respiratory and biochemical responses (Bradbury et al. 1989).

The research of Könemann and Musch (1981) as well as Saarikoski and Viluksela (1982) pointed out the importance of electronic/dissociation parameter in explaining the relative toxicity of phenols that are partially ionized at physiological pH. Lipnick and co-workers (1986), as well as Schultz (1987), and Schultz and others (1987) have reaffirmed the relevance of using Hammett electronic substituent constant (σ) or the dissociation constant (pK_a) in conjugation with log K_{OW} in modeling toxicity of polar narcotic phenols.

The relative toxicity of selected monosubstituted phenols has been assessed by Kramer and Trümper (1986) in the *Chlorella vulgaris* assay. They examined population growth inhibition of this simple green algae under short-term static conditions for 33 derivatives. However, efforts to develop a strong predictive quantitative structure-activity relationship (QSAR) met with limited success because they modeled across modes of toxic action or segregated derivatives such as positional isomers (i.e., ortho-, meta-, para-).

In an effort to further our understanding of the relationships of ecotoxic effects of phenols, we have evaluated the same derivatives reported by Kramer and Trümper (1986) in the *Tetrahymena pyriformis* population growth assay (Schultz 1983), compared the responses in both systems and developed QSARs for the *Chlorella vulgaris* data based on mechanisms of action.

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MATERIALS AND METHODS

Two sets of toxicity data were examined. One set came from Kramer and Trümper (1986). These data were collected for the autotrophic *Chlorella vulgaris* population growth inhibition assay developed by Böhm (1973). The biological response was defined as the inverse of the 50% *Chlorella* growth inhibitory concentration (CGI_{50}). This endpoint, static, axenic culture population density following 6-h exposure, was measured spectrophotometrically by absorbance at 680 nm and 750 nm. The second data set was collected using the heterotrophic *Tetrahymena pyriformis* population growth impairment assay which has recently been reviewed by Schultz et al. (1990). This test uses as its endpoint axenic population densities following 48-h static exposure measured spectrophotometrically by absorbance at 540 nm. The inverse of 50% *Tetrahymena* growth inhibitory concentration (TGI_{50}) described the biological response.

Log K_{ow} values were secured from CLOGP version 3.34, a software based on the paper of Leo et al. (1975). The Hammett sigma values (σ) were secured from the tabulations of Hansch and Leo (1979). The pK_a values were calculated using the computer assisted version (Hunter 1988) of the Perrin et al. (1981) method. QSARs were developed by simple and multiple regression of SAS software on an IBM 3081 computer.

RESULTS AND DISCUSSION

Table 1 is a summary of the data used in the analyses. Because results from *Chlorella* assay for the two series, 750 nm and 680 nm, were collinear [log CGI_{50} at 680 nm = 0.911 (log CGI_{50} at 750 nm) + 0.406; n = 33, r^2 = 0.946, s = 0.129, f = 572.49, Pr > f = 0.0001], we examined only one, 680 nm.

For comparative study, three derivatives, the 2-, 3- and 4-COOH, were not included because the buffering capacity of the *Tetrahymena* medium lessened their toxic response (Schultz et al. 1990). Linear regression analysis of CGI_{50} and TGI_{50} yielded the model,

$$\log CGI_{50} = 0.891 (\log TGI_{50}) + 5.522 \qquad (1)$$

n = 30, r^2 = 0.771, s = 0.283, f = 94.45, Pr > f = 0.0001.

Examination of the residual values revealed the 2-nitro derivative to be a statistical outlier, residing outside the upper 95% confidence level. This is inconsistent with the results of Gaur and Beevers (1959) who showed that the toxicity of chloro- bromo- and nitro-substituted phenols in plants is substitution position dependent, the order of effectiveness being para > meta > ortho. For the remaining chloro-, bromo- and nitrophenols the order of effectiveness is maintained. Removal of 2-nitrophenol and reevaluation yielded,

$$\log CGI_{50} = 0.856 (\log TGI_{50}) + 5.397 \qquad (2)$$

n = 29, r^2 = 0.842, s = 0.219, f = 144.35, Pr > f = 0.0001.

For structure-toxicity relationship in the *Chlorella* part of the data, the proelectrophiles, the 2-NO_2, 4-NO_2, 2-OH and 4-OH derivatives (Roberts 1987) were not included. Based on the remaining 29 derivatives, all thought to act as polar narcotics, the following results were obtained from linear regression of log CGI_{50} versus log K_{ow},

Table 1. Toxicity and Molecular Descriptor Data for Selected Phenols.

Derivative	CAS [a] No.	log [b] CGI_{50}	log [c] CGI_{50}	log [d] TGI_{50}	log [e] K_{ow}	pK_a [f]	σ [g]
H	108-95-2	2.30	2.17	-3.43	1.48	9.92	0.00
2-CH_3	95-48-7	2.60	2.65	-3.27	2.12	10.20	-0.17
2-CH_2-CH=CH_2	1745-81-9	3.23	3.10	-2.65	2.65	9.92	-0.07
2-$C(CH_3)_3$	88-18-6	3.85	3.75	-1.76	3.45	11.10	-0.20
2-F	367-12-4	2.65	2.50	-2.72	1.65	8.72	0.06
2-Cl	95-57-8	3.12	3.00	-2.72	2.20	8.40	0.23
2-Br	95-56-7	3.21	3.07	-2.50	2.35	8.36	0.23
2-NO_2	88-75-5	4.40	4.60	-2.33	1.85	6.80	0.78
2-$COCH_3$	18-93-4	3.11	3.09	-2.92	2.08	9.92	0.50
2-OH	120-80-9	3.35	2.91	-2.25	0.81	9.62	-0.37
2-OCH_3	90-05-1	2.37	2.22	-3.46	1.59	9.92	-0.27
2-OC_2H_5	94-71-3	2.80	2.72	-3.34	1.85	10.10	-0.24
2-COOH	69-72-7	3.13	3.00	---	2.19	2.98	0.45
3-CH_3	108-39-4	2.61	2.47	-3.06	2.12	10.10	-0.07
3-F	372-20-3	3.16	3.12	-2.53	1.91	9.16	0.34
3-Cl	108-43-0	3.60	3.45	-2.04	2.48	9.09	0.34
3-Br	591-20-8	3.57	3.62	-1.94	2.63	9.05	0.39
3-NO_2	554-84-7	3.70	3.73	-2.49	1.85	8.25	0.71
3-CHO	100-83-4	3.05	2.88	-2.92	1.44	9.12	0.35
3-OH	108-46-3	2.26	2.12	-3.65	0.81	9.33	0.12
3-OCH_3	150-19-6	2.42	2.11	-3.14	1.57	9.67	0.12
3-COOH	99-06-9	2.82	2.68	---	1.56	4.07	0.37
4-CH_3	106-44-5	2.78	2.50	-3.19	1.94	10.23	-0.17
4-$C(CH_3)_3$	98-54-4	3.83	3.65	-2.09	3.45	10.30	-0.20
4-F	371-41-5	2.99	2.82	-2.98	1.91	9.79	0.06
4-Cl	106-48-9	3.62	3.30	-2.46	2.48	9.38	0.23
4-Br	106-41-2	3.57	3.23	-2.32	2.63	9.45	0.23
4-I	540-38-5	3.86	3.42	-2.15	2.89	9.45	0.18
4-NO_2	100-02-7	4.30	4.30	-1.07	1.85	7.15	0.78
4-CHO	123-08-8	2.99	3.00	-2.73	1.44	7.62	0.42
4-OH	123-31-9	2.79	2.53	-2.52	0.81	9.55	-0.37
4-OCH_3	150-76-5	2.52	2.36	-3.14	1.57	10.20	-0.27
4-COOH	99-96-7	2.79	2.63	---	1.56	4.58	0.45

[a] Chemical Abstract Service number
[b] 50% *Chlorella* growth inhibition at 680 nm
[c] 50% *Chlorella* growth inhibition at 750 nm
[d] 50% *Tetrahymena* growth inhibition at 540 nm
[e] 1-octanol/water partition coefficient
[f] ionization constant
[g] Hammett substituent constant

$\log CGI_{50} = 0.661 (\log K_{ow}) + 1.689$ (3)

$n = 30, r^2 = 0.664, s = 0.289, f = 53.33, Pr > f = 0.0001.$

Previous QSAR studies with phenols and the *Tetrahymena* system had shown that the addition of σ (Schultz et al. 1978) or pK_a (Schultz 1987) as a second molecular descriptor improved the predictability of the log K_{ow} dependent QSAR. Multiple regression analysis of log CGI_{50} versus log K_{ow} and σ yields,

$\log CGI_{50} = 0.718 (\log K_{ow}) - 0.836 (\sigma) + 1.451$ (4)

$n = 29, r^2 = 0.870, s = 0.183, f = 87.24, Pr > f = 0.0001$ for log K_{ow} and σ.

Examination of the residual values revealed neither statistical nor visual outliers.

Multiple regression analysis of log CGI_{50} versus log K_{ow} and pK_a for the same group of compounds yields,

$\log CGI_{50} = 0.699 (\log K_{ow}) - 0.047\ pK_a + 2.033$ (5)

$n = 30, r^2 = 0.696, s = 0.281, f = 29.69, Pr > f = 0.0001$ for log K_{ow}, $Pr > f = 0.1124$ for pK_a.

Moulton and Schultz (1986) in describing the comparative toxicity of series of chlorophenols noted that if in different systems slope of log toxicity versus the same descriptor does not significantly differ, the linear regression of log toxicity in one system against log toxicity in the other system should yield a line with slope of about 1 and high r^2. The result obtained in Eq. (2) is consistent with this idea and indicates that the toxicity in the *Chlorella* system can be used to predict the toxicity in the *Tetrahymena* system. While this relationship appears to exist for monosubstituted phenols, caution must be taken in extrapolating this finding to other chemicals, especially those which undergo abiotic alterations or are metabolized in one of the systems.

The term polar narcosis reflects the physical reversible anesthetic phenomenon of chemicals which are aromatic and polar in nature. Recently Bradbury et al. (1989) have defined a FATS for polar narcotics. Aware of potential species differences, but encouraged by the correlation in toxic response between *Chlorella* and *Tetrahymena* for such compounds, we used the term polar narcosis to describe the mode of action for the QSARs developed in Eqs. (3-5).

Limiting our investigation to chemicals which elicit the same mode of action, polar narcosis, we successfully developed a strong predictive QSAR (see Eq. 4). This investigation confirmed the importance of using an electronic parameter in modeling biological activity of phenols, especially those which are partially ionized at physiological pH. However, it is worth noting that pK_a is not a significant descriptor with these data although it was in previous studies (Schultz et al. 1991). Since log K_{ow} and pK_a are not correlated, the possibility of the insignificance of the second parameter due to correlation with the first was excluded (Moulton 1988). However, if data consist of compounds not physicochemically diverse then there is a possibility that parameters selected as descriptors of toxicity will vary little. Such parameters are often determined not to be significant. While this study was limited to the data of Kramer and Trümper (1986), it was initially felt that there was physicochemical diversity, but in the group of derivatives tested, with exceptions, pK_a values varied by only 20%. In comparison, in the

investigation of Schultz et al. (1991) pK_a values varied more than 50% and pK_a was a highly significant descriptor. On the other hand σ, which is linearly correlated with pK_a, varied in the present data set by 150%. We feel that pK_a values were not varied enough for it to be a significant descriptor, whereas σ was a significant descriptor simply because it varied over a much larger range. We feel the improvement in predictability noted in Eq. (4) over Eq. (3) is due to the fact that σ corrects log K_{ow} for the degree of ionization.

REFERENCES

Böhm H (1973) Das Chlorella - Testsystem. Wiss Hefte Päd Inst Köthen 2:9-11

Bradbury SP, Henry TR, Niemi GJ, Carlson RW, Snarski VM (1989), Use of respiratory-cardiovascular response of rainbow trout (*Salmo gairdneri*) in indentifying acute toxicity syndromes in fish. Part 3. polar narcosis. Environ Toxicol Chem 8:247-262

Gaur, BK, Beevers H (1959) Respiratory and associated responses of carrot discs to substituted phenols. Pl Physiol 34:427-432

Hansch C, Leo A (1979) Substituent constants for correlations in chemistry and biology. Wiley Interscience, New York, p 339

Hunter R (1988) Computer calculation of pKa. In: Turner JE, Williams MW, Schultz TW, Kwaak NJ (eds) QSAR 88 Proc 3rd International Workshop on Quantitative Structure-Activity Relationships in Environmental Toxicology (CONF- 880520- -DE88013180) NTIS, Springfield, Virginia pp 33-42

Leo A, Jow P, Silipo C, Hansch C (1975) Calculation of hydrophobic constant (log P) from π and f constants. J Med Chem 18:865-868

Moulton M, Schultz TW (1986) Comparison of several structure-toxicity relationships for chlorophenols. Aquat Toxicol 8:121-128

Moulton M (1988) Regression analysis in QSAR: Potential pitfalls in modeling polar narcotic phenols. In: Turner JE, Williams MW, Schultz TW, Kwaak NJ (eds) QSAR 88 Proc 3rd International Workshop on Quantitative Structure-Activity Relationships in Environmental Toxicology (CONF- 880520- -DE88013180) NTIS, Springfield, Virginia pp 1-11

Könemann H, Musch A (1981) Quantitative structure-toxicity relationships in fish toxicity study Part 2: Influence of pH on the QSAR of chlorophenols. Toxicology 19:223-228

Kramer C, Trümper L (1986) QSARs for the autotrophic growth inhibition of synchronous *Chlorella vulgaris* suspensions by monosubstituted phenols. Biochem Physiol Pflanzen 181:81-89

Lipnick RL, Bickings CK, Johnson DE, Eastmond DA (1986) Comparison of QSAR predictions with fish toxicity screening data for 110 phenols. In: Bahuer RC, Hansen DJ (eds) Aquatic Toxicology and Hazard Assessment: 8th Symposium, ASTM STP-891. Amer Soc Testing & Materials, Philadelphia, pp 153-176

Perrin DD, Dempsey B, Sergeant EP (1981) pKa predictions for organic acids and bases, Chapman and Hall, London, p 146

Roberts DW (1987) An analysis of published data on fish toxicity of nitrobenzene and aniline derivatives. In: Kaiser KLE (ed) QSAR in Environmental Toxicity - II, Reidel, Dortrecht, Holland, pp 295-308

Saarikoski J, Viluksela M (1982) Relation between physiochemical properties of phenols and their toxicity and accumulation in fish. Ecotox Environ Saf 6:501-512

Schultz TW (1983) Aquatic toxicology of nitrogen heterocyclic molecules: Quantitative structure-activity relationships. In: Nriagu JO (ed) Aquatic Toxicology, John Wiley and Sons, New York, pp. 579-612

Schultz TW (1987) Relative toxicity of parasubstituted phenols: log K_{ow} and pK_a dependent structure-activity relationships. Bull Environ Contam Toxicol 38:994-999

Schultz TW, Holcombe GW, Phipps GL (1986) Relationships of quantitative structure-activity of selected phenols in the *Pimephales promelas* and *Tetrahymena pyriformis* test systems. Ecotox Environ Saf 12:146-153

Schultz TW, Lin DT, Wesley SK (1991) QSARs for monosubstituted phenols and the polar narcosis mechanism of toxicity. Quant Struct-Act Relat (in press)

Schultz TW, Lin DT, Wilke TS, Arnold LM (1990) Quantitative structure-activity relationships for the *Tetrahymena pyriformis* population growth endpoint: A mechanism of action approach In: Karcher W, Devillers J (eds) Practical Applications of Quantitative Structure-Activity Relationships (QSAR) in Environmental Chemisrty and Toxicology, Kluwer Academic Publishers, Dordrecht, Holland, pp 241-262

Schultz TW, Riggin GW, Wesley SK (1987) Structure-activity relationships for para-substituted phenols. In: Kaiser KLE (ed) QSAR in Environmental Toxicity - II, Reidel, Dortrecht, Holland, pp 333-346

Veith GD, Broderius SJ (1987) Structure-toxicity relationships for industrial chemicals causing type (II) narcosis syndrome. In: Kaiser KLE (ed) QSAR in Environmental Toxicity - II, Reidel, Dortrecht, Holland, pp 385-391

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