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Structure-Toxicity Relationships for *Tetrahymena:* Aliphatic Aldehydes

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Previous studies have used partition coefficient dependent structure-toxicity relationships, as a basis of predicting toxic effects for a given mechanism of action. These have included models for nonpolar narcosis (Veith et al. 1983; Schultz et al. 1990a; Schultz et al. 1990c), polar narcosis (Schultz et al. 1986; Veith and Broderius 1987; Schultz et al. 1990b), respiratory uncoupling (Schultz et al. 1986; Cajina-Quezada and Schultz 1990) and proelectrophic bioreactivity (Schultz and Tichy 1993). Still, many simple industrial organic chemicals, especially direct-acting bioreactive electrophiles, do not fall within any of these mechanisms and QSARs. The aliphatic aldehydes are included among these chemicals. Such bioreactive compounds are more toxic at a lower concentration than their unreactive counterparts of equal hydrophobicity. Nucleophilic groups in proteins and nucleic acids are the most likely sites of action. In the case of aldehydes, interaction with these nucleophiles is via addition at the carbonyl group (Hermens 1990).

The purposes of this investigation were to determine the biological response in the *Tetrahymena* population-growth impairment assay of exposure to selected aliphatic aldehydes and to develop a log K_{OW}-dependent QSAR. Based on the results, six derivatives were then selected for further evaluation in the *Tetrahymena* mortality assay. The results were compared for these two endpoints, along with the activity of similar aldehydes quantitated in three other test systems. Each system was selected to represent a different protocol.

MATERIALS AND METHODS

The chemicals selected for testing form a series of alkyl aliphatic aldehydes. Each chemical was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin and had a purity of 95% or better. The population growth impairment evaluations were done with *Tetrahymena pyriformis* (Schultz et al. 1990b). This 2-d assay used population density which was measured spectrophotometrically at 540 nm as its endpoint. Each chemical was tested in a range-finder, followed by testing in duplicate for at least three replicates. Each replicate was a six-to-

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eight step arithmetic concentration series using freshly prepared stock solutions. Only replicates with control absorbance from 0.6 to 0.9, late log-growth-phase, were used in the analyses. The 50% growth inhibitory concentration, IGC_{50}, was determined using Probit Analysis of Statistical Analysis System (SAS) software (SAS Institute Inc. 1989), with Y as the absorbance normalized as percentage of control and X as the toxicant concentration in part per million.

In addition, 1-d 100% mortality was assessed for selected aldehydes. The *Tetrahymena* mortality test used was modeled after one developed by Rogerson et al. (1983). Stock cultures were essentially infinitely diluted in Hank's basic salt solution to discourage any interaction of media with bioreactive chemicals. These cultures were then allowed to complete a cell cycle before continuation of the experiment. A small aliquot of the washed stock culture, along with the toxicant appropriately diluted with Hank's solution, was drawn into a gas-tight Hamilton 10-mL syringe to fill the syringe. Syringes were set up in a graded arithmetic concentration series. Following 24 hr of incubation at 20 ± 1°C, the contents of the syringes were examined with the aid of a microscope and mortality was assessed.

For comparative purposes, relative toxicity data was collected from the literature for the static 3-d *Lactuca sativa* (lettuce) germination assay (Reynolds 1977), the semi-static 14-d *Poecilia reticulata* (guppy) mortality assay (Deneer et al. 1988), and the flow-through 4-d *Pimephales promelas* (fathead minnow) mortality assay (Nendza and Russom 1991).

For quantitative structure-activity relationship (QSAR) development, the log of the inverse of the 50 or 100% effect value in mM was used as the measurement of relative toxicity (Y) and the hydrophobic parameter log 1-octanol/water partition coefficient (log K_{OW}) was used as the molecular descriptor (X). The log K_{OW} value of each aldehyde was obtained from MEDCHEM CLOGP version 3.53 (Leo and Weininger 1988). The QSAR was generated from General Linear Model for simple regression analysis from SAS.

RESULTS AND DISCUSSION

A summary of the hydrophobicity and toxicity data used in this investigation is listed in Table 1. The log K_{OW} values were distributed over more than six orders of magnitude. Static 2-d *Tetrahymena* population growth impairment (log IGC_{50}^{-1}) varied for almost three orders of magnitude. Of the aldehydes tested, only 1-tetradecylaldehyde did not show a toxic response. Regression analysis of log K_{OW} versus log IGC_{50}^{-1} resulted in the equation,

$$\log \textit{Tetrahymena}\ IGC_{50}^{-1} = 0.52\ (\log K_{OW}) - 1.00;$$
$$n = 14,\ r^2 = 0.961,\ s = 0.168,\ f = 294.51,$$
$$Pr > f = 0.0001 \qquad [1].$$

In the *Tetrahymena* population growth assay, the presence of enriched medium, containing a wealth of nucleophiles, may alter the bioavailability of bioreactive chemicals. Therefore, the static 1-d *Tetrahymena* mortality assay was used to

Table 1. Log K_{ow} and toxicity data for aldehydes

	Compound	CAS number[a]	Log K_{ow}[b]	Log $1/IGC_{50}$[c]	Log $1/LC_{100}$[d]	Log $1/EC_{50}$[e]	Log $1/LC_{50}$[f]	Log $1/LC_{50}$[g]
1.	formaldehyde	50-00-0	-0.35	----	----	-1.29	0.04	0.10
2.	acetaldehyde	75-07-0	-0.22	----	----	-1.34	0.10	0.12
3.	propionaldehyde	123-38-6	0.31	-0.75	-0.38	-1.14	0.59	----
4.	butyraldehyde	123-72-8	0.88[m]	-0.48	----	-0.98	0.72	0.69
5.	valeraldehyde	110-62-3	1.36	-0.11	0.16	-0.61	0.82	0.83
6.	hexylaldehyde	66-25-1	1.78[m]	-0.25	----	-0.26	1.01	0.76
7.	heptylaldehyde	111-71-7	2.42	-0.09	----	-0.11	1.11	----
8.	octylaldehyde	124-13-0	2.95	0.36	0.56	0.13	1.21	----
9.	nonylaldehyde	124-19-6	3.48	0.73	----	----	----	----
10.	decylaldehyde	112-31-2	4.01	1.20	----	----	1.69	----
11.	undecylaldehyde	112-44-7	4.54	1.61	1.23	----	----	----
12.	dodecylaldehyde	112-54-9	5.07	1.68	----	----	----	----
13.	tetradecylaldehyde	124-25-4	6.12	NTAS[h]	NTAS[h]	----	----	NTAS[h]
14.	isobutyraldehyde	78-84-2	0.61	-0.53	----	-1.04	0.43	----
15.	2-methylbutyraldehyde	96-17-3	1.14	-0.41	----	----	0.46	0.94
16.	2-ethylbutyraldehyde	97-96-1	1.67	-0.14	0.18	----	0.81	----
17.	isovaleraldehyde	590-86-3	1.23	-0.42	----	----	----	1.42
18.	2-methylvaleraldehyde	123-15-9	1.67	----	----	----	----	0.73

[a] Chemical Abstract Services registry number.
[b] Measured (m) or calculated values from the CLOGP version 3.53 Program.
[c] 2-d mM *Tetrahymena* growth inhibition.
[d] 1-d mM *Tetrahymena* mortality.
[e] 3-d mM *Lactuca* germination inhibition from Reynolds (1977).
[f] 14-d mM *Poecilia* mortality from Deneer et al. (1988).
[g] 4-d mM *Pimephales* mortality from Nendza and Russom (1991).
[h] NTAS - not toxic at saturation.

eliminate such alteration and evaluate six aliphatic aldehydes (3, 5, 6, 8, 11 and 14 carbon-chained derivatives). The 1-tetradecyl derivative was again, not toxic at saturation. Regression analysis of data for the other aldehydes resulted in the following equation,

$$\log \textit{Tetrahymena}\ LC_{100}^{-1} = 0.36\ (\log K_{ow}) - 0.44;$$
$$n = 5,\ r^2 = 0.986,\ s = 0.081,\ f = 212.85,$$
$$Pr > f = 0.0007 \qquad [2].$$

In comparing Eqs. [1] and [2], the mortality assay was shown to be more sensitive than the sublethal population growth impairment assay. This, we feel reflects the bioavailability of the toxicants. Least-squares regression of the relative toxicities of the aldehydes common to Eq. [1] and Eq. [2] resulted in the following equation,

$$\log \textit{Tetrahymena}\ IGC_{50}^{-1} = 1.47\ (\log \textit{Tetrahymena}\ LC_{100}^{-1}) - 0.32;$$
$$n = 5,\ r^2 = 0.981,\ s = 0.141,\ f = 154.20,$$
$$Pr > f = 0.0011 \qquad [3].$$

Least-squares regression analysis of log K_{ow} versus log EC_{50}^{-1} from the static 3-d study on *Lactuca* seed-germination impairment resulted in the following equation,

$$\log \textit{Lactuca}\ EC_{50}^{-1} = 0.47\ (\log K_{ow}) - 1.25;$$
$$n = 9,\ r^2 = 0.972,\ s = 0.098,\ f = 241.02,$$
$$Pr > f = 0.0001 \qquad [4].$$

Simple regression of the common relative toxicity points between Eq. [1] and Eq. [4] resulted in the following equation,

$$\log \textit{Tetrahymena}\ IGC_{50}^{-1} = 0.67\ (\log \textit{Lactuca}\ EC_{50}^{-1}) + 0.12;$$
$$n = 7,\ r^2 = 0.838,\ s = 0.161,\ f = 25.93,$$
$$Pr > f = 0.0038 \qquad [5].$$

Regression analysis of log K_{ow} versus log LC_{50}^{-1} from semi-static 14-d *Poecilia* mortality study, in which solutions were renewed daily, resulted in the following equation,

$$\log \textit{Poecilia}\ LC_{50}^{-1} = 0.35\ (\log K_{ow}) + 0.26;$$
$$n = 12,\ r^2 = 0.934,\ s = 0.127,\ f = 141.26,$$
$$Pr > f = 0.0001 \qquad [6].$$

Regression of the common points between Eq. [1] and Eq. [6] resulted in the following equation,

$$\log \textit{Tetrahymena}\ IGC_{50}^{-1} = 1.33\ (\log \textit{Poecilia}\ LC_{50}^{-1}) - 1.29;$$
$$n = 10,\ r^2 = 0.845,\ s = 0.232,\ f = 43.48,$$
$$Pr > f = 0.0002 \qquad [7].$$

Analysis of log K_{ow} versus log LC_{50}^{-1} from the flow-through 4-d *Pimephales* mortality system with constant renewal of the chemical results in the following

equation,

$$\log \textit{Pimephales}\ LC_{50}^{-1} = 0.40\ (\log K_{OW}) + 0.32;$$
$$n = 8,\ r^2 = 0.569,\ s = 0.305,\ f = 7.93,$$
$$Pr > f = 0.0342 \qquad [8].$$

Analysis of the aldehydes common to both Eq. [1] and Eq. [8] resulted in the following equation,

$$\log \textit{Tetrahymena}\ IGC_{50}^{-1} = -0.13\ (\log \textit{Pimephales}\ LC_{50}^{-1}) - 0.21;$$
$$n = 5,\ r^2 = 0.064,\ s = 0.169,\ f = 0.21,$$
$$Pr > f = 0.6808 \qquad [9].$$

The manifestation of toxicity is preceded by two events. First, the penetration of the toxicant to the site of action, and second, the interaction of the toxicant with that site. Penetration is predicted by hydrophobicity, whereas interaction is predicted by stereoelectronic processes. For toxicants where the rate limiting step is penetration, K_{OW} has been a high quality molecular descriptor with these toxicants. The interaction of the toxicant with the site of action is null or constant. For chemicals where the rate limiting step is the interaction of the toxicant with the site of action, K_{OW} is not a quality molecular descriptor. Such chemicals are most often thought to be bioreactive. Bioreactivity encompasses a variety of reactions, the most common of which is soft electrophilicity. Bioreactive electrophiles may cause toxicity by three different mechanisms: nucleophilic displacement, addition at a carbon-oxygen double bound, and addition at a carbon-carbon double bond (Hermens 1990). Kamlet et al. (1986, 1987) suggested that the toxicity of aldehydes is due to their participation in Schiff-based formation with amine moieties of proteins.

While differences between organisms can not be ruled out, we feel the differences observed between the above noted QSARs was due to test systems or protocols. Specifically, the results of Eqs. [1, 2, 4 and 6], where log K_{OW} alone is a good predictor of toxic response indicate that with the static and semi-renewal systems, the uptake of the aldehyde is the rate-limiting step in toxicity. This is consistent with the findings for nonreactive mechanisms of action modeled with data from the *Tetrahymena* growth impairment (Schultz et al. 1986; Schultz et al. 1990c; Cajina-Quezada and Schultz 1990). The poor r^2 value for Eq. [8] suggests that with the fathead minnow flow-through system, uptake is not the rate-limiting step. This is in contrast with what has been observed for nonreactive mechanisms of action (Veith et al. 1983; Schultz et al. 1986; Veith and Broderius 1987; Cajina-Quezada and Schultz 1990). This comparison, based upon test design, suggests that static and possibly semi-renewal assays may give conflicting results, when compared with flow-through systems with bioreactive chemicals such as aldehydes.

Eqs. [3], [5] and [7] reveal strong collinearity between toxicities measured with the static and semi-renewal protocols. This collinearity represents lateral validation of aldehyde toxicity.

A further comparison revealed that those protocols where an organic-enriched mixture was present, the ciliate growth and lettuce germination impairment

systems, both the slopes and the intercepts of the respective QSARs, Eq. [1] and Eq. [4], were very similar. Moreover, as indicated by the intercepts, these systems are less sensitive than the simpler salt solution-based protocols of the ciliate mortality, Eq. [2], or guppy mortality, Eq. [6], systems. With the latter two QSARs, similarities were found again in both slopes and intercepts. These results suggest that the organic components of the growth medium modulate the toxic response to bioreactive aldehydes by altering their bioavailability.

In summary, it has been shown that toxicity of bioreactive aldehydes in static and semi-renewal systems can be modeled adequately by simple log K_{OW} dependent QSARs. This was in contrast to what was observed with the flow-through system. The strong collinearity of log K_{OW} and relative toxicity in static and semi-renewal systems demonstrates lateral validation of aldehyde toxicity. Moreover, organic-rich systems with their plethora of readily available nucleophiles have a potential for affecting bioavailability of electrophiles.

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