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Structure–Toxicity Relationships for Nonpolar Narcotics: A Comparison of Data from the Tetrahymena, Photobacterium and Pimephales Systems[1]

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Previous studies have demonstrated that there are several mechanisms of toxic action in aquatic species (McKim et al 1987; Bradbury et al 1989). Several of these mechanisms have been used as the basis for 1-octanol/water partition coefficient (log K_{OW}) dependent quantitative structure-activity relationships (QSAR). These have included nonpolar narcosis or type I narcosis (Könemann 1981; Veith et al 1983; Schultz and Moulton 1984; Schultz et al 1988), polar narcosis or type II narcosis (Schultz et al 1986; Veith and Broderius 1987; Schultz 1987; Schultz et al 1988) and uncoupling of oxidative phosphorylation or respiratory uncoupling (Schultz et al 1986; Cajina-Quezada 1988).

Veith et al (1983) noted that narcosis is the most common mode of toxic action for industrial organic chemicals. It is considered a reversible physical toxicity where equal potency is the result of equal molar concentration at the site of action. Overton (1901) in his classic monograph, stated the narcosis theory and noted that narcotic effects were directly related to oil/water partitioning. Nonpolar narcosis QSARs using fish lethality data have been developed for both *Poecilia reticulata* (Könemann 1981) and *Pimephales promelas* (Veith et al 1983).

Similar QSARs for *Tetrahymena pyriformis* growth impairment have also been elucidated (Schultz and Moulton 1984; Schultz et al 1988), however, the significance of the latter studies are limited by the fact that these investigations did not include known industrial nonpolar narcotics (e.g., aliphatic alcohols and ketones). Moreover, while data is available (Kaiser and Ribo 1988), no nonpolar narcosis QSAR has been developed for the *Photobacterium phosphoreum* bioluminecent inhibition endpoint (i.e., Microtox test).

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It was the purpose of this study: to determine the relative biological response in the *Tetrahymena* toxicity bioassay to each of a series of aliphatic alcohols and ketones, to develop the corresponding log K_{ow} dependent QSAR, and to develop similar QSARs from literature data for the *Pimephales* and *Photobacterium* systems and compare these QSARs.

MATERIALS AND METHODS

Toxicity testing was performed using *Tetrahymena pyriformis* under static conditions (Schultz 1983). This assay uses 48-h population densities of axenic cultures measured spectrophotometrically with absorbance at 540 nm as its endpoint. Each chemical was tested in duplicate for at least three replicates following range finding experiments. Each replicate was a five-step graded concentration series using freshly prepared stock solutions. Only replicates with control cultures in the late log-growth-phase (absorbance of 0.6 to 0.9) were used in these studies. The 50% growth inhibitory concentration, IGC_{50}, was determined for each chemical using Probit Analysis of SAS [Statistical AnalysisÉ System] (SAS Institute Inc. 1985) with Y being the absorbance normalized to percent control and X being the concentration of the toxicant.

The chemicals selected for testing form a series of aliphatic alcohols and ketones containing one to 13 carbon atoms. Each was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, and each had a purity of 95% or better.

For development of a QSAR, the log of the inverse of the IGC_{50} value in mM/L was used as a measurement of relative toxicity (Y) and log K_{ow} was used as a molecular descriptor (X). The hydrophobic constant, log K_{ow}, for each alcohol or ketone used in the analyses was retrieved from the recent review of Hansch et al (1989) as a measured or computer calculated value. The General Linear Model routine for simple regression analysis from SAS was used to generate the QSAR.

Chemical persistence studies were undertaken with the aid of gas chromatography (GC) (Veith et al 1983). The GC analyses were performed on a Hewlett-Packard model 5840A instrument (Palo Alto, California) equipped with an electron capture detector. Packing in the columns was 80-100 mesh Parapak Q. Chromatographic conditions were varied to optimize the analysis. Each test chemical was analyzed at t = 0 and t = 48-h and the percent change in peak area was taken as the abiotic loss.

For comparative purposes relative toxicity data for these chemicals, noted as the log inverse of the 50% effect level in mM/L, was collected for both the 5-min *Photobacterium* bioassay (Kaiser and Ribo 1988) and the 96-h *Pimephales* bioassay (Veith et al 1983). These data were used to similarly develop log K_{ow} dependent QSARs.

RESULTS AND DISCUSSION

A summary of the molecular descriptor and toxicity data used in this investigation is listed in Table 1. The log K_{ow} values were distributed

uniformly over the range of -0.66 to 5.51. *Tetrahymena* toxicity varied over five orders of magnitude. The data for each compound fit their respective regression models extremely well with the $p > \chi^2$ greater than 0.9, based on at least 30 data points.

Regression analysis of $1/\log ICG_{50}$ versus $\log K_{ow}$ resulted in Eq. [1] (Table 2). The coefficient of determination (r^2) for Eq. [1] was near one and no tested chemical was a statistical or visual outlier.

The GC analyses showed that the alcohols had minimal abiotic loss over the duration of the toxicity test. In contrast, the ketones had between 20 and 60% loss over 48-h. However, abiotic loss was not considered a factor in determining Eq. [1]. This assumption was based on the extremely high r^2 observed for this QSAR which used both the alcohol and ketone toxicity data.

We consider Eq. [1] to be the nonpolar narcosis QSAR in the *Tetrahymena* test system. This statement is based on the facts that it was generated with data for aliphatic alcohols and ketones, compounds included in the QSAR studies of both Könemann (1981) and Veith et al (1983), and includes 1-octanol, a model nonpolar narcotic, used in the fish acute toxicity syndrome (FATS) investigations of McKim and co-workers (McKim et al 1987). The slope of the *Tetrahymena* narcosis QSAR presented here in Eq. [1] is strikingly similar to that of the QSAR of Könemann (1981) (i.e., 0.871). The intercepts of these two QSARs are different. This represents a difference in experimental protocols.

Two previous QSAR studies using the *Tetrahymena* test system have attempted to model narcosis or nonspecific membrane perturbation. These were the investigations of Schultz and Moulton (1984) and Schultz et al (1988). In the former, alkyl substituted azaarenes, primary aromatic amines and nitroaromatics were modeled (Schultz and Moulton 1984). In the latter, para-positioned alkyl and halogen substituted pyridines, cyanobenzenes and nitrobenzenes were modeled (Schultz et al 1988). In both cases the slopes (0.826 and 0.822, respectively) were very similar to that for Eq. [1]; however, while the intercepts for the two previous investigations were similar (-1.633 and -1.789, respectively), they were less than that for Eq [1]. It is worth noting that while both of these earlier QSARs show excellent correlation between toxic response and $\log K_{ow}$, more recent investigations (Veith and Broderius 1987; Bradbury et al 1989), have shown that most anilines, like phenols, act as polar narcotics.

These results question whether Schultz and Moulton (1984) were modeling a single mode of toxic action.

Regression analysis of *Pimephales* toxicity ($\log LC_{50}^{-1}$) versus $\log K_{ow}$ for selected alcohols and ketones gave Eq. [2]. The r^2 for Eq. [2] was very high and no compound was an outlier.

Simple regression of *Photobacterium* toxicity ($\log EC_{50}^{-1}$) versus $\log K_{ow}$ for similar alcohols and ketones yielded Eq. [3]. The r^2 value for Eq. [3] was high and no chemical was an outlier.

Table 1. Log K_{ow} and toxicity data for selected aliphatic alcohols and ketones.

No.	Chemical	CAS number	Log K_{ow}[a]	1/Log ICG_{50}[c]	1/Log LC_{50}[d]	1/Log EC_{50}[e]
1.	methanol	67-56-1	-0.77	-2.77	-2.94	-3.42[f]
2.	ethanol	64-17-5	-0.31	-2.41	-2.51[f]	-2.98[f]
3.	acetone	67-64-1	-0.24	-2.31	-2.12[f]	-2.56[f]
4.	2-propanol	67-63-0	0.05	-1.99	-2.20[f]	-2.81[f]
5.	2-butanone	78-93-3	0.29	-1.84	-1.64	-1.85
6.	3-pentanone	96-22-0	0.84[b]	-1.53	-1.25	NT
7.	1-butanol	71-36-3	0.88	-1.52	-1.37	-1.97[f]
8.	1-pentanol	71-41-0	1.56	-1.12	NT	NT
9.	4-heptanone	123-19-3	1.98	-0.76	NT	NT
10.	1-hexanol	111-27-3	2.03	-0.47	0.02	0.40
11.	2-octanone	111-73-7	2.52[b]	-0.23	0.55	0.86
12.	1-heptanol	111-70-6	2.57	0.02	NT	0.90
13.	5-nonanone	502-56-7	3.06[b]	-0.01	0.66	NT
14.	1-octanol	111-87-5	3.15	0.50	0.98	1.32
15.	2-decanone	693-54-9	3.60[b]	0.50	1.50[f]	1.30
16.	1-nonanol	143-08-8	3.69[b]	0.77	1.40	NT
17.	1-decanol	112-30-1	4.23[b]	1.10	1.82	2.13[f]
18.	1-undecanol	112-42-5	4.77[b]	1.87	2.22	NT
19.	1-dodecanol	112-53-8	5.13	2.07 [h]	2.27	NT
20.	1-tridecanol	112-70-9	5.67[b]	2.28 [i]	NTAS	NT

a measured values from Hansch et al (1989)

b calculated value from Hansch et al (1989)

c 48-h mM/L *Tetrahymena*

d 96-h mM/L *Pimephales* from Veith et al (1983)

e 5-min mM/L *Photobacterium* from Kaiser and Ribo (1988)

f average value

g for 2-decanol

h due to the narrow activity range 1/log ICG_{50} was calculated as the mean of the maximum no effect level (1.15 mg/L) and the minimum total effect level (2.00 mg/L)

i due to the narrow activity range 1/log ICG_{50} was calculated as the mean of the maximum no effect level (0.5 mg/L) and the minimum total effect level (1.6 mg/L)

NT, not tested; NTAS, not toxic at saturation

Table 2. Linear regression analyses.

Equation	Dependent Variable	Independent Variable	Slope	Intercept	n	r^2	s	f
1	log ICG_{50}^{-1}	log K_{ow}	0.795	-2.169	20	0.992	0.143	2177.65
2	log LC_{50}^{-1}	log K_{ow}	0.918	-2.051	16	0.989	0.197	1253.29
3	log EC_{50}^{-1}	log K_{ow}	1.168	-2.476	12	0.969	0.379	310.20
4	log LC_{50}^{-1}	log ICG_{50}^{-1}	1.263	0.595	11	0.988	0.200	743.64
5	log EC_{50}^{-1}	log ICG_{50}^{-1}	1.501	0.690	11	0.976	0.337	367.36
6	log LC_{50}^{-1}	log EC_{50}^{-1}	0.830	0.004	11	0.986	0.218	621.95

An examination of Eqs. [1], [2] and [3] suggested minimal differences. Moulton and Schultz (1986) in describing comparative toxicities noted that if the slope of log toxicity versus log K_{ow} for a given set of toxicants in different test systems does not significantly differ, then a regression of the log toxicities should give a slope of one and a high r^2 value. This premise was evaluated by a series of simple regressions of the data for the eleven aliphatic alcohols and ketones common to all three data sets, Eqs. [4], [5] and [6] (Table 2). These equations have shown the toxic response in these three bioassays to be covariant. Eq. [4] was consistent with one previously made when compared with the toxicity of phenols eliciting either the polar narcosis or respiratory uncoupling mode of toxic action (Schultz et al 1986). An examination of the toxicity data in Table 1 revealed best agreement between the *Tetrahymena* and *Pimephales* systems for the low molecular weight (C1 to C3) chemicals. On the other hand, best agreement was observed between the *Pimephales* and *Photobacterium* systems for the higher molecular weight (C8 to C10) compounds.

While literature concerning the actual site and mechanism of action for nonpolar narcotics is extensive, this issue is unresolved. The lipid solubility theories (Miller 1985) state that narcosis is the result of the toxicant accumulating in the lipid portion of membranes. The protein binding theory (Franks and Leib 1978) states that narcosis is the result of toxicants binding to hydrophobic regions of membrane proteins. Despite obvious phylogenetic differences between test species and dramatic differences between test protocols, there was very good quantitative agreement between relative toxicities in the systems compared in this study. This finding is consistent with that of Hansch et al (1989) for saturated aliphatic alcohols. These results suggest that the specific site of action for nonpolar narcosis is hydrophobic in nature. Moreover, while these results do not nullify the protein binding theory, the parallels in toxic response over the diversity of bioassays is more consistent with the lipid solubility theories.

This investigation has formulated the log K_{ow} dependent nonpolar narcosis QSAR for both the *Tetrahymena* and *Photobacterium* bioassays. In addition, these results

have shown that the toxic responses to aliphatic alcohols and ketones in *Tetrahymena* covaries with those in *Pimephales* and *Photobacterium.*

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