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Structure–Toxicity Relationships for Mono Alkyl- or Halogen-Substituted Anilines

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Recent quantitative structure activity relationship (QSAR) investigations suggest that for each mechanism of toxic action there is an individual 1-octanol/water partition coefficient (log K_{ow})-dependent model. Konemann (1981) and Veith et al. (1983) have shown that narcosis, the reversible, non-specific arresting of general cytoplasmic activity, is caused by a myriad of chemicals which have in common the fact that they are both nonreactive and nonpolar. Recently, Schultz et al. (1986) working with a heterogenous series of phenols have demonstrated two other log K_{ow}-dependent QSAR. One for polar narcotic chemicals (narcosis II) and the other for uncouplers of oxidative phosphorylation. More recently Veith and Broderius (1987) have elaborated on the idea of polar narcosis and have shown that selected anilines elicit their toxic response via this mechanism. In the present investigation we have determined the relative toxicity of a series of mono alkyl- or halogen-substituted anilines using the 48-h *Tetrahymena* population growth inhibition test and explored QSAR.

MATERIALS AND METHODS

Tetrahymena pyriformis strain GL-C under static conditions was the assay used. This test system, which uses population density of axenic cultures as its end-point, has been described by Schultz (1983). Each aniline derivative 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. *Tetrahymena* cultures without test aniline acted as controls. Cell population levels were estimated spectrophotometrically as absorbance at 540 nm. Only replicates with control absorbances of 0.6 to 0.9 were used in the analyses. The 50 percent inhibitory growth concentration (IGC_{50}) and 95 percent fiducial limits were determined for each test compound using probit analysis of the Statistical Analysis System (SAS) software. In these analyses, Y was the absorbance normalized to percent control, X was the concentration of tested compound in mg/L, n was > 30 and $P < \chi^2 < 0.9$.

The compounds tested were ortho-, meta- or para-position alkyl or halogen derivatives of aniline. Each was purchased commercially (Aldrich Chemical Co., Milwaukee, Wisconsin, USA) in sufficient purity (i.e., 95% or better) for direct testing. Individual stock solutions were prepared in dimethylsulfoxide (DMSO). In all cases, the volume of stock solution added to the culture medium was

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limited so DMSO did not exceed a final concentration of 0.75 percent, a level which does not affect *Tetrahymena* growth (Schultz and Cajina-Quezada 1982).

For QSAR determination, the log BR (Biological Response) was generated for each aniline by taking the log of the inverse of the IGC_{50} expressed in mM/L. Log K_{ow} values were calculated by the fragment method using the CLOGP version 3.34 software or retrieved as measured values listed for comparison. Mathematical models were generated using the general linear model of regression analysis of SAS with log BR as the dependent variable and log K_{ow} as the independent variable. Model adequacy was measured as the coefficient of determination.

Chemical persistence studies were undertaken for each aniline 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 so as to make the final concentration of the aniline approximately equal to that of its IGC_{50} value. For analysis a Waters Model 840 HPLC 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 anilines 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 and the absorbance detector set at 254 nm and recorded directly onto a Digital Professional 350 microprocessor. At t = 0 h a 10-20 μL aliquot was injected into the HPLC and eluted for 10 min. 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 h, 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 aqueous persistence twice or, in the case of those showing a loss greater than 10 percent, three times.

RESULTS AND DISCUSSION

Table 1 is a compilation of the Chemical Abstract Service registry number (i.e., CAS number), toxicity (i.e., log BR) and hydrophobicity (i.e., log K_{ow}) of each aniline used in these analyses. One compound, 4-decylaniline, did not elicite the measured response at saturation.

Regression analysis of log BR versus log K_{ow} results in Eq. [1].

$$\log BR = 0.6019 (\log K_{ow}) - 0.7451 \qquad [1]$$
$$n = 26;\ r^2 = 0.630;\ s = 0.450$$

For Eq. [1] log K_{ow} is a highly significant descriptor ($P > F = 0.0001$; 1,24). One derivative, 4-fluoro, is a statistical outlier having its toxic response fall outside the upper 95% confidence limit of the model. In addition three other compounds, the 4-chloro, 4-bromo and 4-iodo derivatives, are visual outliers. Each derivative having its toxic response greater than estimated by Eq. [1].

Deletion of the statistical outlier and subsequent regression analysis results in Eq. [2].

$$\log BR = 0.6599 (\log K_{ow}) - 0.9212 \qquad [2]$$
$$n = 25;\ r^2 = 0.736;\ s = 0.384$$

Table 1. Log K_{ow} and biological response (log BR) in the 48 hour *Tetrahymena* population growth assay for select anilines

Derivative	CAS number	log BR	log K_{ow}
Aniline	62-53-3	0.241	0.90
4-methyl	106-49-0	-0.023	1.39
4-ethyl	589-16-2	0.044	1.96
4-propyl	2696-84-6	0.493	2.49
4-butyl	104-13-2	1.045	3.15
4-hexyl	33228-45-4	2.039	4.21
4-octyl	16245-79-7	2.432	5.27
4-decyl	37529-30-9	no effect at saturation	6.33
4-phenyl	92-67-1	0.951	2.80
4-fluoro	371-40-4	1.102	1.15
4-chloro	106-47-8	1.372	1.83
4-bromo	106-40-1	1.191	2.26
4-iodo	540-37-4	1.335	2.34
3-methyl	108-44-1	-0.159	1.40
3-ethyl	587-02-0	-0.124	2.09
3-phenyl	2443-47-2	0.775	2.80
3-fluoro	372-19-0	0.180	1.30
3-chloro	108-42-9	0.297	1.88
3-bromo	591-19-5	0.517	2.10
3-iodo	626-01-7	0.350	2.32
2-methyl	95-53-4	-0.159	1.32
2-ethyl	578-54-1	-0.254	1.74
2-phenyl	90-41-5	0.862	2.84
2-fluoro	348-54-9	-0.313	1.26
2-chloro	95-51-2	-0.086	1.90
2-bromo	615-36-1	0.458	2.11
2-iodo	615-43-0	0.91	2.32

For Eq. [2] the 4-chloro derivative is a statistical outlier. Its deletion followed by a reanalysis of the remaining data results in Eq. [3].

$$\log BR = 0.6815 (\log K_{ow}) - 1.0150 \qquad [3]$$
$$n = 24;\ r^2 = 0.825;\ s = 0.313$$

For Eq. [3] the 4-bromo and 4-iodo derivatives are statistical outliers. Their deletion followed by the reanalysis of the remaining data yields Eq. [4].

$$\log BR = 0.6781 (\log K_{ow}) - 1.0721 \qquad [4]$$
$$n = 22;\ r^2 = 0.904;\ s = 0.229$$

The observed toxicity of all 22 derivatives included in Eq. [4] lies within its 95% confidence limits.

The results of the chemical persistence evaluations are presented in Table 2. For both the alkyl and halogen series abiotic loss is inversely related to molecular weight of the substituent. Typically losses are less than 8%, however, greater losses are observed for the ortho-position halogen derivatives. In any case the loss of test aniline is not considered to have affected toxicity test results.

Eq. [4] in the present investigation is similar to Eq. [5], log BR = 0.5898 (log K_{ow}) - 1.0323, from the study by Schultz et al. (1986) which models polar narcotic phenolic compounds assayed in the *Tetrahymena* system. This likeness is not unexpected since the amino group present in anilines is a strong electron donating moiety as is the hydroxy group of phenols. Veith and Broderius (1987) developed a QSAR for polar industrial chemicals (i.e., anilines and phenols) which cause the type (II) narcosis syndrome (i.e., polar narcosis) in fish. Their partition coefficient-dependent model is also in good agreement with Eq. [4] of the present investigation. The slopes of these two relationships, while different in sign because of the reciprocal transformation of the *Tetrahymena* data, are not significantly different in absolute value.

Recent efforts in this laboratory have included examining the use of pKa as a basis for selecting mechanisms of toxic action of phenols (Schultz 1987). Equation [1], log BR = 0.6128 (log K_{ow}) - 1.1297, of that study is not significantly different from Eq. [4] of the present study. Regression analysis of the aniline data used in Eq. [4] along with those data for the polar narcotic phenols evaluated by Schultz (1987) yields Eq. [5].

$$\log BR = 0.6291 (\text{Log } K_{ow}) - 1.043 \qquad [5]$$
$$n = 36;\ r^2 = 0.909;\ s = 0.237$$

In Eq. [5] log K_{ow} is a highly significant descriptor ($P > F = 0.0001$; 1, 34) and no compound is a statistical or visual outlier.

As previously noted the notion that a separate log K_{ow}-dependent QSAR exists for each mechanism of toxic action is gaining in acceptance. Similarly compounds eliciting their toxic response by the same mechanism of action are strictly additive in joint toxicity experiments (Broderius and Kahl 1985). Therefore, since aniline and phenol have strictly additive joint toxicity (Veith and Broderius 1987) and selected anilines and phenols can be modeled by the same QSAR (see Eq. [5]) it is reasonable to assume that these selected anilines and phenols elicite their toxic response by the same mechanism of action, polar narcosis.

Table 2. Chemical persistence results for select anilines

Derivative	Percentage Loss ± standard deviation
Aniline	11.3 ± 0.9
4-methyl	8.8 ± 0.2
4-ethyl	9.1 ± 1.3
4-propyl	3.7 ± 1.4
4-butyl	2.9 ± 0.2
4-hexyl	-0-
4-octyl	-0-
4-decyl	not tested
4-phenyl	-0-
4-fluoro	11.0 ± 1.1
4-chloro	7.4 ± 0.6
4-bromo	2.6 ± 0.6
4-iodo	1.4 ± 0.3
3-methyl	7.8 ± 2.4
3-ethyl	7.9 ± 0.7
3-phenyl	1.0 ± 1.0
3-fluoro	10.6 ± 1.4
3-chloro	7.7 ± 1.5
3-bromo	4.4 ± 1.6
3-iodo	1.2 ± 1.2
2-methyl	7.7 ± 1.8
2-ethyl	10.1 ± 0.8
2-phenyl	-0-
2-fluoro	21.0 ± 0.5
2-chloro	19.9 ± 2.5
2-bromo	14.1 ± 1.5
2-iodo	10.3 ± 1.3

While the specific reason for the enhanced observed toxicity of the 4-position halogen substituted anilines in the *Tetrahymena* system is beyond the scope of this paper, it is nevertheless interesting to note that this is not the case with fish. Veith and Broderius (1987) note that both 4-chloroaniline and 4-bromoaniline model well as polar narcotic chemicals.

The present investigation reveals that the toxic response of alkyl and 2- and 3-position halogen substituted anilines in the *Tetrahymena* system can be modeled by a log K_{ow}-dependent QSAR. This QSAR is the same as for select phenols and represents the polar narcosis mode of toxic action.

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