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Structure-Toxicity Relationships for Aliphatic Isothiocyanates to *Tetrahymena pyriformis*

T. W. Schultz, J. L. Comeaux

College of Veterinary Medicine, The University of Tennessee, P.O. Box 1071, Knoxville, Tennessee 37901-1071 USA

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Previous studies have used 1-octanol/water partition coefficient (log K_{ow}) dependent quantitative structure-activity relationships (QSAR) as a means of predicting toxic potency measured as *Tetrahymena pyriformis* population growth inhibition by nonreactive, nonpolar narcotics including aliphatic alcohols (Schultz and Tichy 1993), basic ketones (Schultz et al. 1995), and esters (Jaworska et al. 1995). However, select aliphatic chemicals including primary propargylic alcohols (Schultz and Tichy 1993), aldehydes (Schultz et al. 1994), as well as alkenones and alkynones (Schultz et al. 1995) were shown to be bioreactive.

Bioreactive chemicals, especially those with low hydrophobicity, exhibit excess toxicity (T_e). T_e has been defined as a ratio of predicted-to-observed toxicities (Lipnick et al. 1987) > 2.0 where predicted toxicity is calculated with the baseline or nonpolar narcosis QSAR. Bioreactive chemicals often cause problems in the application of QSAR methods for evaluating the effects and hazard of chemicals. McFarland's probabilistic model of toxicity (log 1/potency = A [log the probability of movement to the site of action] + B [log the probability of interacting with the site of action] + C; McFarland 1970) has been suggested as an approach to predicting potency of bioreactive toxicants, especially soft electrophiles (Veith and Mekenyan 1993). Previous work (Schultz and Tichy 1993; Schultz et al. 1995) has demonstrated that α - β (alpha - beta) unsaturation is a toxicophore associated with the soft electrophilicity mechanism of toxic action.

The aims of this study were to: (1) determine the response in the *Tetrahymena* population growth impairment assay to a group of alkyl isothiocyanates, R-N=C=S, and (2) compare the observed toxicity to that predicted by the baseline nonpolar narcosis QSAR.

METHODS AND MATERIALS

Aliphatic isothiocyanates were purchased from either Aldrich Chemical Co., Milwaukee, Wisconsin, USA or MTM Research Chemicals Lancaster Synthesis Inc., Windham, New Hampshire, USA. Each compound had a purity of 95 % or better and was not repurified prior to testing. Stock solutions of each toxicant were prepared in dimethyl sulfoxide at a concentration of 5 or 10 wt/vol immediately

Correspondence to: T. W. Schultz

prior to use. *Tetrahymena pyriformis* population growth impairment testing followed the protocol described by Schultz et al. (1990). This 2-d assay uses population density measured spectrophotometrically at 540 nm as its endpoint. Each chemical was tested in a range-finding assay prior to testing in duplicate for three additional replicates. Replicates consisted of a six or more step concentration series. Each replicate was set up using freshly prepared stock solutions. Only replicates with control-absorbance values from 0.6 to 0.8 were used in analyses.

The 50% growth inhibitory concentration, IGC

RESULTS AND DISCUSSION

Hydrophobicity and toxicity data for each tested isothiocyanate are reported in Table 1. Log K_{ow} values uniformly covered a range of 1.47 to 4.24. A comparison of potencies for n-propyl isothiocyanate and allyl isothiocyanate (i.e., derivatives 2 and 7, respectively) showed that a decrease in hydrogen saturation results in an increase in toxicity. A similar comparison of toxicity values for n-propyl isothiocyanate and 1,3-propylene diisothiocyanate (i.e., derivatives 2 and 8, respectively) disclosed that the introduction of a second isothiocyanate group increases toxicity ten-fold. Furthermore, a comparison of potencies between derivatives 4 and 6 (i.e., the n-amyl- and t-amyl-derivatives, respectively) demonstrates that hydrocarbon branching near the isothiocyanate moiety results in a dramatic decrease in toxicity. In the case of the t-amyl-derivative, the branching is thought to cause a steric hindrance of the reactive isothiocyanate moiety.

Simple regression analysis of log IGC_{50}-1 versus the log K_{ow} for the n-alkane isothiocyanates (i.e., derivatives 1 - 5) yields the equation,

$$\log IGC_{50}\text{-}1 = 0.037 (\log K^{ow}) + 1.593;$$
$$n = 5, r^2 = 0.847, s = 0.020, f = 16.55$$
$$Pr > f = 0.0268 \qquad [1].$$

Table 1. Hydrophobicity and toxicities of aliphatic isothiocyanates

Derivative	CAS number	Log K_{ow}	Log IGC_{50}^{-1} (mM)	T_e[a]
1. ethyl	542-85-8	1.47[b]	1.63	317.9
2. n-propyl	628-30-8	2.13[c]	1.69	108.4
3. n-butyl	592-82-5	2.92[b]	1.72	26.6
4. n-amyl	629-12-9	3.58[d]	1.71	7.5
5. n-hexyl	4404-45-9	4.24[d]	1.75	2.5
6. t-amyl	NA[e]	3.58[f]	0.35	0.3
7. allyl	57-06-7	1.58[g]	2.06	605.0
8. propylene di	109704-32-7	2.28[h]	2.74	822.2

[a] excess toxicity (predicted IGC_{50}/observed IGC_{50}).
[b] measured value.
[c] estimated as the mean of the ethyl and n-butyl values.
[d] estimated from the n-butyl-value + 0.66 (π for $-CH_2-$) for each additional ($-CH_2-$).
[e] not available.
[f] estimated as equal to the n-amyl value.
[g] estimated as log K_{ow} for the n-propyl-derivative + 0.88 (π for $-CH=CH_3$), - 1.43 (π for $-CH_2CH_3$).
[h] estimated as 2 times the log K_{ow} for the ethyl-derivative - 0.66 (π for $-CH_2-$).

Analysis of residual values based on Eq. [1] disclosed a distribution not significantly different from normal. The slope of Eq. [1] is extremely shallow and potency was interpreted as being a constant (i.e., 0.0202 mM ± 0.0023).

T_e values are reported in Table 1. Simple regression analysis of log T_e versus molecular weight for the n-alkane isothiocyanates (i.e., derivatives 1 - 5) yields the relationship,

$$\log T_e = -0.038 \text{ (molecular weight)} + 5.86; \quad n = 5,\ r^2 = 0.998,\ s = 0.042,\ f = 1632.42,\ Pr > f = 0.0001 \qquad [2].$$

Eq. [2] revealed that within the congeneric series of n-alkane-substituted isothiocyanates, T_e was inversely related to alkyl chain length. A comparison of T_e values for derivatives 2, 7, and 8 indicates that T_e was also directly related to the degree of hydrogen unsaturation.

McFarland (1970) hypothesized that the manifestation of chemical toxicity must be preceded by two events: 1) the movement of the toxicant to the site of action, and 2) the interaction of the toxicant with the molecular site of action. Perfusion of the chemical to the site of action typically requires the crossing of one or more biological membranes. This process, one of passive diffusion, is best quantitated by log K_{ow}. In contrast, interaction of the chemical with the site of action is more elaborate. Such interactions are quantified by electronic and/or steric parameters.

This approach results in the generic QSAR, log 1/T = A (M) + B (I) + C where T is toxic potency, M is movement of the toxicant to the site of action, and I is the interaction of the toxicant with the site of action.

With baseline narcosis, the slope of log 1/T versus log K_{ow}

In the quantitative modeling of bioreactive toxicants, $B \neq 0$. Chemicals which are bioreactive elicit toxic responses with the aid of forces other than hydrophobic ones. Thus, the term bioreactive infers that a toxicant will have a positive steric or electronic interaction with macromolecules in living systems. These interactions are either immediate or may follow activation of the parent molecule into a reactive metabolite. As supported by the present study bioreactivity is structurally associated with hydrogen unsaturation (Hermens 1990), especially α-β unsaturated compounds where at least one of the unsaturated moieties includes a heteroatom (i.e., O, N, or S).

For some bioreactive toxicants, chemicals for which the limiting step is the ability to reach the site of action, $A \neq 0$. However, because they are bioreactive, these toxicants also must have an electronic and/or steric interaction at the molecular site of action, so $B \neq 0$. With bioreactive toxicants that have identical mechanisms of action, A or the slope of the log K_{ow} term is < 1.00 and the interaction term, B, is constant. Thus in the modeling of certain bioreactive toxicants, especially within congeneric series, interaction terms are excluded, resulting in high quality, simple log K_{ow}-dependent relationships. This leads to a family of hydrophobicity-dependent linear relationships with different slopes, as evidenced by the results obtained for the proelectrophiles 2-alkyn-1-ols (slope = 0.64; Schultz and Tichy 1993) and aliphatic aldehydes (slope = 0.52; Schultz et al. 1994).

For other bioreactive toxicants, potency is not limited by the ability to reach the site of action and A = 0. For example, α - β unsaturated carbonyl compounds, such as alkynones and alkenones, exhibit a toxicity that is independent of hydrophobicity (Schultz et al. 1995). Because such toxicants are bioreactive, they must have an interaction at the molecular site of action and $B \neq 0$. However, because bioreactive toxicants with identical mechanisms of action have an identical interaction term and B is constant, both the A and B term drop out of the equations and potency is a constant. We feel this is the case with the saturated n-isothiocyanates examined in the course of this investigation.

The trend of T_e being inversely related to hydrocarbon-chain length is in agreement with the suggestion of Ferguson (1939) that there is a transition from "chemical toxicity" (i.e., bioreactivity) to "physical toxicity" (i.e., narcosis) as hydrophobicity increases within a homologous series of electrophiles. Moreover, this observation is consistent with the observations for epoxides (Lipnick et al. 1987) and primary propargylic alcohols (Schultz and Tichy 1993).

In summary, the toxicity of eight aliphatic isothiocyanates was evaluated in the static *Tetrahymena pyriformis* population growth assay. A comparison of potency of ally1 isothiocyanate and n-propyl isothiocyanate showed that a decrease in hydrogen saturation results in an increase in toxicity. Similarly, a comparison of

1,3-propylene diisothiocyanate with n-propyl isothiocyanate disclosed that the introduction of a second isothiocyanate group sharply increases toxicity. A comparison of potency between n-amyl isothiocyanate and t-amyl isothiocyanate revealed a decrease in toxicity due to steric hindrance. Although correlated with log K_{ow}, toxicity of the saturated straight-chain hydrocarbon-substituted isothiocyanates was interpreted as being a constant (0.0202 mM ± 0.0023). Excess toxicity, an indicator of bioreactivity, was associated with both lower molecular weight and lower hydrogen saturation.

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