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Structure-Toxicity Relationships for Selected Naphthoquinones to *Tetrahymena pyriformis*

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Naphthoquinones are reactive compounds that contain conjugated carbonyl groups. Based on their interaction with cellular components, quinones may act in various ways. However, recent investigations have identified soft electrophilic addition to biological nucleophiles such as proteins and nucleic acids and, to a lesser extent, redox cycling oxidation as the most likely mechanisms of quinone toxicity in *Tetrahymena* (Schultz et al. 1997). These molecular mechanisms of quinones have been discussed in the reviews of O'Brien (1991) and Monk et al. (1992).

Quinones may be reduced enzymatically by one-electron or two-electron processes. The outcome is a semiquinone or hydroquinone, respectively. Semiquinones as radicals may be toxic themselves. However, they also may be reoxidized to the parent quinone and reduce oxygen to superoxide anion. The reduction of O_2 to a superoxide anion can lead to oxidative stress. The net process, regeneration and reduction, is a cyclic procedure.

Quinones may also covalently react with macromolecular nucleophiles. Most of these reactions proceed via Michael-type addition. Whether a quinone acts as an arylating electrophile or a redox cycler is dependent on a number of factors (Monk et al. 1992). Among the investigated quinones are the 1,4-naphthoquinones. Studies with isolated hepatocytes (O'Brien 1991) have related naphthoquinone toxicity and flavoprotein-mediated reduction of these chemicals to their one-electron redox potentials.

Bioassay with *Tetrahymena pyriformis* allows, in a short time period, the examination of a large number of independent organisms that concomitantly possess features of both single eucaryotic cells and whole organisms (Schultz 1996). Because of these attributes, *Tetrahymena* has been used extensively to generate toxicity data to explore its use as a non-vertebrate model for toxicological investigations and for the development of structure-toxicity relationships.

This purpose of this study was to examine the aquatic toxicity of selected naphthoquinones. To this end, the specific aims were to: (1) determine the

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biological response to each quinone in the *T. pyriformis* population growth impairment assay; (2) compare the observed toxicity to that predicted by the baseline toxicity model for neutral organics, and (3) examine toxic potency QSARs developed with selected molecular descriptor data.

METHODS AND MATERIALS

A group of eight naphthoquinones were purchased from Aldrich Chemical Co., Milwaukee, WI, USA. Chemicals had a purity of 95% or better and were not repurified prior to use. Stock solutions of each quinone were prepared in dimethyl sulfoxide.

Tetrahymena pyriformis population growth impairment testing was executed following the protocol described by Schultz (1996). This static 40-hr assay used population density measured spectrophotometrically at 540 nm as its endpoint. Test conditions allow for 8-9 cell cycles in control cultures. Each quinone was tested in a range finder prior to testing in duplicate for three additional replicates. Two controls, one with no test material but inoculated with *T. pyriformis*, and the other, a blank which had neither toxicant nor ciliates, were used to provide a measure of the acceptability of the test and a basis for interpreting treatment data. Each definitive test replicate consisted of six to eight different concentrations with duplicate flasks of each concentration. Only replicates with control-absorbency values > 0.60 but < 0.75 were used in the analyses.

The 50% growth inhibitory concentration, IGC_{50}, was determined for each quinone by Probit Analysis of Statistical Analysis System (SAS) software (SAS Institute Inc. 1989). The effect levels were not analytically measured for quinone concentrations. Logarithms of the 1-octanol/water partition coefficients (log K_{OW}) values were secured from CLOGP for Windows (BIOBYTE Corp., Claremont, CA) software. One-electron reduction potential (E/mV) values were secured from Wardman (1989). Since concentrations were nominal, aquatic solubility (S) was estimated from log K_{OW} and melting point (MP) values, Log S = -log K_{OW} –0.01 MP + 1.05 (Yalkowsky and Valvani 1980). All IGC_{50} values were below estimated solubility.

Quantitative structure-toxicity relationships were examined using log of the inverse of the IGC_{50} (log $(IGC50^{-1})$) in mM as the dependent variable, and the log K_{OW} or E/mV values as the independent variable. Data were modeled using least squares regression (general linear model procedure of SAS). Model adequacy was quantified with the coefficient of determination (r^2 value). The root of the mean square for error (s value), the Fisher statistic (F value), and the probability greater than the F value (Pr > F) were also noted.

RESULTS AND DISCUSSION

A computation of the Chemical Abstract Service registry numbers, toxicity, and selected molecular descriptors values are given in Table 1. The plot of log K_{OW}

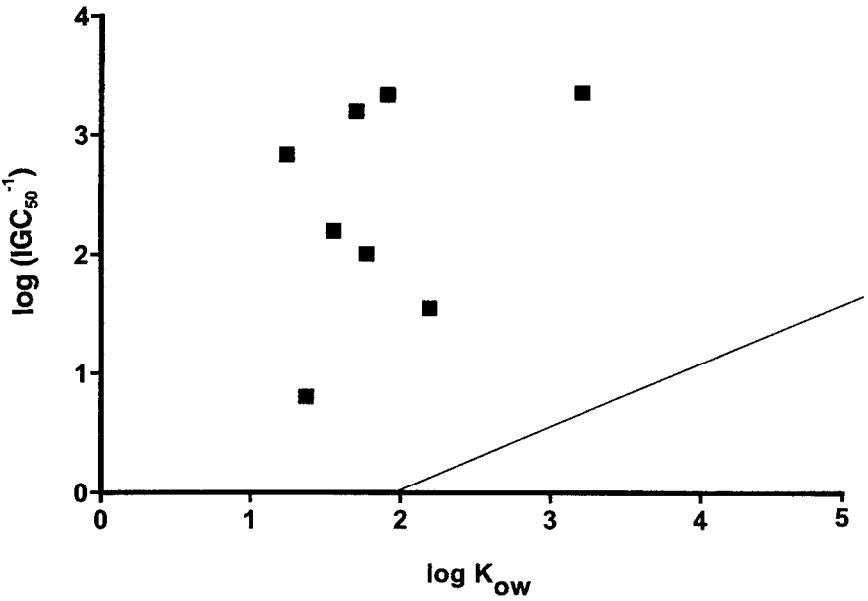

Figure 1. Plot of toxicity to *T. pyriformis* (log IGC50^{-1}) of naphthoquinones against hydrophobicity (log K_{ow}). The baseline QSAR for neutral organics, log (IGC50^{-1}) = 0.8 (log K_{ow}) - 2.0, (Schultz 1996) is also shown.

Table 1. Toxicity and molecular descriptor values for selected naphthoquinones.

Compound	CAS Number	log 1/IGC$_{50}$	log K_{ow}[a]	E/mV[b]
1,2-naphthoquinone	524-42-5	3.20	1.71	-89
1,4-naphthoquinone	130-15-4	2.00	1.78[m]	-140
2-methyl-1,4-naphthoquinone (menadinone)	58-27-5	1.54	2.20[m]	-203
2-hydroxy-1,4-naphthoquinone (lawsone)	83-72-7	0.80	1.38[m]	-415
5-hydroxy-1,4-naphthoquinone (juglone)	481-39-0	3.33	1.92[m]	-93
5,8-dihydroxy-1,4-naphthoquinone (naphthazarin)	475-38-7	2.83	1.25	-110
5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin)	481-42-5	2.20	1.56[m]	-156
2,3-dichloro-1,4-naphthoquinone (dichlone)	117-80-6	3.36	3.22	-36

[a]1-octanol/water partition coefficient from CLOGP for windows; m = measured value. [b]one-electron redox potential from Wardman (1989)

versus the log (IGC_{50}^{-1}) for the eight naphthoquinones (Figure 1) shows all eliciting a toxic response more potent than predicted from the baseline log K_{OW} dependent QSAR. Moreover, Figure 1 demonstrates that quinone toxicity is not correlated with hydrophobicity. As noted previously, toxicity of quinones is due to, at minimum, two competing mechanisms-- the capacity to produce oxidative stress and/or the ability to arylate. With the oxidative stress mechanism, the initiation of cell damage lies in the ability of the quinone to form free-radical metabolites (e.g., semiquinones), that in turn may react with O_2 to generate toxic superoxide anions.

Whether or not a quinone is able to undergo redox cycling depends, in large part, on its one-electron redox potential. However, this ability to redox cycle is complicated by the fact that different reductases have different redox potentials and can use different quinones as electron acceptors. As noted by Powis and Appel (1980), a primary element in determining the rate of flavoprotein-mediated reduction is the one-electron reduction potential at pH = 7 of the quinone to semiquinone process. The importance of the one-electron reduction potential is probably due to the fact that flavin-containing enzymes exhibit little substrate specificity for artificial electron acceptors. *Tetrahymena*, as well as higher animals, contain a microsomal membrane electron transport system (Sasaki et al. 1985) that encompasses a variety of flavoproteins, including NADPH-cytochrome c reductase (Fukushima et al. 1983). As previously noted, quinones with one electron, redox potentials between -240 E/mV and -170 E/mV or -50 E/mV and 25 E/mV may elicit their toxic response via oxidative stress by acting as redox cyclers (Powis and Appel 1980; O'Brien 1991). Seven of the eight naphthoquinones tested have one-electron reduction potentials within the range noted above.

A plot of toxicity versus the one-electron redox potential is observed in Figure 2. A linear relationship was observed for those quinones with potentials within the "window" of redox cycling (i.e., -240 to 25 E/mV). This relationship is captured in Eq. [1];

$$\log (IGC_{50}^{-1}) = 0.0127\ (E/mV) + 4.142;$$

$$n = 7,\ r^2 = 0.883,\ s = 0.273,\ F = 37.71,\ Pr > F = 0.0017. \qquad \text{Eq. [1]}$$

The single tested quinone whose one-electron redox potential lies outside the above noted range, 2-hydroxy- 1,4-naphthoquinone (one-electron redox potential of -415 E/mV), is more toxic that predicted by Eq. [1]. It is hypothesized that this compound elicits an electrophilic toxic mechanism. Most electrophilic reactions involve soft interactions. In soft electrophilic reactions, electron density is donated or accepted by molecular orbitals (Klopman 1974). The result is alkylation or arylation to thiol and amino moieties associated with proteins. One group of soft electrophiles is the α,β-unsaturated ketones (Schultz et al. 1995) which contain polarized double bonds and act as Michael-type acceptors (Karabunarliev et al. 1996). Naphthoquinones are aromatic diketones. Their carbonyl groups are conjugated with aromatic double bonds. The net result of this

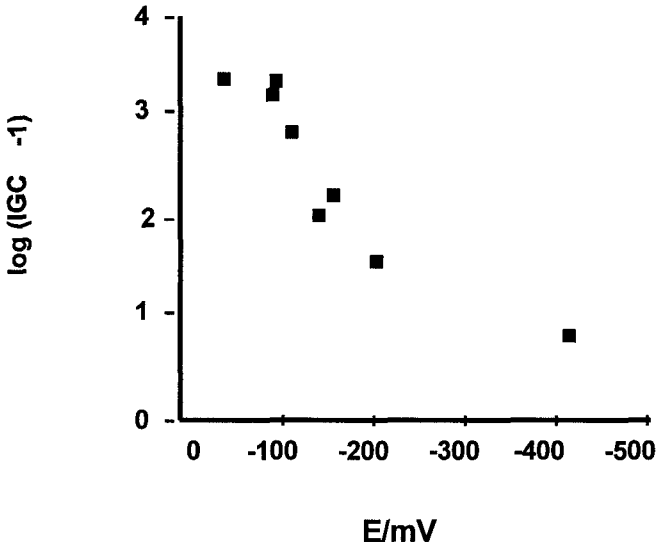

Figure 2. Plot of toxicity to *T. pyriformis* (log IGC_{50}^{-1}) of naphthoquinones versus the one-electron reduction potential (E/mV).

conjugation are polarized double bonds that allows for a Michael-type addition. Therefore, in respect to electrophilic molecular mechanisms, 2-hydroxy-1,4-naphthoquinone is similar to α,β-unsaturated ketones.

The molecular orbital energy E_{LUMO} was shown to be important in modeling toxic potency of α,β-unsaturated ketones (Schultz et al. 1995). Acceptor superdelocalizability was shown to be important in modeling aquatic toxicity of aromatic electrophiles (Veith and Mekenyan 1993). Neither of these parameters model naphthoquinone toxicity (data not shown).

In summary, naphthoquinones can act via the redox cycling and/or a soft electrophilic mechanism of toxic action. Selection of the most prevalent mechanism is one-electron redox potential-dependent.

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