

To the Graduate Council

I am submitting herewith a thesis written by Adam DeWeese entitled "Quantitative-Structure-Activity Relationships for Acute Toxicity of Selected Halogenated Monoesters and Carboxylic Acids to *Tetrahymena pyriformis*." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

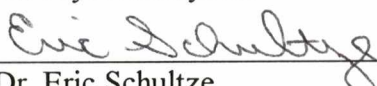


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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS
FOR ACUTE TOXICITY OF SELECTED HALOGENATED
MONOESTERS AND CARBOXYLIC ACIDS TO
TETRAHYMENA PYRIFORMIS

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DEDICATION

I would like to dedicate this thesis to my wife Ruth for her love and encouragement, and to my parents Jerion and Dr. David DeWeese who taught me the value of perseverance in following a dream.

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ABSTRACT

Halogen-substituted esters and carboxylic acids are thought to exhibit toxicity via a variety of mechanisms that are electro(nucleo)philic in character. The carbonyl group in these compounds has an oxygen atom with both a partially negative charge and a pair of unshared electrons in a π cloud which make it susceptible to nucleophilic addition reactions. Because halogen atoms are good leaving groups in S_N2 reactions, substitution of a halogen atom on esters and carboxylic acids with a cellular nucleophile increases toxic potency above that of basic narcosis. The *Tetrahymena pyriformis* multigenerational growth inhibitory assay was used to test the toxicity of a suite of halogenated basic esters and halogenated carboxylic acids. Structure-toxicity and property-toxicity relationships were examined for each set of haloesters and haloacids. The structure-toxicity relationships of halogen-substituted esters demonstrated several trends. α -Haloesters are more toxic than ones with the halogens separated from the carboxyl group by either methylene or ether moieties. Hydrocarbon substitution on the carbon bonded with the halogen diminishes toxicity. Multiple halo-substitution enhances haloester toxic. Bromo-substituted esters are more potent toxicants than chloro-substituted ones. The property-toxicity relationships for halogen-substituted esters revealed a number of points. Haloesters are more toxic than narcotic simple esters. Toxic potency of haloesters is poorly modeled by the electrophilic parameter, the energy of the lowest unoccupied molecular orbital (E_{LUMO}) and even less so by the hydrophobic parameter, the 1-octanol/water partition coefficient (log

K_{OW}). However, with noted exceptions, a combination of the above two terms explained better than 85% of the variability in toxic potency. The major exceptions were three carbon atom-containing esters that were more toxic than predicted. The structure-toxicity relationships for halogen-substituted carboxylic acids revealed five trends. α -Haloacids are more toxic than those with the halogen separated from the carboxyl group by one or more methylene groups. Hydrocarbon substitution on the carbon atom substituted with the halogen atom diminishes toxicity. Toxic potency for haloacids is directly proportional to alkyl chain-length as long as the compound is greater than three carbon-atoms in length. Multiple halogen-substitution enhances haloacid toxicity. Chloro-substituted acids are more toxic than bromo-substituted ones. The property-toxicity relationships for halogen-substituted carboxylic acids demonstrated a number of trends. The toxic potency of haloacids is greater than non-halogenated acids. Toxic potency of haloacids is modeled poorly by the ionization constant pK_a , and even less so by E_{LUMO} . Log K_{OW} models haloacids reasonably well. However, small molecules of three or less carbon atom could not be modeled in the same grouping as larger compounds of four or more carbon atoms. Based on relative hydrophobicity the smaller haloacids were more toxic than the remaining haloacids.

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LIST OF ABBREVIATIONS

A_{ave} , Average acceptor superdelocalizability

CAS, Chemical Abstract Service

E_{HOMO} , Energy of the Highest Occupied Molecular Orbital

E_{LUMO} , Energy of the Lowest Unoccupied Molecular Orbital

F, Fischer statistic

IGC₅₀, 50% Growth Inhibitory Concentration

K_{OW} , 1-octanol/water partition coefficient

pK_a , Ionization constant

$Pr > F$, Probability greater than the F value

QSAR, Quantitative Structure-Activity Relationship

r^2 , Coefficient of determination

s, Square root of the mean square for error

SAS, Statistical Analysis System

SMILES, Hydrogen-suppressed molecular structure

S_N^2 , Electro(nucleo)philic substitution reaction requiring 2 molecules

CHAPTER 1

INTRODUCTION

Aliphatic esters and carboxylic acids are economically important and pervasive groups of industrial organic chemicals. They are used in the manufacture of a variety of products including polyester fibers, film resins, cosmetics and foodstuffs. Because of their structural diversity and variations in toxic mechanisms (Jaworska et al., 1995) it is important to have high quality, concentration-dependent toxicity data with which to evaluate ecological endpoints of concern. In recent years, considerable effort has been made to standardize ecotoxicological assays, including the *Tetrahymena pyriformis* multigeneration growth impairment assay (Larsen et al., 1997; Schultz, 1997). Because generating toxicological data is lengthy and costly, predictive models in the form of Quantitative Structure-Activity Relationships (QSARs) have become a necessary tool to fill data gaps for environmental risk assessment and regulatory concerns.

For more than a century, attempts to quantitate relationships between chemical structure and toxic potency have been part of scientific literature. The traditional approach to quantitative structure-toxicity relationships for acute toxicity has been the congeneric series approach. Typically, this approach uses a series of homologous compounds, which have a single functional group or toxicophore and an alkyl moiety of varied size. The basis for this approach is that chemical reactivity is attributable to the functional group whereas the hydrocarbon moiety alters hydrophobicity (i.e., effect penetration or uptake). Therefore, potency is

directly related to hydrophobicity; reactivity can be ignored. A more complex approach uses analogous data sets. Analogous data sets can be developed by combining homologous data sets. As the chemical set becomes more diverse in structure (i.e., the type, number and position of substituents become more complex), the probability of altering chemical reactivity becomes more likely.

Currently the paradigm is that one QSAR should model only a single mechanism of action. However, this doctrine only requires the *a priori* identification of a mechanism of action from molecular structure which can be difficult (Bradbury, 1994). The reason for this difficulty dwells in the notion that the activity of toxicants in a biophase depends upon a variety of interactions, the majority of which are poorly understood (Nendza and Russom, 1991). An important distinction in ecotoxicology is between mechanism and mode of toxic action. While the terms "mechanism" and "mode" are often used interchangeably, they mean something quite different. Mode of toxic action refers to the response at the organismal level, while mechanism refers to the response at the chemical level. Historically research has measured the mode of action and attempted to explain these results by the mechanism(s) of action.

Acute toxicity can be compiled into two general categories: reversible narcoses (i.e., non-specific toxicity) and irreversible reactivity (i.e., specific toxicity) (Schultz, 1997). As many as 70% of all industrial organic chemicals are thought to exhibit a narcosis mode of toxic action (Bradbury and Lipnick, 1990). Narcosis is the general term that describes the interaction between a xenobiotic and the cellular membrane, the theoretical site of action. Historically, narcotic effects

were estimated by the ability of a chemical to partition into the cellular membrane as quantitated by the 1-octanol/water partition coefficient (K_{OW}). Narcotic effects are thought to result in non-covalent interactions, such as the disruption of van der Waals interactions between lipid and/or protein components within the membrane (Abernethy et al., 1988; Franks and Lieb, 1990). Conversely, non-reversible (i.e., specific) toxicity is related to the ability of a toxicant to elicit a covalent interaction with a biological system. Among the specific toxicities electro(nucleo)philicity is the most common (Hermens, 1990). It is reasonable to assume a continuum between these two extremes. In other words, a robust data set is likely to contain compounds that impart their toxicity via reversible narcosis, as well as compounds exhibiting non-reversible mechanisms.

According to McFarland (1970), chemical toxicity is a combination of penetration into the biological membrane and the interaction of the toxicant with the site of action. McFarland's approach is represented mathematically as follows:

$$\text{Log (toxicity)}^{-1} = A (\text{log penetration}) + B (\text{log interaction}) + C \quad [1].$$

"C" in this equation is the Y-intercept

This approach is toxicokinetic- and toxicodynamic-based and does not require separation of electronic and steric effects, which is a difficult task. The toxicokinetic phase includes chemical uptake and metabolic activation. Toxicokinetics can be thought of as the ability of the toxicant to reach the molecular site of action. The toxicodynamic phase includes the molecular/biochemical

responses, which lead to cellular or physiological responses and ultimately an organismal response.

The approach modeled by McFarland, equation [1], is a surface, which in its simplest configuration takes the shape of a plane. The "X" and "Z" axes in this plane are comprised of descriptors for the penetration and interaction terms, which are independent variables. The "Y" axis quantitates toxic potency. To maximize the versatility of such a plane will require the use of global parameters to quantify both penetration and interactions. $\log K_{OW}$ is the universal descriptor for biouptake (i.e., penetration). There is no universal descriptor of interaction. However, since soft electrophilicity is the second most common mode of action (Hermens, 1990) and such electrophilic interactions are orbitally controlled (Mekenyan and Veith, 1993), frontier orbital energies, especially E_{LUMO} , are the logical choice for the interaction term. Soft electrophiles differ from hard electrophiles in that the former are orbitally controlled, and the latter are charge controlled.

The response-plane to describe potency of toxic chemicals requires the separation of baseline nonpolar narcotics from electro(nucleo)philic toxicants. The rationale for this separation is the assumption that, in baseline nonpolar narcosis, the stereoelectronic interaction is nil (i.e., B equals zero in equation [1]). If this assumption is true, then it is possible to overestimate toxicity by the inclusion of descriptors for reactivity. Since the response-plane approach uses only broad terms for electro(nucleo)philic interactions, two types of outliers can be expected. First, outliers can be in the form of toxicity greater than predicted (i.e., above the surface).

In such cases, the reactivity of the chemical will be greater than quantified by E_{LUMO} . Secondly, outliers can be in the form of toxicity less than predicted (i.e., below the surface). In such cases, either the uptake will be less than quantified by $\log K_{\text{OW}}$, or the reactivity of the chemical will be less than quantified by E_{LUMO} .

In general, bioreactive compounds which result in covalent bonds are involved in electro(nucleo)philic interactions that encompass addition or substitution of electron-rich atoms or substituents to nucleophilic sites in cellular amino acids or their polymers (Hermens, 1990; Mekenyan and Veith, 1993, 1994; Veith and Mekenyan, 1993). Examples include Schiff-base formation and Michael-type addition (Karabunarliev, 1996a). While historically modeling approaches for such soft electrophiles used electronic substituent constants, more recent studies, including those by Cronin and Schultz (1998) and Akers et al. (1999) focused on the use of quantum chemical descriptors, such as E_{HOMO} and E_{LUMO} (see below).

An understanding of covalent reactivity is founded in the principles of chemical bonds and reactions. Chemical bonds are formed when the atomic orbital of one atom interacts with the atomic orbital of another atom to form molecular orbitals. The electrons in molecular orbitals are shared between the two atoms making a molecular bond. The frontier orbitals are those that are involved in bond formation. These orbitals are the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). An electron from the HOMO orbital of a nucleophile fills the LUMO orbital of an electrophile forming a bond.

Soft electrophiles have low-energy LUMO values (e.g., < 0.5 mV). Soft nucleophiles have high-energy HOMO values (e.g., > -10.0 mV).

The electrons brought by each atom to the bond are shared between them. Sigma (σ) bonds occur by electron overlap along the axis joining the two nuclei. Pi (π) bonds occur by electron overlap above and below the internuclear axis. Because of the patterns of electron overlap, an individual π bond is not as strong as a σ bond. The net result is a π bond is stronger and shorter than a σ bond but less than twice as strong. From a reactivity standpoint, the important feature of π bonds is that because there is no electron density between the two nuclei, the electrons are concentrated in “clouds” above and below the plane of the bond. As such these electrons are held less tightly by the nuclei than electrons of σ bonds.

Mono-esters are structurally asymmetrical chemicals having the general formula $R(=O)-O-R'$ (where R means alkyl group). While previous studies have determined that basic esters exhibit excess acute toxicity in fish (Veith et al., 1985; Kamlet et al., 1987), basic ester toxicity to *T. pyriformis* is via a non-polar narcotic mechanism of action (Jaworska et al., 1995). More recently Akers et al., (1999) determined the relative toxicity of halogenated alcohols and nitriles in the *Tetrahymena* population growth assay. They noted that modification of the molecular structure with the addition of one or more halo-groups alters the mechanism of toxic action from that of non-polar narcosis to that of reactive electrophiles (Akers et al., 1999).

Aliphatic monocarboxylic acids are also structurally asymmetrical having the general formula $R(=O)OH$. They contain a polar hydroxy group directly adjacent to a lone carbonyl moiety and are structurally similar to esters. Because of this structural similarity it can be hypothesized that they will behave in a similar fashion to esters when halogenated. That is, if halogen-substitution of an ester alters the mechanism of toxic action from non-polar narcosis to reactive electrophiles it should follow that the same scenario should be observed with a carboxylic acid. However, the toxic potency of carboxylic acids will be altered by the ionizability of the polar hydroxyl moiety at physiological pH.

The purpose of this study was to evaluate the aquatic toxicity of selected halogen-substituted esters and carboxylic acids, in particular bromine-containing compounds. The specific aims of this study were the following: 1) to perform concentration-response, aquatic ecotoxicity tests using the *Tetrahymena pyriformis* population growth impairment assay, and 2) to evaluate structure-toxicity and property-toxicity relationships for these esters and carboxylic acids.

CHAPTER 2

MATERIALS AND METHODS

All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, USA. Each had a purity of 95% or greater and was not repurified prior to use. Stock solutions of each were prepared in dimethyl sulfoxide at concentrations less than 0.75% (Schultz, 1997)

Tetrahymena pyriformis population growth impairment testing was performed following the protocol described by Schultz (1997). Tests were conducted in foam-stoppered 250 ml Erlenmeyer flasks containing 50 ml of sterile, semi-defined proteose peptone-based medium. Test conditions (static, 40-hrs at $27 \pm 1^\circ\text{C}$) allowed for 8-9 cell cycles in control cultures. The assay measures population density spectrophotometrically as optical density at 540 nm. Light with a wavelength of 540 nm is passed through a standard path and population density is measured by the amount of this light that is able to pass through. The greater the quantity of light absorbed by the *Tetrahymena pyriformis* population the denser the population is. A less dense population will allow for a larger portion of light to pass completely through.

Each halo-compound was tested in a range-finder prior to testing in duplicate for three additional replicates. Each definitive test consisted of six to eight serial concentrations. Two quality control checks were run; a control and a blank. The control contained no test material but was inoculated with *T. pyriformis*; the blank contained neither toxicant nor inoculum. These checks provided a measure of

the acceptability of the replicate and a basis for interpreting treatment data. Only controls with absorbency values > 0.6 but < 0.75 were used in the analyses.

The 50% growth inhibitory concentration, IGC_{50} , was determined for each toxicant by Probit Analysis of Statistical Analysis System (SAS) software (SAS Institute Inc., 1989). All statistical analysis was performed on nominal concentrations as chemical analysis of concentrations was not performed.

Logarithms of the 1-octanol/water partition coefficients ($\log K_{OW}$) values were secured from CLOGP for Windows (BIOBYTE Corp., Claremont, CA) software. The energy of the lowest unoccupied molecular orbital (E_{LUMO}) was calculated. Initially, each molecule was built and its energy minimized with NEMESIS for PC molecular modelling software (Oxford Molecular Limited, Oxford, UK). The structure was then converted into a MOPAC internal file via the BABEL shareware file conversion program. Subsequently, each compound was geometrically optimized and molecular orbital quantum chemical calculations performed using the AM1 Hamiltonian in the MOPAC6 program (Stewart, 1996). The pK_a of each carboxylic acid was determined using the MICROQSAR software (Hunter, 1988) and the hydrogen-suppressed molecular structure of each halo-substituted acid. The hydrogen-suppressed molecular structure of a chemical is, as the name would suggest, the chemical structure of a molecule minus its hydrogen atom constituents.

Quantitative structure-toxicity relationships were examined using log of the inverse of the IGC_{50} ($\log (IGC_{50}^{-1})$) in mM as the dependent variable, and the log

K_{ow} , pK_a , or E_{LUMO} 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). In addition, 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 noted.

CHAPTER 3

RESULTS

Structure-Toxicity Relationships of Halogen-Substituted Esters

The Chemical Abstract Service registry number and hydrogen-suppressed molecular structure of each ester evaluated in this study is presented in Table 1.* Structurally these esters have in common that they are all aliphatic, contain only one ester group and one or two halogen moieties (i.e., bromine- or chlorine atoms).

α -Haloesters are straight-chained (i.e., non-branched) esters with a halogen atom in the two-position. The toxic potency of these compounds is listed in Table 2. An examination of these data reveal the toxicity of this type of ester is related to alkyl-chain length in a parabolic manner with potency being greatest in the largest and smallest molecules (Figure 1). This relationship of decreasing toxicity with increasing hydrocarbon number is reversed when the chain reaches six-carbons in length.

In Table 3 straight-chained α -haloesters are evaluated against their isoconformer. An isoconformer has in addition to the halogen a methyl group bonded to the carbon next to the carbonyl group. In both of these cases, the isoconformer is less toxic than its straight-chained counterpart.

*All tables and figures may be found in the appendix

Straight-chain esters with the bromine atom at the end of the chain are listed in Table 4. In this case toxicity is directly proportional to chain-length and does not have any discernible relationship to the distance of the halogen from the ester toxicophore (Figure 2).

Esters, as stated previously, are asymmetrical having the general formula $R(=O)-O-R'$ (where R means alkyl group). In Table 5 esters with the halogen on the carbonyl-side [$R(=O)$] of the molecule were evaluated against esters of the same chain-length with the halogen on the ether-side [$O-R'$]. In both cases, the chemical having the halogen on the ether-side was more toxic than its counter part with the halogen on the carbonyl-side.

Table 6 lists toxicity data for mono- and dibromine-substituted derivatives. The 2,3-dibromoesters were more toxic than either the 2- or 3-bromo-substituted analog of equal chain length. Moreover, ethyl-2,3-dibromopropionate was more toxic than ethyl-2,3-dibromobutyrate.

The toxicity data for the three chlorinated esters in this study as well as their brominated counterparts are reported in Table 7. In each of these cases, the bromoester was more toxic than its associated chlorinated derivative.

The structure-toxicity relationships of halogen-substituted esters demonstrate four trends. 1) Halo-substituted esters with the leaving group (i.e., halogen) next to the carboxyl group (i.e., α -haloesters) are more toxic than ones with the halogens separated from the carboxyl group by either methylene or ether moieties. 2) Hydrocarbon branching (i.e., isoconfiguration) on the carbon substituted with the halogen group diminishes toxicity. 3) Multiple halo-substitution enhances haloester

toxic. 4) Bromine-substituted esters are more potent toxicants than chlorine-substituted esters.

Property-Toxicity Relationships of Halogen-Substituted Esters

Since esters can elicit an acute toxic response via narcotic or electro (nucleophilic) mechanisms, property-toxicity relationships focused on hydrophobicity and frontier orbital parameters. Toxic potency and property data for all haloesters tested are presented in Table 8. They are listed in increasing order of E_{LUMO} . A plot of toxicity ($\log \text{IGC}_{50}^{-1}$) versus hydrophobicity ($\log K_{\text{OW}}$) shown in Figure 3 revealed all the halo-substituted esters exhibited an acute toxicity in excess of that predicted by the baseline, nonpolar narcosis QSAR [$\log \text{IGC}_{50} = 0.828 (\log K_{\text{OW}}) - 1.924$] (Jaworska et al., 1995). Moreover, regression analysis of these data showed no relationship with the r^2 being 0.127.

Regression analysis of toxicity ($\log \text{IGC}_{50}^{-1}$) versus reactivity measured by the electrophilic index (E_{LUMO}) yielded:

$$\log \text{IGC}_{50}^{-1} = -1.30 (E_{\text{LUMO}}) + 0.88; \quad [2]$$

$$n = 21, r^2 = 0.539, s = 0.557, F = 22, \text{Pr} > F = 0.0002.$$

Three of the isoelectronic windows represented by these data (Table 8) had enough chemicals (i.e., 4 or more) to run toxicity versus hydrophobicity regression analyses. Groups 2 (short-chained 2-bromoalkanoates) and 3 (long-chained 2-

bromoalkanoates) showed no relationship with $\log K_{OW}$ ($r^2 = 0.000$). Group 6 (end-carbon bromo-substituted alkanoates) showed a weak relationship with $\log K_{OW}$ ($r^2 = 0.697$).

When toxicity of all the chemicals was regressed using both $\log K_{OW}$ and E_{LUMO} as descriptors a weak relationship was observed:

$$\log IGC_{50}^{-1} = 0.16 (\log K_{OW}) - 1.22 (E_{LUMO}) + 0.53 \quad [3]$$

$$n = 21, r^2 = 0.557, s = 0.560, F = 11, Pr > F = 0.0007.$$

Figure 4 shows a scatter plot of the observed values vs. the predicted values for equation [3]. Significant positive outliers included propylbromoacetate, and ethyl-2,3-dibromopropionate. The only significant negative outlier was ethyl-2-bromoisovalerate. When the outliers are removed with subsequent reanalysis, the following equation was derived:

$$\log IGC_{50}^{-1} = 0.38 (\log K_{OW}) - 0.80 (E_{LUMO}) - 0.10 \quad [4]$$

$$n = 18, r^2 = 0.740, s = 0.331, F = 21, Pr > F = 0.0001.$$

Significant negative outliers for equation [4] included ethyl-2-bromoisobutyrate, and ethyl-2-chloropropionate. Their removal followed by an additional analysis yielded;

$$\log IGC_{50}^{-1} = 0.34 \log K_{OW} - 0.84 (E_{LUMO}) + 0.04 \quad [5]$$

$$n = 15, r^2 = 0.848, s = 0.255, F = 33, Pr > F = 0.0001.$$

A comparison of Equations [4] and [5] reveals that while the data fit improved, neither slope term nor the intercept changed significantly.

The property-toxicity relationships for halogen-substituted esters demonstrate four points. 1) Halosters are more toxic than narcotic basic esters. 2) Toxic potency of haloesters is poorly modeled by the electrophilicity parameter (E_{LUMO}) and, 3) even less so by hydrophobicity. 4) With noted exceptions a combination of the above two terms explained better than 85% of the variability in toxic potency. The major exceptions were small molecules (i.e., three carbon atom-containing compounds) which were more toxic than predicted.

Structure-Toxicity Relationships of Halogen-Substituted Carboxylic Acids

The Chemical Abstract Service registry number and hydrogen-suppressed molecular structure of each halogen-substituted acid in this study is presented in Table 9. Structurally these halogen-substituted acids have in common that they are all aliphatic, contain only one carboxylic acid group (i.e., $-C(=O)OH$), and contain one or two halogens (i.e., bromine or chlorine-atoms).

α -Position, halo-substituted carboxylic acids are straight-chained acids with a bromine atom in the two-position. The toxic potency of these compounds is listed in Table 10. An examination of the toxicity of α -haloacids reveals that potency is directly related to alkyl chain-length in a parabolic manner with potency being greatest in compounds having the longest and shortest alkyl-chain (Figure 5). The smallest, 2-bromobutyric acid is most toxic.

In Table 11 two straight-chain α -position, halo-substituted carboxylic acids are evaluated against their isoconformer. In both cases the straight-chained derivative was more toxic than its branched isomer.

Straight-chained acids with the bromine on the end carbon atom are evaluated in Table 12. The acids in this series show a direct relationship between toxic potency and the number of methyl groups in the chain (Figure 6). The exception is the smallest of these acids, 3-bromopropionic acid. It was more toxic than either 4-bromobutyric acid or 5-bromovaleric acid.

Table 13 compares the toxicity for 3-bromopropionic acid with that for 2,3-dibromopropionic acid. The 2,3-dibromoninated acid is nearly two-orders of magnitude more toxic than the 3-bromo-derivative.

The toxicity data for the three chloro-substituted acids examined in this study as well as each bromo-homolog are listed in Table 14. In each comparison the chloro-isomer was more toxic than the brominated homolog.

The structure-toxicity relationships for halogen-substituted carboxylic acids revealed four trends. 1) Hydrocarbon branching (i.e., isoconfiguration) on the carbon atom substituted with the halogen atom diminishes toxicity. 2) Toxic potency for halo-substituted carboxylic acids is directly proportional to alkyl chain-length as long as the compound is greater than three carbon-atoms in length. 3) Multiple halogen-substitution enhances toxicity. 4) Chloro-substituted acids are more toxic than bromo-substituted acids.

Property-Toxicity Relationships of Halogen-Substituted Carboxylic Acids

Toxic potency and property data for all halogen-substituted carboxylic acids are presented in Table 15. They are listed in increasing order of E_{LUMO} . Regression analysis of toxicity ($\log \text{IGC}_{50}^{-1}$) versus hydrophobicity ($\log K_{\text{OW}}$) showed only a very poor relationship with the r^2 being 0.348. Visual inspection of the scatter plot of $\log \text{IGC}_{50}^{-1}$ versus $\log K_{\text{OW}}$ (Figure 7) revealed all halogen-substituted acids to be in excess of that predicted by the baseline nonpolar narcosis QSAR [$\log \text{IGC}_{50} = 0.30 (\log K_{\text{OW}}) - 0.70$] (unpublished data from Seward and Schultz). In addition, two distinct, nearly linear groupings of chemicals were observed. The three propionic acids make up the more toxic upper group, whereas, those acids with more than three carbon atoms make up the lower group. Regression analysis of $\log \text{IGC}_{50}^{-1}$ versus $\log K_{\text{OW}}$ for the twelve acids containing greater than three carbon atoms yielded:

$$\log \text{IGC}_{50}^{-1} = 0.67 (\log K_{\text{OW}}) - 1.15 \quad [6]$$

$$n = 12, r^2 = 0.756, s = 0.326, F = 31.05, \text{Pr} > F = 0.0002$$

Two outliers were apparent in equation [6]. Both 2-bromooctanoic acid and 2-bromoisovaleric acid were less toxic than the model predicted.

No linear relationship (r^2 values of 0.104 and 0.085, respectively) was observed with either pK_{a} or E_{LUMO} for the complete data set of fifteen acids. Even

less of a relationship was observed when the three carbon acids were dropped from the model ($r^2 = 0.003$ for pK_a and 0.004 for E_{LUMO} , $n = 12$).

Adding pK_a to the hydrophobicity model in equation [6] added very little predictive power to the model and yielded the following equation:

$$\log IGC_{50}^{-1} = 0.69 (\log K_{OW}) + 0.09 (pK_a) - 1.52 \quad [7]$$

$$n = 12, r^2 = 0.771, s = 0.333, F = 15.15, Pr > F = 0.0013$$

Adding E_{LUMO} to the hydrophobicity model in equation [6] also added very little predictive power to the model and yielded the following equation:

$$\log IGC_{50}^{-1} = 0.70 (\log K_{OW}) + 0.20 (E_{LUMO}) - 1.25 \quad [8]$$

$$n = 12, r^2 = 0.778, s = 0.327, F = 15.79, Pr > F = 0.0011$$

Only one isoelectronic window was found in the halo-substituted carboxylic acid data (Table 15). This group was the 2-bromoacids. Regression analysis of toxicity versus hydrophobicity showed no relationship with $\log K_{OW}$ ($r^2 = 0.000$).

The property-toxicity relationships for halogen-substituted carboxylic acids revealed four trends. 1) The toxic potency of halogenated carboxylic acids is greater than non-halogenated carboxylic acids. 2) Toxic potency of haloacids is modeled poorly by the ionization constant pK_a , and even less so by the electrophilicity quantum chemical parameter E_{LUMO} . 3) The hydrophobicity parameter $\log K_{OW}$ models haloacids reasonably well. 4) Small acids (i.e., three carbon atom compounds) could not be modeled in the same grouping as larger acids (i.e., four or

more carbon atoms). Based on relative hydrophobicity the smaller halogen-substituted carboxylic acids were more toxic than the rest of the data set.

Comparison of Structure-Toxicity Relationships for Halogen-Substituted Esters and Carboxylic Acids

Esters with a bromine atom substituted in the α -position show a similar trend in toxicity to α -position, bromine-substituted, carboxylic acids (Figures 1, and 5; Tables 2, and 10). In both cases toxic potency is directly related to alkyl chain-length in a parabolic manner with potency being greatest in compounds having the shortest and longest alkyl-chain.

Straight-chained, α -position, bromoesters and straight-chained, α -position, bromoacids exhibit the same relationship when their toxicity is compared with that of their branched-chained, isoconformer (Tables 3, and 11). In all cases the straight-chained derivative was more toxic than the isoconformer.

Straight-chained, end-position, bromo-substituted esters and straight-chained, end-position, bromo-substituted carboxylic acids showed the same direct relationship between potency and alkyl chain-length (Figures 2, and 6; Tables 4, and 12). The one exception to this rule is 3-bromopropionic acid. It was more toxic than the next longest chained acid.

Since carboxylic acids do not have an ether moiety, a comparison between esters and carboxylic acids with the bromine substitution on the ether versus carbonyl side of the molecule is not possible. The mono versus di-bromo-

substituted comparison for esters and carboxylic acids yielded the same results (Tables 6, and 13). In each case the dibromo-substituted compound was more toxic than the mono-substituted analogs.

Differing results were observed in the comparison between the toxic potencies of chlorine and bromine-substituted esters, and chlorine and bromine-substituted carboxylic acids. In the case of the esters the bromo-homologs were more toxic than the chloro-containing one (Table 7). The opposite result was seen with the carboxylic acids (Table 14).

The structure-toxicity relationship comparison for halogen-substituted esters and carboxylic acids revealed two common trends. 1) Hydrocarbon branching diminishes toxicity. 2) Multiple halogen-substitution enhances toxicity. The major difference in structure-toxicity relationships between halogen-substituted esters and carboxylic acids was in the comparison of bromine and chlorine homologs. Bromine-substituted esters are more toxic than their chloro-substituted homologs, and chlorine-substituted carboxylic acids are more toxic than their bromo-substituted homologs.

Comparison of Property-Toxicity Relationships for Halogen-Substituted Esters and Carboxylic Acids

A relationship with $\log K_{OW}$ could not be established for the complete data sets of either haloesters or haloacids ($r^2 = 0.127$, and 0.348 respectively).

Haloesters showed a slight relationship with E_{LUMO} ($r^2 = 0.539$) whereas, no such relationship was evident with the haloacids ($r^2 = 0.085$).

Both data sets contained positive outliers in the form of small three carbon compounds. Their removal followed by regression analysis with both $\log K_{OW}$ and E_{LUMO} created quality distinct models for both the haloesters ($\log IGC_{50}^{-1} = 0.34 \log K_{OW} - 0.84 (E_{LUMO}) + 0.04$; $n = 15$, $r^2 = 0.848$), and haloacids ($\log IGC_{50}^{-1} = 0.70 (\log K_{OW}) + 0.20 (E_{LUMO}) - 1.25$; $n = 12$, $r^2 = 0.778$).

A comparison of the two models reveals that each has a similar predictive power in the neighborhood of 80%. A comparison of the y-intercepts (0.04, and -1.25, respectively) revealed the haloesters to be significantly more toxic than haloacids. While, the $\log K_{OW}$ slope of the haloacid model was twice as steep as that of the halo-ester model ($= 0.70$, and 0.34 , respectively), A comparison of the E_{LUMO} slopes (-0.84 , and 0.20 respectively) shows that haloacid toxicity is negatively correlated with electrophilicity whereas, haloester toxicity is positively but to a lesser extent correlated with electrophilicity.

In summary four trends were found in the comparison of the property-toxicity relationship results between halogen-substituted esters, and halogen-substituted carboxylic acids. 1) A hydrophobicity-dependent relationship was not evident in either of the complete data sets. 2) Although a slight relationship with E_{LUMO} was observed for the esters it was still a poor one, and no relationship was observed with E_{LUMO} for the carboxylic acids. 3) The combination of the hydrophobic parameter $\log K_{OW}$, and the electronic parameter E_{LUMO} did produce

quality QSARs for both haloesters and haloacids but not until the smaller molecules were removed from the analysis. 4) Halogen-substituted esters are more toxic than halogen-substituted carboxylic acids.

CHAPTER 4

DISCUSSION

Halogen Substituted Esters

The complexity of the structure-toxicity relationships and property-toxicity relationships suggest that the toxicity of haloesters is by multiple and /or mixed mechanisms of action which are electro(nucleo)philic in character. The following discussion will elaborate on why this is so.

The toxicity of the ethyl-2-bromoalkanoates (see Table 2) is explained by a combination of steric hindrance and hydrophobicity. The potential to be involved in electro(nucleo)philic reactions is fairly constant with α -halogen-substituted esters as indicated by a constant E_{LUMO} value. However, as seen in Figure 1 there is a parabolic relationship between toxicity and molecular size. This relationship is the net result of two physicochemical effects. First the increased length of the straight-chained hydrocarbon moieties reduces electro(nucleo)philic reactivity due to steric hindrance between the nucleophile and the halogen. Second, this steric hindrance is offset by the increase in biouptake resulting from the increase in hydrophobicity from the increased length of the hydrocarbon moieties.

Hydrocarbon branching, especially near the carbonyl-group, causes further steric hindrance. This further hindrance explains the reduced potency observed in

the branched isoconformer as compared with its straight-chained derivative (see Table 3).

The toxicity observed in straight-chained esters with the bromine atom in the terminal position (see Table 4) is better explained by hydrophobicity than electro(nucleo)philic reactivity. Once the β -position is halogenated, or more assuredly the χ -position, reactivity becomes more consistent, as indicated by consistence in E_{LUMO} values. However, the increase in toxicity resulting from the increase in number of methylene groups increases hydrophobicity (i.e., biouptake).

Not only are there toxicity differences between the position of the halogen, but also the proximity of the halogen to the carbonyl group. The carbonyl moiety adjacent to the leaving group (i.e., the halogen) stabilizes negative charges. Negative charge builds up in the transition-state and the reaction takes place faster. Proximity to the carbonyl moiety should increase the ability to donate an electron to the π bond; thereby making the compound more toxic. As seen in comparing the potency of ethyl-2-bromopropionate and ethyl-3-bromopropionate this is indeed the case. In Table 5 compounds with the bromine atom on the carbonyl-side of the molecule were compared against those with the bromine atom on the ether-side. Both ethyl-5-bromovalerate and 5-bromopentylacetate have the bromine atom more than two atoms away from the carbonyl group; therefore the constant difference in their toxicity is minimal. However, there does exist a significant difference in the toxicities of 4-bromobutyl acetate and ethyl-4-bromobutyrate. The reason for this difference probably lies in the fact that the electronegativity of oxygen, even in the

ether form, is polarized in the direction of the oxygen. Thus, the halogen leaving group is more reactive.

The chemistry of halogenated organic compounds is related to both the properties of the leaving group and to electron-withdrawing properties. As shown in Table 6 the addition of a second bromine atom in the β -position to α -haloesters increases toxicity more than can be accounted for by hydrophobicity. The addition of a second bromine atom improves the leaving properties of the molecule and increases the rate of the substitution reaction. Additionally, bromine is a better leaving group than chlorine. As shown in Table 7, bromoesters are more toxic than chloroesters.

The structure-toxicity and property-toxicity relationships observed in this study for halogen-substituted esters indicate that multiple properties such as hydrophobic and electronic properties control the toxicity of esters. However the predictive power of multivariate models using hydrophobic and electrophilic properties are not without exceptions, especially small, highly reactive molecules.

Halogen Substituted Carboxylic Acids

Similar to haloesters, the complexity of the structure-toxicity relationships and property-toxicity relationships of haloacids suggest that toxicity is by multiple and /or mixed electro(nucleo)philic mechanisms. However, carboxylic acid toxicity is more complicated due to ionization. All of the acids in this study have pK_a values between 2.26 and 4.74. As such they are mostly ionized at physiological pH.

Toxicity can be expected to be influenced by the presence of ionic components. Acidity of the carboxylic acid is enhanced by the presence of the halogen atom which introduces an inductive effect increasing acid strength. Currently good descriptors for modeling ionic species do not exist.

As with the esters there is a parabolic relationship between toxicity and molecular size of α -halogen-substituted acids. The difference in toxicity of α -halogen-substituted acids (see Table 10), like with the esters, is explained by a combination of steric hindrance and hydrophobicity. With the halo-atom in the 2-position, the acid's potential to undertake an electro(nucleo)philic reaction is fairly constant, as indicated by the E_{LUMO} values. However, the increased length of the hydrocarbon moieties reduces reactivity by steric hindrance of the interaction of the nucleophile and the halogen. This steric hindrance is offset by the increase in hydrophobicity resulting from hydrocarbon groups. As with the esters, hydrocarbon branching, especially near the carbonyl-group, increases steric hindrance. This increased hindrance reduces toxicity. Therefore, the branched conformers are less toxic than straight-chained acids (see Table 11). As with the haloesters steric hindrance is clearly evident in the toxicity of the isoconformer 2-bromisovaleric acid. It was less toxic than its hydrophobicity would predicted.

As shown in Table 15, bromo-substituted acids with the halogen on the end carbon atom have varied reactivity. Yet as shown in Figure 7 their toxicity exhibits a hydrophobicity dependent relationship. The smaller acids 3-bromopropionic acid, 3-chloropropionic acid, and 2,3-dipropionic acid do not follow this hydrophobic relationship. Each was more toxic than their four-carbon counterparts.

As noted above bromine is a better leaving group than chlorine and as such compounds with bromine substitution in general are more toxic than derivatives with a chlorine substitution. This general statement does not hold true for chemicals ionized at physiological pH. The QSARs using the log K_{OW} parameter for hydrophobicity fail to take into account the anisotropic nature of lipid bilayer biological membranes. Chemicals that are ionized at physiological pH can have a significantly greater affinity for biological membranes than the 1-octanol-water partition coefficient describes (Escher and Schwarzenbach, 1996), but less of an affinity than the neutral form. Electron withdrawing groups have an inductive effect on the resonance stabilized carboxyl groups in carboxylic acids and increase its ionization potential. Chlorine atoms have a greater capacity for electron withdrawal than bromine atoms. Due to this increased electron withdrawal capacity, the chlorinated acids are more ionizable. Because of the leaving ability of the chlorine and the increased toxicity of the chloroacids, nucleophilic addition to the double bond in the carbonyl group is likely to be the dominant reaction for this type of compound.

The structure-toxicity and property-toxicity relationships observed in this study for halogen-substituted acids indicate that multiple properties including hydrophobic and electronic control the toxicity of esters. However the predictive power of multivariate models using hydrophobic and electrophilic properties are not without exceptions, especially small highly reactive molecules.

Halogen-substituted esters and carboxylic acids are electrophiles with the possibility of multiple electro(nucleo)philic mechanisms of reactivity. An

electrophile is any chemical species that will react with a nucleophile. Nucleophiles are centered on heteroatoms (i.e., S, O, and N). Thus, amino acids and peptides are common biological nucleophiles. Because they possess a π bond, carbonyl-containing compounds such as esters and carboxylic acids are among the most important organic electrophiles. One of the most common reactions in chemistry is addition to a π bond. There are three mechanisms for such an addition: electrophilic, nucleophilic, and free radical. The only difference between electrophilic and nucleophilic addition is the sequence of steps. In both mechanisms, an electrophile and a nucleophile are added to a π bond.

Carbon atoms with leaving groups such as halogen atoms also can react as electrophiles. In such substitution reactions one group is replaced by another. This may be direct, addition followed by elimination, or visa versa. Aliphatic substitution is usually nucleophilic. Substitution at a saturated carbon is often S_N2 in nature. As noted in Figure 8, the mechanism of electro(nucleo)philic toxicity with α -haloesters and acids can be S_N2 . In such a reaction the halogen acts as the electron source and the carbonyl group as the electron sink. The net result is covalent bonding to a nucleophile and the loss of the halogen ion. Since the halogen ion is lost it is known as a leaving group. In the case of non- α -haloesters and acids, the mechanism of electro(nucleo)philic reactivity can be S_N2 via the halogen-substituted carbon or addition at the π bonded carbonyl group.

A comparison of the trends revealed by the structure-toxicity and property-toxicity relationship for halogen-substituted esters and halogen-substituted

carboxylic acids reveals one significant difference. Specifically differing results were seen in the comparison between the potencies of chlorine and bromine-substituted compounds. In the case of the esters the bromo-substituted compounds were more toxic than the chloro-homologs. The opposite result was seen in the carboxylic acids (see Tables 6, and 14). In the case of the esters the increased toxicity that the halogen brings to the compound is most likely due to an increase in S_N2 reactivity due to the leaving ability of the halogen. As the bromine atom is a better leaving group than the chlorine atom it makes sense that the bromo-substituted esters would be more toxic than the chloro-substituted homologs.

In the case of the carboxylic acids the enhanced toxicity that the halogen brings to the compound is most likely due to an increase in additivity at the carbonyl group. As the chlorine atom is more electronegative than the bromine it has a greater ability to induce nucleophilic addition at the carbonyl moiety.

In conclusion, potency of halogenated esters and carboxylic acids is by multiple mechanisms that are electro(nucleo)philic in nature. Halo-substituted esters are more toxic than the halo-substituted acids (equations 5, and 8). Ionizable species such as haloacids have a more difficult time penetrating the lipid membrane. While the predictive power of the models derived in this study using the response surface approach offer encouraging results further consideration will be required in the future as novel descriptors are generated.

The Response-Surface Approach to Quantitative Structure-Toxicity Analysis

Veith and Mekenyan (1993) revealed that the acute toxic effects to the fathead minnow could be satisfactorily explained as a plane in a three-dimensional "toxic space". They described the plane by using the $\log K_{OW}$ to model the toxicodynamics (i.e., penetration) and the molecular orbital properties, E_{LUMO} and average acceptor superdelocalizability (A_{ave}) to model the toxicokinetics (i.e., interaction). Soft electrophilicity was presented as a continuum. Narcotic chemicals showing no evidence of reactive toxicity (i.e., nonpolar narcosis) were distributed along the toxicity- $\log K_{OW}$ face. Chemicals of moderate electrophilicity (i.e., polar narcosis) were distributed in the central area of the plane. Whereas, chemicals with properties for electrophilic reactive toxicity and respiratory uncoupling were observed in upper part of the plane characterized with the lowest E_{LUMO} or highest A_{ave} values.

The response-surface approach was employed by Karabunarliev et al. (1996b) to model benzenes in a fish toxicity database. Initially, all benzenes were screened and compounds whose reactivity was likely due solely to the electrophilicity of a substituent were eliminated. It was surmised that the resulting planes in the toxicity/hydrophobicity/reactivity space combine different modes of toxic action into a single QSAR. Cronin and Schultz (1996) modeled *Tetrahymena* toxic potency data for 120 phenols, representing several modes of action using a response-surface approach. Most recently, Cronin and Schultz (1998) developed

QSARs with logarithm of the inverse of the 15-min toxicity data (pT_{15}) to *Vibrio fischeri* (the acute Microtox™ test). A statistically robust response-plane ($r^2 = 0.892$) was developed for a combination of alkanones acting by the non-specific, baseline narcosis mechanism of action, aldehydes acting by the Schiff-base formation mechanism of electrophilicity, and alkenals acting by the Michael-type acceptor mechanism of electrophilicity. Cronin et al. (1998) developed a similar plane for nitrobenzene derivatives including highly reactive skin sensitizing agents.

Since the response-plane approach uses only broad terms for electro(nucleo)philic interactions, two types of outliers can be expected. First, outliers can be in the form of toxicity greater than predicted (i.e., above the surface). In such cases, the reactivity of the chemical will be greater than quantified by E_{LUMO} . Secondly, outliers can be in the form of toxicity less than predicted (i.e., below the surface). In such cases, either the uptake will be less than quantified by $\log K_{OW}$, or the reactivity of the chemical will be less than quantified by E_{LUMO} . In the former case, ionization may alter uptake. In the latter case, toxicity may be reduced due to steric inhibition. As noted by Karelson et al., (1996) a weakness of quantum-chemical parameters such as E_{LUMO} is the failure to address bulk related effects.

The results presented here mimic previous studies on halogenated aliphatic alcohols and nitriles (Akers et al., 1999). Non-halogen-substituted aliphatic alcohols and nitriles act by non-polar narcosis (Schultz and Tichy, 1993; Schultz, 1997). The study of Akers et al. (1999) found that the addition of a halogen atom

on aliphatic alcohols and aliphatic nitriles altered the mode of toxicity to soft electrophilicity.

Summary

In summary, the structure-toxicity relationships and the property-toxicity relationships examined in this study have revealed several parameters controlling haloester and haloacid acute toxicity. Haloesters are more toxic than narcotic basic esters. Haloesters are thought to exhibit toxicity via the S_N2 mechanism of the soft electrophilic mode of toxic action. This toxicity is imparted by the leaving ability of the halogen, which is enhanced when placed in close proximity to the carbonyl group. This leaving ability allows haloesters, especially α -haloesters, to undergo an S_N2 or addition-elimination substitution electro(nucleo)philic reaction. The rate of such reactions is inversely related to E_{LUMO} . However, the ability to predict toxic potency of haloesters by E_{LUMO} met with only limited results. This limitation is a reflection of the fact that non- α -haloesters have more limited reactivity. In addition, hydrophobicity modeled by $\log K_{OW}$ is an important factor in the explanation of their toxicity. With noted exceptions a combination of the above two terms explained better than 85% of the variability in toxic potency. The major exceptions were small α -haloesters, which were more toxic than predicted. Molecules that exhibit steric hindrance also did not model well. The latter were less toxic than the model predicted.

Halogen-substituted carboxylic acids are more toxic than narcotic basic carboxylic acids. In the case of α -haloacids this toxicity is imparted by the electronegativity of the halogen, when placed in close proximity to the carbonyl group. This allows α -haloacids, to undergo a nucleophilic addition at the carbonyl group. The rate of such reactions is directly related to E_{LUMO} . However, non- α -haloacids undergo an S_N2 or addition-elimination substitution electro(nucleo)philic reaction at the halogen-substituted carbon atom. Because of multiple mechanisms of action and ionization the ability to predict toxic potency of halo-substituted carboxylic acids by E_{LUMO} met with very poor results.

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APPENDIX

TABLE 1
CHEMICAL ABSTRACT SERVICES REGISTRY NUMBER AND HYDROGEN-SUPPRESSED
MOLECULAR STRUCTURE OF HALO-SUBSTITUTED ESTERS

Chemical	CAS#	SMILES Notation
Ethyl-2,3-dibromobutyrate	609-11-0	<chem>CC(Br)C(Br)C(=O)OCC</chem>
Ethyl-2,3-dibromopropionate	3674-13-3	<chem>BrCC(Br)C(=O)OCC</chem>
Propylbromoacetate	35223-80-4	<chem>BrCC(=O)OCCC</chem>
Ethyl-2-bromoisobutyrate	600-00-0	<chem>CC(C)(Br)C(=O)OCC</chem>
Methyl-2-bromobutyrate	319-15-4	<chem>CCC(Br)C(=O)OC</chem>
Ethyl-2-bromopropionate	535-11-5	<chem>CC(Br)C(=O)OCC</chem>
Ethyl-2-bromooctanoate	544-29-4	<chem>CCCCCCC(Br)C(=O)OCC</chem>
Ethyl-2-bromobutyrate	533-68-6	<chem>CCC(Br)C(=O)OCC</chem>
Ethyl-2-bromovalerate	615-83-8	<chem>CCCC(Br)C(=O)OCC</chem>
Ethyl-2-bromohexanoate	615-96-3	<chem>CCCCC(Br)C(=O)OCC</chem>
Ethyl-2-bromoisovalerate	609-12-1	<chem>CCC(C)(Br)C(=O)OCC</chem>
Ethyl-2-chloropropionate	535-13-7	<chem>CC(Cl)C(=O)OCC</chem>
Ethyl-3-bromopropionate	539-74-2	<chem>BrCCC(=O)OCC</chem>
Ethyl-4-bromobutyrate	2969-81-5	<chem>BrCCCC(=O)OCC</chem>
Ethyl-5-bromovalerate	14660-52-7	<chem>BrCCCCC(=O)OCC</chem>
Methyl-5-bromovalerate	5454-83-1	<chem>BrCCCCC(=O)OC</chem>
4-bromobutylacetate	4753-59-7	<chem>CC(=O)OCCCCBr</chem>
Ethyl-6-bromohexanoate	25542-62-5	<chem>BrCCCCC(=O)OCC</chem>
5-bromopentylacetate	15848-22-1	<chem>CC(=O)OCCCCCBr</chem>
Ethyl-3-chloropropionate	623-71-2	<chem>ClCCC(=O)OCC</chem>
Ethyl-4-chlorobutyrate	3153-36-4	<chem>ClCCCC(=O)OCC</chem>

TABLE 2
COMPARISON OF TOXICITY OF STRAIGHT-CHAINED 2-POSITION BROMO-SUBSTITUTED
ESTERS

Chemical	log IGC ₅₀ ⁻¹
Ethyl-2-bromopropionate	1.06
Ethyl-2-bromobutyrate	0.76
Ethyl-2-bromovalerate	0.70
Ethyl-2-bromohexanoate	0.86
Ethyl-2-bromooctanoate	1.50

TABLE 3
COMPARISON OF TOXICITY OF STRAIGHT-CHAINED AND BRANCH-CHAINED BROMO-SUBSTITUTED ESTERS

Chemical	Log IGC ₅₀ ⁻¹
Ethyl-2-bromobutyrate	0.76
Ethyl-2-bromisobutyrate	0.15
Ethyl-2-bromovalerate	0.70
Ethyl-2-bromoisovalerate	0.02

TABLE 4
COMPARISON OF TOXICITY OF STRAIGHT-CHAINED, END-POSITION, BROMO-SUBSTITUTED ESTERS

Chemical	log IGC ₅₀ ⁻¹
Ethyl-3-bromopropionate	-0.13
Ethyl-4-bromobutyrate	-0.03
Ethyl-5-bromovalerate	0.22
Ethyl-6-bromohexanoate	0.59

TABLE 5
COMPARISON OF TOXICITY OF CARBONYL-SIDE AND ETHER-SIDE BROMO-SUBSTITUTED ESTERS

Chemical	log IGC ₅₀ ⁻¹
ethyl-5-bromovalerate	0.22
5-bromopentylacetate	0.29
ethyl-4-bromobutyrate	-0.03
4-bromobutylacetate	0.39

TABLE 6
COMPARISON OF TOXICITY OF MONO- AND DI-BROMO-SUBSTITUTED ESTERS

Chemical	Log IGC ₅₀ ⁻¹
ethyl-2,3-dibromopropionate	2.21
ethyl-2-bromopropionate	1.06
ethyl-3-bromopropionate	-0.13
ethyl-2,3-dibromobutyrate	1.43
ethyl-2-bromobutyrate	0.76

TABLE 7
COMPARISON OF TOXICITY OF CHLORO- AND BROMO-SUBSTITUTED ESTERS

Chemical	Log IGC ₅₀ ⁻¹
ethyl-2-chloropropionate	-0.43
ethyl-2-bromopropionate	1.06
ethyl-3-chloropropionate	-0.50
ethyl-3-bromopropionate	-0.13
ethyl-4-chlorobutyrate	-0.28
ethyl-4-bromobutyrate	-0.03

TABLE 8.

TOXICITY AND MOLECULAR PROPERTIES OF HALO-SUBSTITUTED ESTERS

Chemical Name	log IGC ₅₀ ⁻¹	Log K _{ow}	E _{LUMO}	Isoelectronic Group
Ethyl-2,3-dibromobutyrate	1.44	2.52	-0.49066	1
Ethyl-2,3-dibromopropionate	2.21	1.98	-0.52746	1
Propylbromoacetate	2.31	1.49	-0.12497	2
Ethyl-2-bromoisobutyrate	0.15	1.80	-0.10730	2
Methyl-2-bromobutyrate	1.02	1.49	-0.10270	2
Ethyl-2-bromopropionate	1.06	1.49	-0.10248	2
Ethyl-2-bromooctanoate	1.50	4.14	-0.06771	3
Ethyl-2-bromobutyrate	0.76	2.02	-0.06650	3
Ethyl-2-bromovalerate	0.70	2.55	-0.06643	3
Ethyl-2-bromohexanoate	0.86	3.08	-0.06577	3
Ethyl-2-bromoisovalerate	0.02	2.42	-0.02879	4
Ethyl-2-chloropropionate	-0.43	1.35	0.35960	5
Ethyl-3-bromopropionate	-0.13	1.44	0.43954	5
Ethyl-4-bromobutyrate	-0.03	1.62	0.60844	6
Ethyl-5-bromovalerate	0.22	2.15	0.67569	6
Methyl-5-bromovalerate	-0.08	1.62	0.68466	6
4-bromobutylacetate	0.39	1.62	0.69477	6
Ethyl-6-bromohexanoate	0.59	2.68	0.72963	6
5-bromopentylacetate	0.29	2.15	0.73932	6
Ethyl-3-chloropropionate	-0.50	1.30	0.75456	6
Ethyl-4-chlorobutyrate	-0.28	1.48	0.93833	7

TABLE 9.
CHEMICAL ABSTRACT SERVICES REGISTRY NUMBER AND HYDROGEN-SUPPRESSED
MOLECULAR STRUCTURE OF HALO-SUBSTITUTED CARBOXYLIC ACIDS

Chemical Name	CAS#	SMILES Notation
2,3-dibromopropionic acid	600-05-5	<chem>BrCC(Br)C(=O)O</chem>
2-bromoisobutyric acid	2052-01-9	<chem>C(C)C(Br)C(=O)O</chem>
2-bromobutyric acid	80-58-0	<chem>CCC(Br)C(=O)O</chem>
2-bromovaleric acid	584-93-0	<chem>CCCC(Br)C(=O)O</chem>
2-bromohexanoic acid	616-05-7	<chem>CCCCC(Br)C(=O)O</chem>
2-bromooctanoic acid	2623-82-7	<chem>CCCCCCC(Br)C(=O)O</chem>
2-bromoisovaleric acid	565-74-2	<chem>CC(C)C(Br)C(=O)O</chem>
3-bromopropionic acid	590-92-1	<chem>BrCCC(=O)O</chem>
4-bromobutyric acid	2623-87-2	<chem>BrCCCC(=O)O</chem>
3-chloropropionic acid	107-94-8	<chem>ClCCC(=O)O</chem>
5-bromovaleric acid	2067-33-6	<chem>BrCCCCC(=O)O</chem>
6-bromohexanoic acid	4224-70-8	<chem>BrCCCCCC(=O)O</chem>
8-bromooctanoic acid	17696-11-6	<chem>BrCCCCCCCC(=O)O</chem>
4-chlorobutyric acid	627-00-9	<chem>ClCCCC(=O)O</chem>
5-chlorovaleric acid	1119-46-6	<chem>ClCCCCC(=O)O</chem>

TABLE 10
COMPARISON OF TOXICITY OF STRAIGHT-CHAINED 2-POSITION BROMO-SUBSTITUTED
CARBOXYLIC ACIDS

Chemical Name	Log IGC ₅₀ ⁻¹
2-bromobutyric acid	0.12
2-bromovaleric acid	-0.04
2-bromohexanoic acid	0.45
2-bromooctanoic acid	0.49

TABLE 11
COMPARISON OF TOXICITY OF STRAIGHT-CHAINED AND BRANCH-CHAINED BROMO-
SUBSTITUTED CARBOXYLIC ACIDS

Chemical Name	Log IGC ₅₀ ⁻¹
2-bromobutyric acid	0.12
2-bromoisobutyric acid	-0.58
2-bromovaleric acid	-0.04
2-bromoisovaleric acid	-0.55

TABLE 12
COMPARISON OF TOXICITY OF STRAIGHT-CHAINED, END-POSITION, BROMO-SUBSTITUTED CARBOXYLIC ACIDS

Chemical Name	Log IGC ₅₀ ⁻¹
3-bromopropionic acid	-0.04
4-bromobutyric acid	-0.77
5-bromovaleric acid	-0.69
6-bromohexanoic acid	0.03
8-bromooctanoic acid	1.32

TABLE 13
COMPARISON OF TOXICITY OF MONO- AND DI-BROMO-SUBSTITUTED CARBOXYLIC ACIDS

Chemical Name	Log IGC ₅₀ ⁻¹
3-bromopropionic acid	-0.04
2,3-dibromopropionic acid	1.32

TABLE 14
COMPARISON OF TOXICITY OF CHLORO- AND BROMO-SUBSTITUTED CARBOXYLIC ACIDS

Chemical Name	Log IGC ₅₀ ⁻¹
3-bromopropionic acid	-0.04
3-chloropropionic acid	-0.33
4-bromobutyric acid	-0.77
4-chlorobutyric acid	-0.68
5-bromovaleric acid	-0.69
5-chlorovaleric acid	-0.29

TABLE 15
TOXICITY AND MOLECULAR PROPERTIES OF HALO-SUBSTITUTED CARBOXYLIC ACIDS

Chemical	Log IGC ₅₀ ⁻¹	Log K _{ow}	Pk _a	E _{LUMO}
2,3-dibromopropionic acid	1.32	1.03	2.26	-0.49215
2-bromoisobutyric acid	-0.58	0.86	2.83	-0.24523
2-bromobutyric acid	0.12	1.42	2.95	-0.21035
2-bromovaleric acid	-0.04	1.61	2.97	-0.20069
2-bromohexanoic acid	0.45	2.14	3.05	-0.19472
2-bromooctanoic acid	0.49	3.19	3.05	-0.19312
2-bromoisovaleric acid	-0.55	1.48	3.01	-0.17098
3-bromopropionic acid	-0.04	0.50	4.11	0.23482
4-bromobutyric acid	-0.77	0.68	4.49	0.46030
3-chloropropionic acid	-0.33	0.41	4.15	0.57437
5-bromovaleric acid	-0.69	1.21	4.64	0.61138
6-bromohexanoic acid	0.03	1.74	4.70	0.67589
8-bromooctanoic acid	1.32	2.79	4.74	0.74753
4-chlorobutyric acid	-0.68	0.54	4.50	0.76405
5-chlorovaleric acid	-0.29	1.07	4.65	0.93545

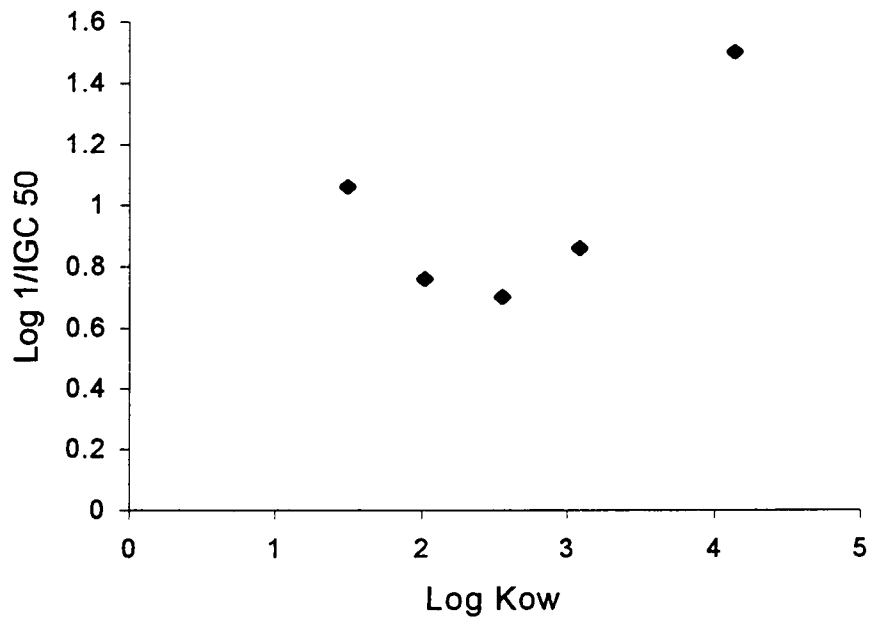


Figure 1. Plot of Toxicity vs. Hydrophobicity of Straight-Chained, 2-Position, Bromo-Substituted Esters

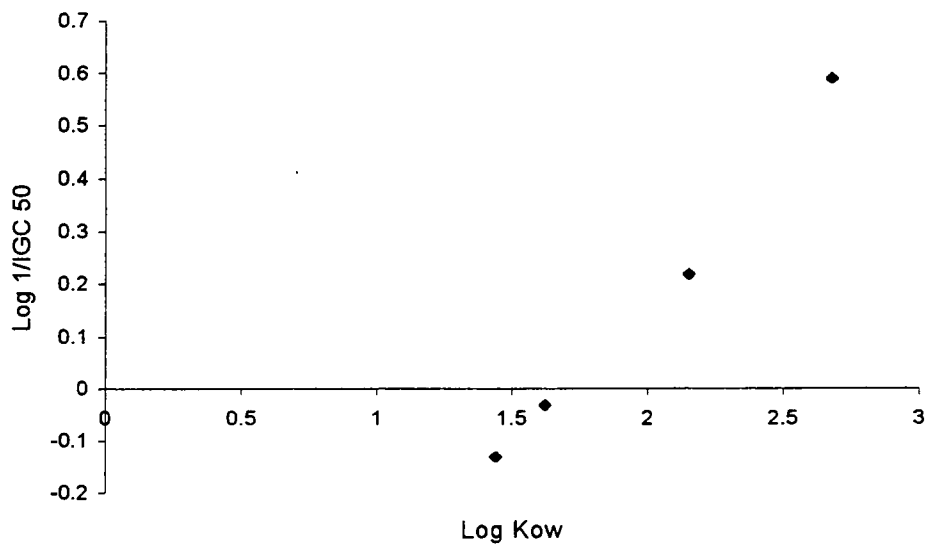


Figure 2. Plot of Toxicity vs. Hydrophobicity of Straight-Chained, End-Position, Bromo-Substituted Esters

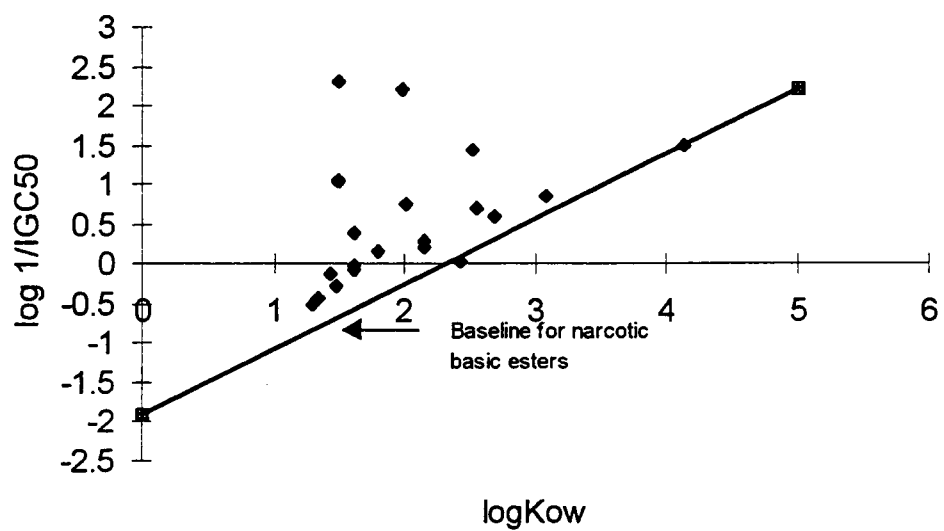


Figure 3. Plot of Toxicity vs. Hydrophobicity for Bromo-Substituted Esters

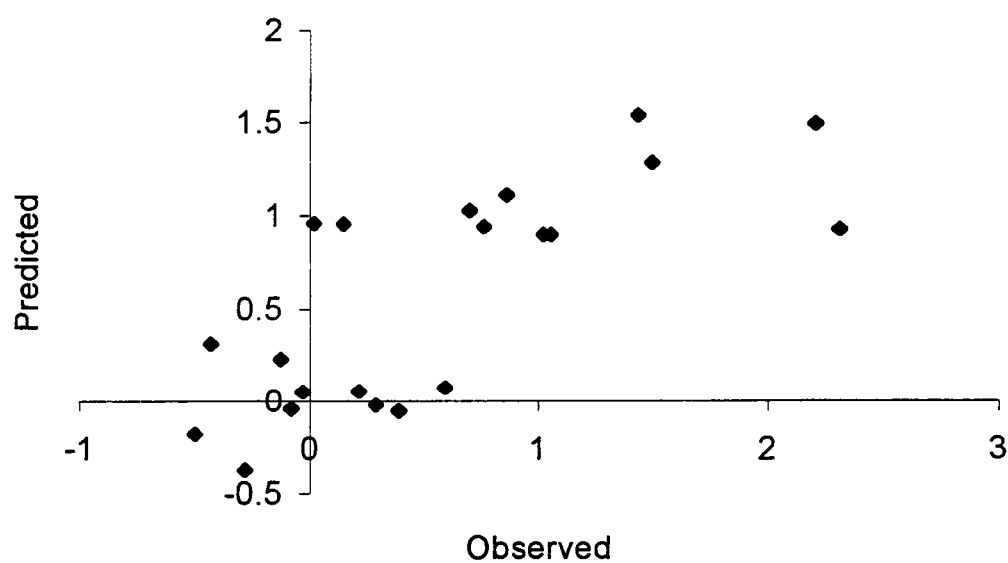


Figure 4. Plot of Observed vs. Predicted Toxicity Values for Equation 3

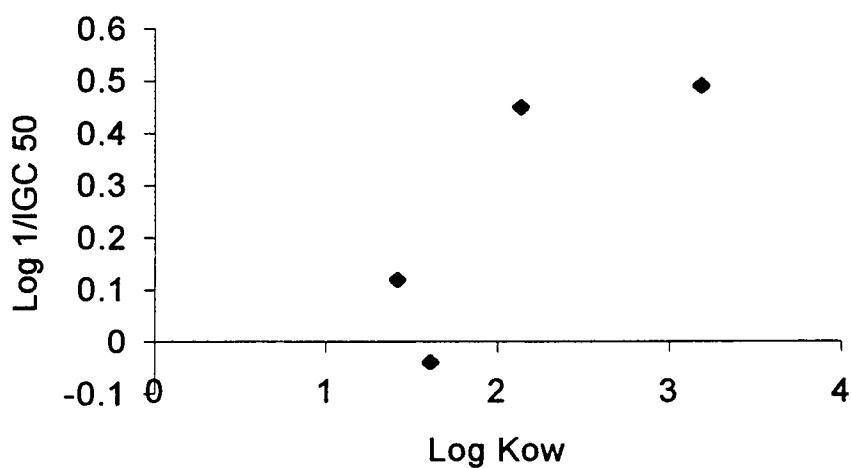


Figure 5. Plot of Toxicity vs. Hydrophobicity of Carboxylic Acids with Bromo-Substitution in the 2-Position

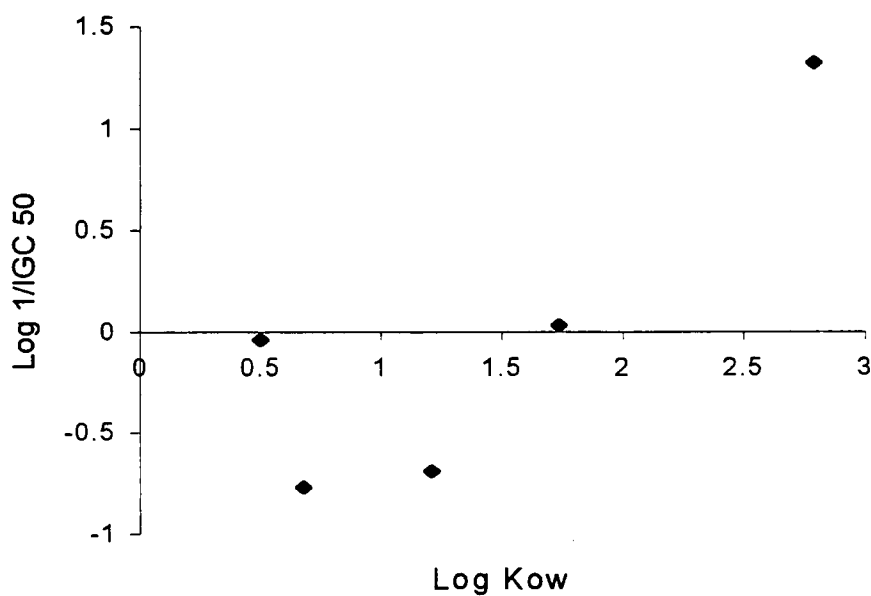


Figure 6. Plot of Toxicity vs. Hydrophobicity of Straight-Chained, End-Position, Bromo-Substituted Carboxylic Acids

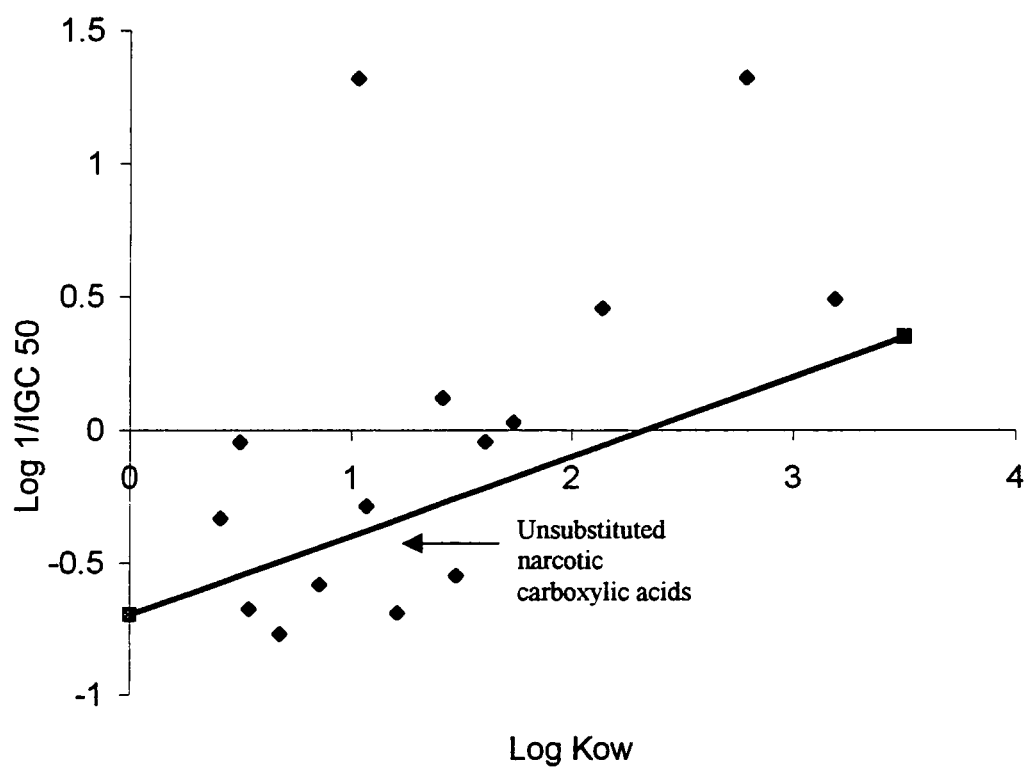


Figure 7. Plot of Toxicity vs. Hydrophobicity for Halo-Substituted Carboxylic Acids

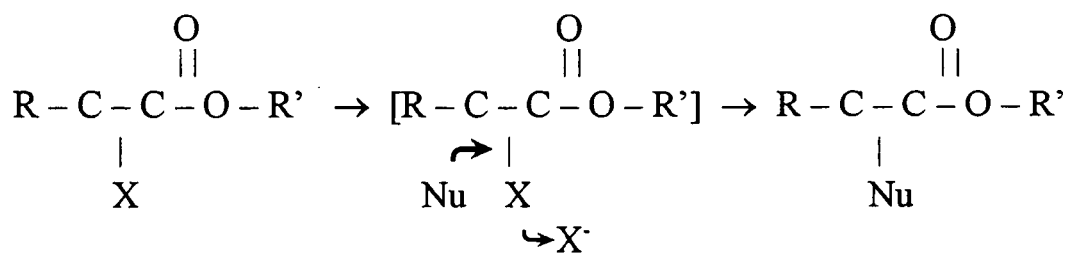


Figure 8.
S_N2 Reaction

VITA

Adam Daniel DeWeese was born in Normal, Illinois on March 7, 1969. At the age of two, he moved with his family to Oak Park, Illinois. In Oak Park he attended elementary school, junior high school, and high school. His father is a high school principal and his mother is a hospital administrator. Adam received an athletic scholarship to the University of Southern Illinois in 1987. He was graduated from East Tennessee State University in 1994 with a Bachelor of Science Degree in Biology. Upon completion of the Master of Science degree he will continue working as an Environmental Scientist with the Tennessee Department of Environment and Conservation where he has been employed since January, 1995.