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# Structure-Toxicity Relationships for Unsaturated Alcohols to *Tetrahymena pyriformis:* 3-Alkyn-1-ols and 2-Alken-1-ols

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The previous study of Schultz and Tichy (1993) evaluated the relative toxicity of selected unsaturated alcohols in the static *Tetrahymena pyriformis* population growth assay. They observed that 2-position unsaturated derivatives were more toxic than other isomers. Moreover, they found triple bond-containing alkyn-1-ols to be more toxic than double bond-containing alken-1-ols. Using toxicity data for 34 basic aliphatic alcohols, Schultz and Tichy (1993) generated an 1-octanol/water partition coefficient (log $K_{ow}$) dependent quantitative structure-activity relationship (QSAR) which they used to predict toxicity defined as the baseline, nonpolar narcosis, mechanism of acute toxic action (Schultz 1989). Using the concept of excess toxicity ($T_e$), defined as the ratio of predicted baseline toxicity to observed toxicity (Lipnick et al. 1987), Schultz and Tichy (1993) also examined in detail the relative toxicity of primary propargylic alcohols and observed $T_e$ for 2-alkyn-1-ols was inversely related to molecular size. A similar relationship was observed with the 96-hr *Pimephales promelas* (fathead minnow) $LC_{50}$ data from Veith et al. (1989). Moreover, Veith et al. (1989) noted that the primary homopropargylic alcohol 3-butyn-1-ol exhibited an $T_e$ of 321. A similar calculation for 2-propen-1-ol, allyl alcohol, using a log $K_{ow}$ value of -0.25 revealed its observed toxicity to fathead minnow, 0.32 mg/L (Center for Lake Superior Environmental Studies University of Wisconsin-Superior 1990), to be 17,500 greater than predicted by the baseline narcosis model of Veith et al. (1983). Similarly, Lipnick et al. (1987) reported an $T_e$ of 16,000 for allyl alcohol to goldfish. The above observations lead to the undertaking of this study, the purpose of which was to determine the biological response in the *Tetrahymena* population growth impairment assay of exposure to selected 3-alkyn-1-ols and 2-alken-1-ols, compare observed and predicted toxicities, and develop QSARs for these two classes of chemicals.

## MATERIALS AND METHODS

The chemicals tested formed parallel series of 3-alkyn-1-ols and 2-alken-1-ols. Each chemical was purchased from either Aldrich Chemical Co., Milwaukee, Wisconsin, USA or MTM Research Chemicals Lancaster Synthesis Inc.,

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Windham, New Hampshire, USA, and each had a purity of 95% or better. The static *Tetrahymena pyriformis* population growth impairment test was performed (Schultz et al. 1990). This 2-d assay uses cell density as its endpoint. Population levels were measured spectrophotometrically as absorbance at 540 nm. Each chemical was assayed in a range-finder, followed by definitive testing as duplicates for three or more replicates. Each replicate was at minimum a six-step arithmetic concentration series using freshly prepared stock solutions. Stock solutions were prepared in dimethyl sulfoxide (DMSO) at concentrations of 5, 10, 25, or 50 g/liter. In every case, the volume of stock solution added to each flask was limited so the final DMSO concentration did not exceed 0.75%, an amount that does not alter *Tetrahymena* reproduction (Schultz and Cajina-Quezada 1982). Only replicates with control absorbance values from 0.6 to 0.9 were used in the analyses. The 50% growth inhibitory concentration, $IGC_{50}$, was determined for each alcohol 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 ppm. This 50% effect concentration was adjusted from pipetted amount to weight concentration by multiplying by density.

The experimental toxicity measured as the density adjusted $IGC_{50}$ in mM was compared with that predicted from the baseline equation developed by Schultz and Tichy (1993) for saturated monoalcohols,

$$\log IGC_{50}^{-1} = 0.80 (\log K_{ow}) - 2.04;$$
$$n = 34,\ r^2 = 0.982,\ s = 0.171,\ F = 1744.53$$
$$Pr > F = 0.0001 \qquad [1].$$

The log $K_{ow}$ value of each unsaturated alcohol was obtained from PROLOGP version 4.1 (CompuDrug USA Inc 1990) and in limited cases compared with measured values.

QSARs were generated using log $IGC_{50}^{-1}$ as the measure of toxicity (Y) and the hydrophobic parameter log $K_{ow}$ as the molecular descriptor (X). The equations were generated using the General Linear Model for regression analysis from SAS.

## RESULTS AND DISCUSSION

A computation of the hydrophobicities, density and relative toxicities for the seven 3-alkyn-1-ols examined in this investigation is given in Table 1. The log $K_{ow}$ values were distributed over more than five orders of magnitude. The log $IGC_{50}^{-1}$ values varied over four orders of magnitude. Regression analysis of calculated log $K_{ow}$ versus the log of the density-adjusted $IGC_{50}^{-1}$ for the 3-alkyn-1-ols resulted in the equation,

$$\log IGC_{50}^{-1} = 0.84 (\log K_{ow}) - 1.80;$$
$$n = 7,\ r^2 = 0.990,\ s = 0.164,\ F = 495.24$$
$$Pr > F = 0.0001 \qquad [2].$$

As expected from the high coefficient of determination ($r^2$), an examination of residual values revealed no outliers. An examination of $T_e$ values showed

Table 1. Hydrophobicity, density and toxicity of primary homopropargylic alcohols

| | alcohol | CAS number[a] | log $K_{ow}$[b] exp.[c] | log $K_{ow}$[b] se[d] | log $K_{ow}$[b] calc.[e] | log $K_{ow}$[b] exp.[c] | density | observed $IGC_{50}$ (mM) | log $IGC_{50}^{-1}$ | predicted[g] $IGC_{50}$ (mM) | $T_e$[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 3-butyn-1-ol | 927-74-2 | -.- | -.- | 0.04 | -.- | 0.927 | 69.106 | -1.84 | 101.859 | 1.47 |
| 2. | 3-pentyn-1-ol | 10229-10-4 | 3.364 | 0.278 | 0.56 | 0.53 | 0.912 | 14.960 | -1.17 | 39.084 | 2.61 |
| 3. | 3-hexyn-1-ol | 1002-28-4 | 9.459 | 1.129 | 1.08 | 0.98 | 0.900 | 10.599 | -1.03 | 14.997 | 1.41 |
| 4. | 3-octyn-1-ol | 14916-80-4 | -.- | -.- | 2.12 | -.- | 0.883 | 1.129 | -0.05 | 2.208 | 1.96 |
| 5. | 3-nonyn-1-ol | 31333-13-8 | -.- | -.- | 2.63 | -.- | 0.884 | 0.455 | 0.34 | 0.863 | 1.90 |
| 6. | 3-decyn-1-ol | 51721-39-2 | -.- | -.- | 3.15 | -.- | 0.871 | 0.075 | 1.12 | 0.331 | 4.41 |
| 7. | 3-tetradecyn-1-ol | 55182-74-6 | -.- | -.- | 5.23 | -.- | 0.865[f] | 0.003 | 2.52 | 0.007 | 2.33 |

[a] Chemical Abstract Service-registry number.

[b] 1-octanol/water partition coefficient.

[c] Experimentally determined by equilibrium method and gas chromatography.

[d] Standard error.

[e] Calculated by PROLOGP version 4.1.

[f] Estimated value.

[g] Predicted from calculated log $K_{ow}$ values and the QSAR, log $IGC_{50}^{-1}$ = 0.80 (log $K_{ow}$) - 2.04.

[h] Excess toxicity parameter defined as $IGC_{50}$ (pred)/$IGC_{50}$ (obs).

primary homopropargylic alcohols exhibited a modest excess toxicity ranging from 4.41 to 1.41 and having a mean $T_e$ value of 2.30 +/- 0.95. These observed $T_e$ values are in sharp contrast with the values reported by Veith and co-workers who showed higher $T_e$ to fish for primary homopropargylic alcohols (Veith et al. 1989).

Primary homopropargylic alcohols are considered proelectrophiles (Veith et al. 1989). They differ structurally from the previously studied primary propargylic alcohols (Schultz and Tichy 1993), containing a methylene moiety between the hydroxyl and acetylenic groups. The presence of this methylene group prevents primary homopropargylic alcohols from undergoing oxidation by way of alcohol dehydrogenase to an alpha-unsaturated aldehyde. Therefore, no Michael-type acceptor electrophile (Lipnick 1985) can be synthesized. Veith et al. (1989) drawing upon earlier studies, including those of Taylor (1967) and Alston et al. (1979), proposed an elaborate proelectrophile mechanism for 3-alkyn-1-ols which included biochemical oxidation, enolization to a conjugated anion, tautomerization to an allene, and nucleophilic attack. The modest $T_e$ observed for the primary homopropargylic alcohols examined in this study suggests this pathway is not as efficient in *Tetrahymena*.

The hydrophobicities, density and relative toxicities for the twelve 2-alken-1-ols examined in this investigation are listed in Table 2. There was fair agreement between calculated and measured log $K_{OW}$ values. As before, the log $K_{OW}$ values were distributed over approximately five orders of magnitude. Similarly, the log $IGC_{50}^{-1}$ values varied over four orders of magnitude. Regression analysis of calculated log $K_{OW}$ versus the log of the density-adjusted $IGC_{50}^{-1}$ for the 2-alken-1-ols resulted in the equation,

$$\log IGC_{50}^{-1} = 0.86 (\log K_{OW}) - 1.82;$$
$$n = 12,\ r^2 = 0.994,\ s = 0.109,\ F = 1574.19$$
$$Pr > F = 0.0001 \qquad [3].$$

As expected from the high coefficient of determination ($r^2$), an examination of residual values showed no outliers. An examination of $T_e$ values showed 2-alken-1-ols exhibited a modest excess toxicity ranging from 3.07 to 1.14 and having a mean $T_e$ value of 2.24 +/- 0.56. These observed $T_e$ values are in sharp contrast with 150 fold higher $T_e$ values noted for allyl alcohol toxicity in fish (Lipnick et al. 1987; Veith et al. 1989).

A mechanism of acute toxic action for 2-alken-1-ols was proposed by Lipnick (1985). It involved the metabolic activation by way of alcohol dehydrogenase to an alpha and beta-unsaturated 2-alkenal with the 2-alkenal acting as a Michael-type acceptor electrophile. This mechanism is not different from the one proposed for primary propargylic alcohols (Veith et al. 1989). Moreover, it is the same mechanism Schultz and Tichy (1993) used to explain the observed $T_e$ for 2-alkyn-1-ols to *Tetrahymena*. One explanation for this lack of higher $T_e$ values in *Tetrahymena* for any of the 2-alken-1-ols is a reduced level of enzymatic activity in *Tetrahymena*.

Table 2. Hydrophobicity, density and toxicity of 2-alken-1-ols

| alcohol | CAS number[a] | log $K_{ow}$[b] exp.[c] | se[d] | log $K_{ow}$[b] calc.[e] | exp.[c] | density | observed $IGC_{50}$ (mM) | log $IGC_{50}^{-1}$ | predicted[g] $IGC_{50}$ (mM) | $T_e$[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. 2-propen-1-ol | 107-18-6 | -.- | -.- | -0.12 | -.- | 0.854 | 82.755 | -1.92 | 136.773 | 1.65 |
| 2. 2-buten-1-ol | 6117-91-5 | -.- | -.- | 0.41 | -.- | 0.848 | 29.749 | -1.47 | 51.523 | 1.73 |
| 3. (cis)2-penten-1-ol | 1576-95-0 | 11.67 | 0.62 | 0.92 | 1.07 | 0.853 | 13.321 | -1.12 | 20.137 | 1.51 |
| 4. (trans)2-hexen-1-ol | 928-95-0 | 57.81 | 7.01 | 1.44 | 1.76 | 0.849 | 2.964 | -0.47 | 7.727 | 2.61 |
| 5. (trans)2-hepten-1-ol | 33467-76-4 | 222.11 | 21.89 | 1.96 | 2.35 | 0.850[f] | 0.901 | 0.05 | 2.119 | 2.35 |
| 6. (trans)2-octen-1-ol | 18409-17-1 | -.- | -.- | 2.48 | -.- | 0.850[f] | 0.468 | 0.33 | 1.138 | 2.43 |
| 7. 2-nonen-1-ol | 22104-79-6 | -.- | -.- | 3.00 | -.- | 0.850[f] | 0.170 | 0.77 | 0.437 | 2.57 |
| 8. 2-decen-1-ol | 22104-80-9 | -.- | -.- | 3.52 | -.- | 0.850[f] | 0.070 | 1.16 | 0.166 | 2.37 |
| 9. (trans)2-undecen-1-ol | 75039-84-8 | -.- | -.- | 4.04 | -.- | 0.850[f] | 0.024 | 1.62 | 0.065 | 2.71 |
| 10. 2-dodecen-1-ol | 22104-81-0 | -.- | -.- | 4.56 | -.- | 0.850[f] | 0.009 | 2.07 | 0.025 | 2.78 |
| 11. 2-methyl-2-propen-1-ol | 513-42-8 | -.- | -.- | 0.40 | -.- | 0.855 | 45.893 | -1.66 | 52.481 | 1.14 |
| 12. 3-phenyl-2-propen-1-ol | 104-54-1 | -.- | -.- | 1.83 | -.- | 1.044 | 1.202 | -0.08 | 3.690 | 3.07 |

[a] Chemical Abstract Service-registry number.

[b] 1-octanol/water partition coefficient.

[c] Experimentally determined by equilibrium method and gas chromatography.

[d] Standard error.

[e] Calculated by PROLOGP version 4.1.

[f] Estimated value.

[g] Predicted from calculated log $K_{ow}$ values and the QSAR, $\log IGC_{50}^{-1} = 0.80 (\log K_{ow}) - 2.04$.

[h] Excess toxicity parameter defined as $IGC_{50}$ (pred)/$IGC_{50}$ (obs).

Since Eqs. [2] and [3] were almost identical, these data were combined and reanalyzed. Regression analysis resulted in the equation,

$$\log IGC_{50}^{-1} = 0.81 (\log K_{OW}) - 1.85;$$
$$n = 19,\ r^2 = 0.992,\ s = 0.124,\ F = 2095.84$$
$$Pr > F = 0.0001 \qquad [4].$$

A comparison of Eqs. [1] and [4] revealed their intercepts to be significantly different, but their slopes not to be significantly different.

In summary, the relative toxicity of selected 3-alkyn-1-ols and 2-alken-1-ols were evaluated in the static *Tetrahymena pyriformis* population growth assay. Both groups of unsaturated alcohols can be modeled by the same QSAR and possess a very modest, yet consistent, excess toxicity when compared to that predicted by the baseline nonpolar narcosis QSAR for saturated monoalcohols. The magnitude of the excess toxicity was not as great as reported for acute fish lethality.

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