THE USE OF QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS IN PREDICTING THE ACUTE TOXICITY OF SELECTED AROMATIC HYDROCARBONS

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CHAPTER I

INTRODUCTION

Thousands of chemicals are introduced into the environment yearly. A 1981 estimate shows that almost six to eight million chemicals existed with an estimated 400,000 being added yearly (Craig and Enslein, 1981). The rapid growth of chemical technology with a concomitant increase in the introduction of chemicals into the environment makes it imperative that methods be developed to use biological, chemical, and physical properties of existing chemicals for predicting the activities of new ones. Such methods should be fast, efficient, and easy to use when compared with existing biological measurements. Maki (1983) noted that the ultimate enforcement of existing regulations requires the techniques to evaluate and predict types and degree of deleterious effects of chemicals. The precision of chemical measurements together with the existing large data base of biological information on existing chemicals offer an attractive possiblity for integrating biochemical data into predictive models.

Quantifying biological activity using physical or chemical properties was first proposed by Meyer (1899) and Overton (1899). They independently observed that the narcotic activity of many substances can be explained by their fat-water partition coefficients. When the relationship between biological, physical, and chemical parameters is

quantified, it is referred to as Quantitative Structure-Activity Relationship (QSAR).

Since the early observations by Meyer and Overton, landmark achievements in QSAR have been observed in different areas of science. Recently, environmentalists have evaluated some QSAR models for predicting fate and effect of contaminants based on physical and chemical parameters.

In 1978, Schultz et al. studied the relationship between partition coefficient (log P) of a compound between octanol and water and toxicity of five organic contaminants associated with aqueous coal conversion effluents, the aromatic compounds containing two or more methyl groups were more toxic than those with one or no alkyl substitutions. A series of 11 nitrogen-containing heterocyclic compounds which may be associated with the effluents from coal conversion technologies were examined by Schultz, Cajina-Quezada, and Dumont (1980). They concluded that an increasing linear relationship existed between octanol/water partition coefficient (log P), molecular weight, and boiling point.

The relationship between LC50 and log P, High Performance Liquid Chromatography (HPLC) ret ntion indices, solubility, and molecular connectivity indices using guppies as test organisms was examined by Konemann (1981). In this study, log P gave the best correlation with the LC50 values. Konemann and Musch (1981) evaluated the influence of pH on the QSAR of chlorophenols. They observed a high correlation between log (1/LC50) and both log P and dissociation constant, pKa. Their observation led to the conclusion that the influence of pKa on toxicity is because the molecular form of an acid can passively diffuse through the membrane faster than the ionic form. These earlier attempts

at predicting toxicity from different parameters using QSAR form the basis for my thesis.

Objective

The major objective of the present study is to develop a method capable of predicting the toxic effect of organic chemicals based upon physicochemical properties of chemicals with a similar mode of action. The Linear Free Energy Relationship (LFER) or Hansch approach to QSAR was adopted because it is relatively fast, efficient, and easy to use (Hansch, Muir, Fujita, Maloney, Geiger, and Streich, 1963). Statistical analyses were performed in order to determine if it was possible to use an apparent relationship between existing biological data and certain molecular properties for predicting the toxicity of a group of substituted and unsubstituted aromatic compounds with anesthetic effects. Hydrophobic consant (π) and molar refractivity, (MR) were chosen as the appropriate parameters to determine the toxicity of nonpolar aromatic compounds. The choice of hydrophobic constant was based on a prior knowledge that log P is not only linearly related to π but that log P can be used to predict toxicity. The major advantage of using π instead of log P is that it eliminates the need to determine the individual partition coefficient of each compound. A knowledge of the presence of a substituent can be used to estimate a compound's toxicity. This approach to QSAR, known as the moiety or group approach, recognizes the contribution of each group to the overall hydrophobicity of a compound.

A comparison of the degree of correlation between MR and π with toxic effects both individually and collectively, will help in determining how accurately toxicity can be predicted through the knowledge of a compound's size or hydrophobicity.

Hypotheses

The hypotheses for this research were as follows:

1. Ho: There is no significant linear relationship between the log of the inverse of oral LD50 (1/log 10 oral LD50) and hydrophobic constant (π).

2. Ho: There is no significant linear relationship between the log of the inverse of oral LD50 (1/log 10 oral LD50) and molar refractivity (MR).

3. Ho: There is no significant linear relationship between the log of the inverse of oral LD50 (1/log 10 oral LD50) and both hydrophobic constant and molar refractivity.

Assumptions and Limitations

The major assumptions of the Hansch-type quantitative structure-activity relationship methodology are the following:

1. A significant relationship exists between the structure of a compound and its biological activity,

2. The relationship between the biological activity and molecular properties can be described mathematically (Martin, 1981),

3. Substituent effects on the biological activities of a compound is additive and constitutive (Fujita, Iwasa, and Hansch, 1964), and

4. The partitioning of a compound between octanol and water simulates its partitioning in lipophilic biological substances,

The limitations of the Hansch-type QSAR methods include:

1. Multicolinearity and cross correlation of independent variables (Cammarata, Allen, Seydel, and Wempe, 1970; Craig, 1971). The

possibility of cross correlation between the two parameters used in this study will be checked through the use of a correlation matrix.

2. The possiblity of chance correlation when too many variables are used to correlate too few biological data. In order to prevent the possiblity of chance correlation, at least 12 compounds were used in the analysis.

3. Inappropriate scaling of parameters. This was avoided by using a well tested scaling scheme for molar refractivity.

The Hansch approach is also inappropriate for correlation of data where compounds fall into many different structural series or no series at all (Cramer, Redl, and Berkoff, 1974).

CHAPTER II

LITERATURE REVIEW

Historical Development

It was shown in 1899 that biological activities of chemicals could be predicted from their physicochemical properties (Meyer, 1899; Overton, 1899). Relating the activity of anesthetics to their lipid-water partition coefficient, Meyer and Overton also proposed that anesthetics induce equal effects at equimolar concentrations in certain cell lipids. For the next six decades, extensive research was directed towards testing the relationship between diverse chemical structures using different biological systems and to defining different parameters which could be used to quantify this relationship. Langley (1905) proposed that the characteristic properties associated with an observed activity in a given biological assay could be correlated with physical properties such as solubility and refractive index. Other investigators have found a correlation between the toxicity of insect fumigants and increasing boiling point and decreasing vapor pressure (Moore, 1917). Tiley and Schaffer (1926) proposed the existence of relationships between toxicity and structures within different homologous series. Clark (1933) observed a rapid decrease in the toxic concentration of alcohol to frogs' hearts. This did not continue indefinitely with an increase in molecular weight. A point existed in a series when a compound of maximum toxicity was reached. While also working with a homologous series, Meyer and Hemmi

(1935) reported a correlation between the concentration of some chemicals within the series and physical properties such as water solubility, vapor pressure, and surface activity. They proposed that such a correlation was indicative of the equilibrium that existed between the concentration of the chemicals and the surroundings.

An outstanding contribution to quantifying structure-activity relationships during this period was from the study of the effect of sterically remote substituents on the equilibrium or rate constants of organic reaction (Hammett, 1935). His observations led to the formulation of the Hammett Sigma (σ) constant. Hammett Sigma constants are measures of the electronic contribution of a substituent attached to a parent compound. The first application of the thermodynamic processes to structure-activity studies in biological systems was by Fergusson (1939). He proposed that the chemical potential of chemicals could be used for determining their toxicity. Bell and Roblin (1942) evaluated the use of pKa on the relative negativity of the SO_2 groups of sulfanilamide compounds, while Albert, Rubbo, Goldcare, Davey, and Stone (1945) demonstrated that the degree of ionization of acridine molecules was important in determining antibacterial activities. Beckett (1956) demonstrated a relationship between pKa and the analgesic potency of tertiary amines and some synthetic analgesics. Brodie and Hogben (1957) observed a relationship between gastrointestinal absorption of drugs, the pKa, and the partition ratio of organic electrolytes in gastric juice and plasma. Vogel (1948) performed extensive studies on measuring of the contribution to refractivity by some substituents. Taft (1952) extended Hammett's concepts to steric effects of substituents on nonaromatic parent compounds. The sustituent was subsequently known as Taft (Es) constant.

Although, it was generally known that hydrophobicity played an important role in determining biological activity, many unsolved problems remained. According to a review by Martin (1978), such problems included:

1. Lack of agreement on the solvent system to be used to simulate biological systems,

2. The nonrecognition of statistical methods,

3. The lack of high speed computers, and

4. Inadequate attention was paid to predicting partition coefficient from structures.

Between 1960 and 1970, major advances in quantitative structure-activity relationships were performed by Hansch et al. (1963), Hansch and Steward (1964), Fujita et al. (1964), and Iwasa, Fujita, and Hansch (1965). Hansch and co-workers proposed the Linear Free Energy Relationship (LFER) or extradynamic approach to quantitifying structure-activity relationships. For the first time, structure-activity relationships were examined for statistical significance. They proposed that the log of octanol water partition coefficient should be taken as a measure of lipophilicity. In addition, they pointed out that the coefficient is additive and constitutive and can, therefore, be calculated from molecular structure. They also defined the hydrophobic parameter, π, which is a measure of the contribution to the hydrophobicity of a parent compound by a substituent. An alternate QSAR method was proposed by Free and Wilson (1964). Known as the de novo or Free-Wilson approach, it assumed that the contribution to biological action by substituents at different positions were additive and independent of the effect of substituents at other positions. Building on the idea first put forward by Hammett, Swain and Lupton (1968) separated

electronic effects into field, F, and resonance, R, effects. Leo, Hansch, and Church (1969) observed that substituent partition coefficient, π, gave better correlations over several series of compounds than those obtained by the use of polarizability, parachor, and molar attraction constants.

Between 1970 and 1980 existing models and parameters were modified (Purcell, Bass, and Clayton, 1970; Canas-Rodriguez and Tute, 1972; Davis, 1973; Nys and Rekker, 1973; Goldfarb, 1973; and Leo, Yow, Silipo, and Hansch, 1975), other statistical methods and parameters were developed (Weiner and Weiner, 1973; Hansch, Leo, Unger, Kim, Nikaitani, and Lien, 1973; Martin, 1978; and Mager, 1980), and extensive computer models in QSAR studies were developed (Verloop, Verloop, Hoogenstraaten, and Tipker, 1976). The Hansch equation was generalized to include other variables such as new parameters, new structural and topographical features, molecular orbital indices, and indicator or dummy variables. Statistical applications included the use of discriminant analysis, principal component and factor analysis, cluster analysis, and combined multivariate analysis (Blankley, 1983). Increasing availability of computers stimulated different applications of computer models. For instance, Kirschner and Kowalski (1979) developed a model based on the pattern recognition called ARTHUR. Verloop et al. (1976) developed the STERIMOL parameter for modelling molecular shape, while Stuper and Jurs (1975) developed the ADAPT system. Most of these early applications of computer modelling in QSAR were in different areas of medicinal chemistry. The first application of computer modelling for predicting toxicity of chemicals was developed by Cramer et al. in 1974, followed two years later by the works of Craig and Waite (1976) and Craig and

Enslein (1981). Craig and Enslein modified the substructural approach first proposed by Cramer for predicting the toxicity of some compounds.

By the 1980s, emphasis had shifted from attempting to explain the observed behavior of a set of compounds to using such a behavior for predictive purposes. The two major QSAR methods that have been widely used for studies were the Linear Free Energy Relationship (LFER), or the Hansch approach, and the substructural approach. The Free-Wilson additivity model was not useful for predictive purposes. Although the substructural approach required a large number of structurally diverse chemicals and involved more complex statistical applications than LFER, it can be applied to a wider range of chemicals when compared to LFER.

The Linear Free Energy Relationship, or Hansch approach, to quantitative structure-activity relationships was considered appropriate for use in my study due to the fact that it is a relatively simple approach to use.

Parameters and Quantitative Structure-Activity Relationship

Different parameters can be used in evaluating the environmental impact of chemicals released into the environment. They can be either biological parameters, such as LD50 (i.e., the lethal dose of a chemical that kills 50% of the organisms present); physical parameters such as vapor pressure; and chemical parameters such as the rate of photolysis. Certain parameters may reflect both the physical and chemical characteristics of a chemical and these are referred to as the physicochemical parameters. In the hazard assessment of chemicals, these different types of parameters can be grouped into three broad categories. These are those used in environmental fate assessment, in predicting environmental

distribution of the chemicals, and in predicting the toxicity of the chemicals.

These three categories are not entirely independent of one another. Parameters used for evaluating the fate of a chemical within an environment can be equally useful for predicting its distribution and toxicity. For instance, the environmental fate of a chemical is governed by it's equilibrium distribution between soil, water, air, and biota (Kenaga, 1982). The distribution of the chemical between water and biota can be assessed through the use of the logarithm of its octanol/water partition coefficient (log P). This parameter is equally useful in predicting the toxicity of the chemical to an organism (Konemann and Musch, 1981).

Parameters for predicting the fate and distribution of chemicals have been extensively studied. Chiou, Virgil, Schmedding, and Kohnert (1977) determined the relationship between the n-octanol/water partition coefficient and a variety of compounds including aliphatic and aromatic hydrocarbons. The relationship between lipophilicity and bioaccumulation potential has been demonstrated in sediments (Southworth, Beauchamp, and Schieder, 1978; Karickhoff, Brown, and Scott, 1979), fish (Kenaga, 1980), earthworms (Lord, Briggs, Neale, and Manlov, 1980), and soil (Kenaga and Goring, 1980). Various environmental models using either the "environmental rate approach" or the "model ecosystem approach" are available for predicting fate and distribution of chemicals within the environment (Branson, 1978).

Parameters that can be used in predicting the toxicity of a compound are referred to as either biological parameters or physicochemical parameters. Biological parameters refer to the effect of the compound, i.e., acute, chronic, or subtle. Acute toxicity is measured by the use of values such as LC50, LD50, or ED50. When compared with the chemical

measurements, inherent difficulties, such as variability and poor reproducibility, may be associated with measurements of biological effects.

The precision of chemical measurements provides an alternative to measurements of biological effects. Physicochemical parameters are intrinsic characteristics of chemicals that can be used in quantifing toxicity of chemicals using the existing data base of toxicity parameters. Various attempts have been made to correlate the toxicity of compounds with their physicochemical properties. McGowan (1966) was able to predict the toxicity of some compounds from their boiling point. Trucco, Engelhardt, and Stacey (1983), correlated molecular weight with toxicity.

Biological Systems and Physicochemical Parameters

Two solvent systems are essential for assessing the behavior of a chemical within biological systems, a polar solvent system and a non-polar solvent system. An example of a polar system is the cytoplasm which is basically a dilute solution of salt in water (Martin, 1978). Enzymes that catalyze essential biochemical synthesis and metabolism are located in the cytoplasm. The membrane is considered a vital non-aqueous system. Not only do membranes surround most living things, but they form a barrier to the movement of chemicals in and out of a cell (Tanford, 1973; Martin, 1978) and selectively permit movement of essential chemicals. They also serve as surfaces for membrane bound enzyme catalyzed reactions (Martin, 1978). According to the fluid mosaic model proposed by Singer (1971), the membrane is composed of a lipid bilayer interrupted by protein rich regions. The lipid layers maintain the integrity and cohesiveness of the membrane while the protein regions

are responsible for the functional properties of the membranes. Membranes are regarded as models for the types of interactions that take place between a chemical and any biological system.

Covalent and noncovalent interactions occur between chemical and biological systems, Noncovalent interactions form the basis for specificity and strength of covalent interactions of a chemical for its site of action. It is chosen over covalent interaction because it represents the first level of interaction of a chemical to its site of action. That is, a loose noncovalent bond preceeds most covalent bonds (Martin, 1978). Noncovalent bonds are important when specific covalent interactions between a chemical and its site of action is obscure. The major types of noncovalent interactions between a chemical and a biological system are steric attraction or repulsion, electrostatic, and hydrophobic interactions. Other forms of noncovalent interactions include solute-solvent and charge transfer interactions and hydrogen bonding.

Two different approaches are available for quantifying the noncovalent interaction of a chemical at its site of action (Ariens, 1971). The first is the integrated approach which considers the molecule as a whole and recognizes the overall physicochemical property of the chemical. An example is the use of log P as a measure of the partition of a molecule between polar and nonpolar phases. The second approach is the group or moiety approach which recognizes the contribution of certain chemicals through the use of substituent constants.

Substituent Constants and Quantitative Structure-Activity Relationship (QSAR)

The Hansch method used in the present study assumes that a variation in certain biological activity of some chemicals can be analyzed in terms of their free energy related physicochemical parameters. The use of the substituent or moiety approach was adopted instead of the integrated approach because it eliminated the necessity for determining separately the properties of individual compounds. Instead, the parameters can be calculated from an existing data base of substituents. The use of substituent constants for correlation analysis has been reviewed by Hansch (1973); Exner (1978); Martin (1978); Hansch and Leo (1979); Craig and Enslein (1981); and Govers, Ruepert, and Aiking (1984).

Different types of substituent parameters have been used for estimating hydrophobic, steric, or electronic effects. Hydrophibic parameter, π, and fragment constant, f have been used for quantifying hydrophobic substituent effects. Taft Es, molecular weight, Verloop L parameter, Parachor, and Molar Refractivity have been used for quantifying substituent steric effects based on bulk, size, or volume. The parameters used in quantifying electronic effects include Hammett σ, Field (F), Resonance parameters (R), Molar Refractivity and Van der Waals radius. Goodforb (1973) has outlined the criteria for determining which of these parameters will be used for predictive purposes.

1. The prior relevance of the parameter,
2. Its predictability,
3. Orthogonality with respect to other parameters,

4. General applicability,

5. Availability of parameter, and

6. Record of success.

Additional guidelines include the type of chemical used in the analysis and the ease of calculating and applying the parameters.

Substituent Parameters

Hydrophobic forces are considered to be "the most important single factor providing the driving force for noncovalent interactions in aqueous solutions" (Jencks, 1969). Martin (1978) defined the hydrophobic bond as the tendency of water molecules to associate with themselves rather than nonpolar substituents. Hydrophobic forces are, therefore, vital in the distribution of a compound within a biological system. Parameters used for quantifying hydrophobic effects of substituents were hydrophobic constant, π, and fragment, f constant. Fujita et al., (1964) defined the hydrophobic constant as:

$$\pi_X = \log P_X - \log P_H$$

where P_X is the partition coefficient of a derivative, P_H is the partition coefficient of the compound and π is the hydrophobic constant for the substituent.

The π parameter, therefore, includes the contribution of individual substituents to the overall hydrophobic effects of a compound. Fujita et al. (1964) showed that the partition coefficient was an additive constitutive property; i.e. multiple substituents exert an influence equal to the sum of individual substituents and the effect of a substituent varies depending on the molecule to which it is attached or its environment (Martin, 1978). Ariens (1971) observed that any

substituent which has a predominant effect on the hydrophobicity of a compound will affect its potency, because passive membrane transport process was based upon partition over different compartments. The use of hydrophobic constant π assumes that the π for hydrogen is zero. This was later shown to be erroneous (Davis, 1973). Nys and Rekker (1973) calculated a new set of hydrophobic parameters called fragment constant, f. Additional measurements of fragment, f constants were undertaken by Hansch and Leo (1979). The hydrophobic constant (π) was chosen over fragment constant (f) because it was relatively easier to calculate and because f did not offer any advantage over π in determining toxicity of nonpolar aromatic compounds. Leo et al. (1969) demonstrated the superiority of π parameter over several nonpolar parameters. Fujita and Bans (1971) observed that hydrophobic constant π inherently includes the effect of hydrogen bonding. Some of the limitations associated with the use of the constants included the approximate nature of any partition coefficient calculation (Canas-Rodriguez and Tute, 1972), and the fact that a steric effect of substituent might be important in predicting the overall biological effect of a compound (Blankley, 1983). The additive constitutive properties of π breaks down when a strong electron withdrawing group is present within the compound. Compounds used in this study were nonpolar and did not have any strong electron withdrawing groups.

Steric attraction or repulsion can occur when two charged molecules come close together. In 1894, Meyer proposed that the atomic weight of ortho substituents determine the ease of esterification of ortho substituted aromatic acids. Taft (1952) proposed that the rate of hydrolysis of substituted carboxylic acids should be used as a measure of steric effects. For nonpolar compounds, molar refractivity (MR),

parachor, or molecular weight could be used as an estimate of bulk or size of substituents. Both parachor and MR were highly correlated and could be used interchangeably (Hansch and Leo, 1979). Molar refractivity was also correlated with molecular weight and could, therefore, be used as a "crude" estimate of size (Hansch et al., 1973b; Hansch et al., 1973). MR was chosen instead of molecular weight as a parameter for quantifying bulk because MR provides a better correlation (Hansch and Leo, 1979). It was also easier to apply MR when compared to both Van der Waals radius and Verloop parameters. Like hydrophobic constant π, molar refractivity was additive and constitutive. MR has been successfully utilized in QSAR (Hansch, Greico, Silipo, and Vittoria 1977; Yoshimoto and Hansch, 1976, and Shah and Coats, 1977).

The effect of a substituent on the overall electronic effect of a compound was first parameterized by Hammett sigma (σ) value (1935). He defined sigma as:

$$\sigma = \log k_x - \log k_H$$

where k_H is the ionization constant for benzoic acid in water at 25°C and k_x is the ionization constant for a meta or para derivation. The Hammett sigma constant is additive (Jaffe, 1953). It was later realized that a need existed to separate the inductive (polar) part of an electronic effect from the resonance component (Taft and Lewis, 1959). Swain and Lupton (1968)developed a completely different approach to determining polar (F) and resonance (R) constants. These substituent constants can be used for polar compounds. In the case of nonpolar compounds, the most appropriate parameter for quantifying electronic contribution of the substituent is molar refractivity (MR). Molar refractivity is the dispersion bond which holds nonpolar molecules together in a liquid phase

when other forms of electrostatic bonds are absent (Martin, 1978). Dispersion bonds occur when an instantaneous dipole induces another dipole in a neighboring molecule. The instantaneous dipole is a result of the vibrations of electrons with respect to the nucleus of molecules with no permanent dipole (Martin, 1978).

Martin (1978) observed that since noncovalent forces are weak, they may require a combination of forces between bonds. The hydrophobic constant π and molar refractivity are selected as the substituent constants to be used in predicting toxicity of nonpolar aromatic compounds. There are conflicting reports about the amount of correlation between the two parameters π and MR. Some reports have indicated a significant correlation (Craig, 1971; Wootlon, Sheppey, Hudson, and Goodforb, 1976), while others have shown little or no correlation between the two parameters (Hansch et al., 1973; Yoshimoto and Hansch, 1975).

CHAPTER III

METHODS

Model Development

The regression analysis at the Oklahoma State University Computer Center was used in elucidating relationships between the biological effects of selected chemicals and their physical and chemical parameters. For predictive purposes, equations were obtained at 95% confidence intervals. The correlation analysis was used in eliminating the problem of colinearity between the parameters used. About 23 compounds were selected initially in the regression equations in order to obtain statistically significant equations. Parameters which were used in this study were chosen in such a way as to reflect some or all of the following effects:

1. The ability of the chemicals to partition themselves in biological systems as measured by π.

2. Electronic interactions of chemicals at the site of action as measured by MR.

3. Steric effects of molecules as measured by MR.

Correlation, linear regression, and multiple linear regression analysis were chosen over other statistical methods. The correlation analysis was used to check for the possiblity of colinearity between π and MR. An all-equation multiple linear regression approach was used. In this approach, the computer calculated all possible equations and

provided a limited output for each. Multiple linear regression analysis was chosen over other statistical analyses because it is a relatively simple statistical procedure for use by nonstatisticians. It can also be performed at most computer installations. Important statistical calculations required for QSAR studies include (Martin, 1978; Blankley, 1983):

1. S, the standard error of estimate (or standard deviation).

2. R^2, the percentage of data variance accounted for by the model. The R^2 values were used with the all-possible regression equation approach. The intent was to obtain an equation where adding more independent variables resulted in only a small increment in R^2 value rather than maximizing R^2 (Neter, Wasserman, and Kutner, 1983).

3. F, a statistic for assessing overall significance of the derived equation including the degree of freedom and confidence level.

The Statistical Analysis System (SAS) available at the Oklahoma State University Computer Center was used. SAS was chosen because it satisfies the conditions required for a successful statistical analysis in QSAR outlined by Martin (1978). In QSAR methodologies, biological data are selected as the dependent variable and the chemical or physical parameters are considered the independent variable. Since biological data are usually more variable.

Calculation of Substituent Constants

The hydrophobic constant π can be calculated or taken from Table VI-I in Hansch and Leo (1979). Due to the additive and constitutive nature of π constants, they can be estimated from Leo et al. (1975) and Fujita et al. (1964).

The equation for calculating π is:

$$\pi_X = \log P_X - \log P_H$$

where P_X is the partition coefficient of a derivative and P_H that of the parent compound. For example,

$$\pi_{Cl} = \log P_{C_6H_5Cl} - \log P_{C_6H_6}$$
$$= 2.84 - 2.13$$
$$= 0.71$$

Octanol/water partition system was chosen as the preferred system for estimating log P because octanol is assumed to simulate biological systems. Log P values were obtained from Appendix II of Hansch and Leo (1979).

MR values were taken from a compilation by Hansch and Leo (1979). The MR values were then scaled by 0.1, thereby making it equiscalar with respect to π (Hansch and Yoshimoto, 1974). When only one substituent is attached to the benzene ring, the value was simply taken and multiplied by 0.1. For example:

MR(Cl) = 0.1 x 6.03 = 0.60
MR(Br) = 0.1 x 8.88 = 0.89

When two or more substituents were present, an additional 1.03 was subtracted for each H that is replaced on the ring. The MR value for H was 1.03. For example:

$$MR(Cl)_3 = 0.1[3x6.03 - 2(1.03)] = 1.6$$

Preliminary Screen of Compounds

Both the substituent parameters and the groups of chemicals were carefully selected to complement each other (Table I). Chemicals and

TABLE I

ORIGINAL LIST OF COMPOUNDS USED IN THE QSAR ANALYSIS

Compound	Oral LD50 (rat) mg/kg	MR	π
Benzene	4894	1.03	0.00
Bromobenzene	2699	0.89	0.86
Butylbenzene	5000	1.96	2.13
Chlorobenzene	2910	0.60	0.71
p Dichlorobenzene	500	1.00	1.42
O-diethybenzene	5000	1.85	2.04
Ethylbenzene	3500	1.03	1.02
Fluorobenzene	4399	0.09	0.14
Hexachlorobenzene	10000	3.00	4.26
Iodobenzene	1799	1.39	1.12
Pentachlorobenzene	1080	2.50	3.55
Toluene (methyl benzene)	5000	0.57	0.56
α Chlorotoluene	1231	1.05	0.17
Xylene (dimethyl benzene)	5000	0.92	1.12
Ethyltoluene	5000	1.39	1.58
$\alpha\alpha\alpha$Trifluorotoluene	15000	0.50	0.88
Propylbenzene	4830	1.50	1.55
1,2,3,4 Tetramethylbenzene	6408	2.00	2.24
Tetrachlorobenzene	1500	2.00	2.84
1,2,4,5 Tetramethylbenzene	6984	1.85	2.24
1,2,4 Trichlorobenzene	756	1.50	2.13
Triethylbenzene	5000	2.78	3.06
Trimethylbenzene	5000	1.39	1.68

their toxicity data were selected from the Registry of Toxic Effects of Chemical Substances (RTECS, 1983). Chemicals were selected such that all contain an identical parent compound, benzene. The substituents present on the parent compound do not have strong electron withdrawing or donating effects on the parent compound. In addition, all chemicals have the same mode of action. Ariens (1971) noted that structure-activity correlation was possible only if the compounds had an identical mode of action. The compounds selected were substituted aromatic compounds with anesthetic effects. Anesthetics are capable of depressing essential functions of all types of cells (Burger, 1960).

This initial group of chemicals was screened with the following procedure in order to obtain a final group of compounds:

1. Scatter plots of the inverse of the log of oral LD50 of the initial group of chemicals against each of the independent variable.

2. A scatter plot of the hydrophobic constants and molar refractivity for the initial list of compounds.

3. A correlation analysis between the two independent variables. High correlation between the two variables were reduced by introducing new less hydrophobic compounds and eliminating obvious outliers.

4. A regression analysis between the biological response (1/log 10 oral LD50) and each independent variable for the initial group of compounds.

Selection of Appropriate Regression Models

The relationship between the biological response (1/log 10 oral LD50) and the independent parameters, hydrophobic constant (π) and molar refractivity (MR) were modeled by the procedure outlined below. The square terms were included in order to investigate the possibility of

quadratic relationships between the dependent and the independent variables. At this point, certain compounds were withheld from the regression equations for the purpose of evaluating selected regression models.

The first step was to identify appropriate regression models through the use of an all possible equation approach. The R^2 criterion was used in selecting the appropriate combination of variables which resulted in an acceptable predictive equation. The R^2 criterion required an examination of the coefficient of multiple determination, R^2. The coefficient measured the appropriate reduction in total variation in the dependent variables explained by the independent variables. The second step involved a correlation analysis between the independent variables.

Homoscedasticity and aptness of the simple and quadratic linear equations were tested through the plots of residuals against each of the independent variables and the plots of the residuals versus predicted variables. The appropriateness of the fitted regression models was tested through the plot of the dependent versus independent variables. F-tests were used to test for the significance of the relationships expressed by the fitted regression models.

CHAPTER IV

RESULTS AND DISCUSSION

In order to obtain an appropriate model that fits a set of data, it is necessary that the set of data be analyzed repeatedly (Neter et al., 1983). A preliminary screen of the original data set, involved linear regression, correlation and residual analysis (Appendix C), and scatter plots of the dependent versus independent variables resulted in the elimination of some chemicals. These plots (Figures 1 and 2) and residual (observed - predicted) values revealed some chemicals which differed sustantially from the rest of the data set and were subsequently eliminated from further regression analysis since their presence would unnecessarily bias the results of regression analysis. These compounds included three alkyl substituted benzene compounds, namely butylbenzene, o-diethylbenzene, and ethylbenzene. These compounds did not show any variation in toxicity with increasing hydrophobic or molar refractivity values. Others included some chlorobenzenes with unusually low LD50 values, namely p-dichlorobenzene, 1,2,4-trichlorobenzene, and α-chlorotoluene. Also eliminated were some chlorobenzenes with unusually high LD50 values, namely ααα-trifluorotoluene and hexachlorobenzene.

An initial high correlation existed between the hydrophobic constant and of molar refractivity (Figure 3). An R value of 0.92287 was obtained through the use of matrix correlation analysis. This initially high value was reduced to 0.8838 through the introduction of less hydrophobic

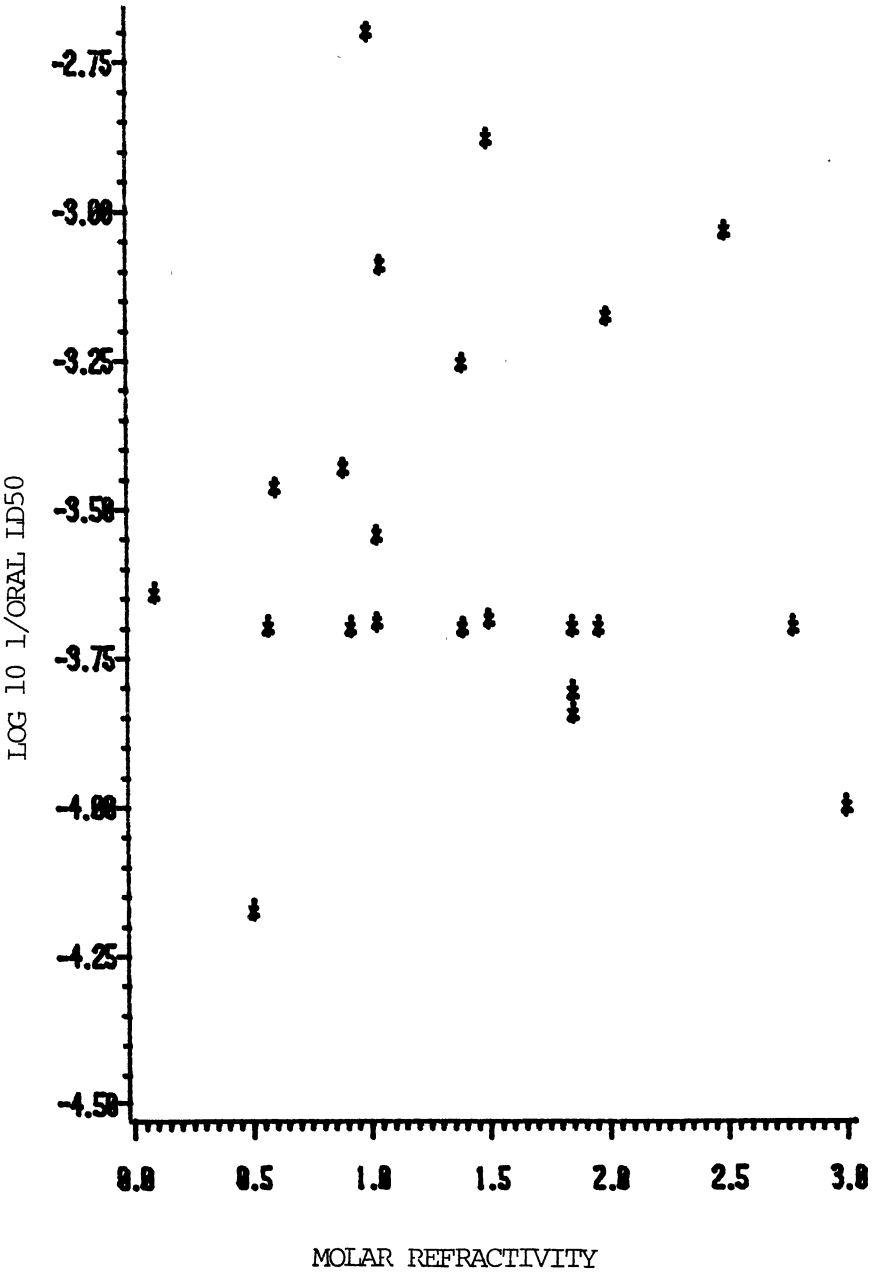

Figure 1. A scatter plot of the relationship between the inverse log of oral LD50 (rat) and molar refractivity for initial group of compounds

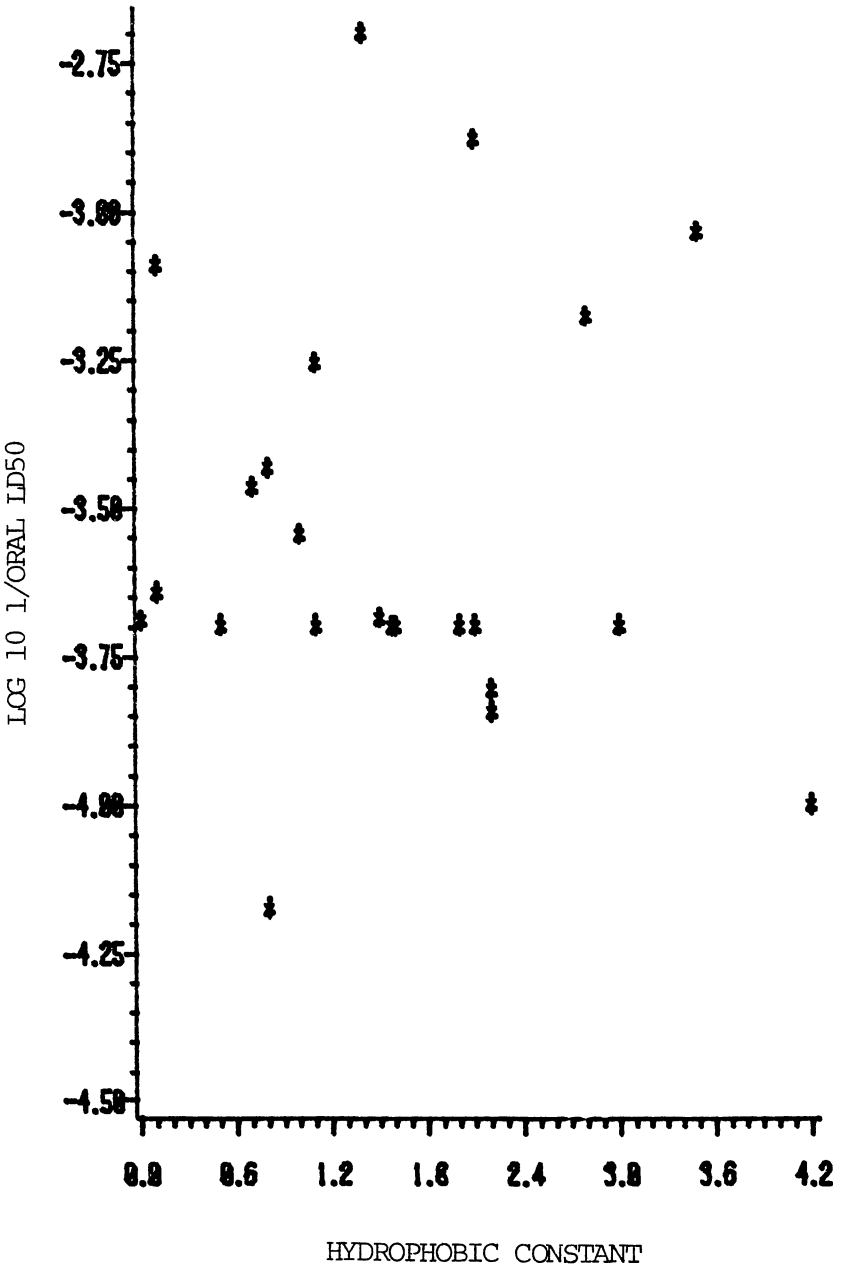

Figure 2. Scatter plot of the relationship between the inverse log of the oral LD50 (rat) and hydrophobic constant for initial group of chemicals

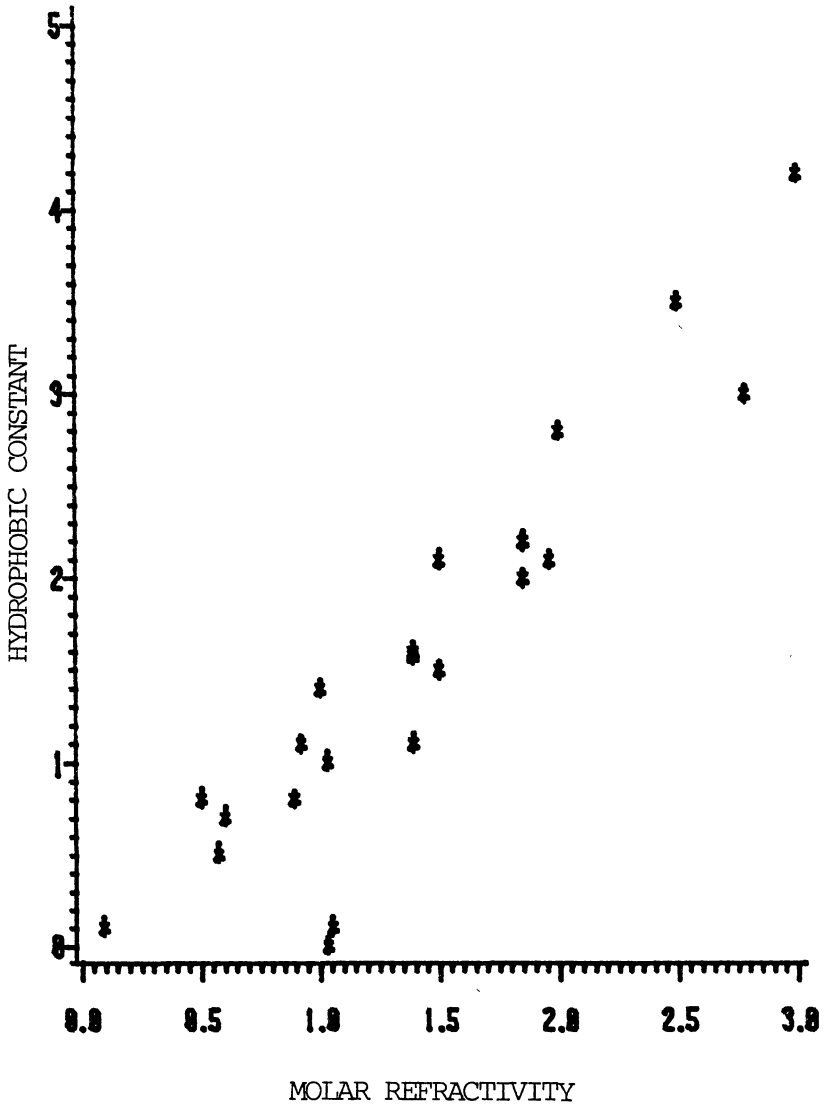

Figure 3. Hydrophobic constant as a function of molar refractivity for the initial group of compounds

chemicals. At this point, some of the chemicals were withheld for evaluating the regression models. The remaining compounds used in subsequent regression analysis are listed in Appendix A. Figure 4 is a scatter plot of the relationship between the two variables for the final group of chemicals. The correlation between the two variables remained high as evident by the plot and the correlation matrix in Table III. This high correlation indicated that the variables can be used interchangeably.

In order to select the appropriate model for the group of chemicals analyzed, the first step involved an all possible equation approach (Table II). The second step involved a correlation analysis of the dependent variables that would be used in the regression analysis. Table III shows the correlation matrix between the two independent variables and their squares. The probability levels at which the relationship is significant is indicated beneath each correlation coefficient. Thus, the lowest corelation coefficient, R, was 0.81659 between π^2 and MR with a probability of 0.0012. This high correlation coefficient also indicates that the two variables could be used interchangeably.

The 12 possible equations in Table II were evaluated based upon 1. the number of chemicals used in the regression models, 2. the result of the R analysis in Table II, and 3. the correlation between the variables in a regression equation. A minimum of five chemicals was required per independent parameter to obtain statistically significant equations. With 12 chemicals used, only equations containing two or less independent variables were considered. In addition to this requirement, the introduction of a new parameter must result in a substantial difference in the R^2 parameter. The high correlation between some variables resulted in eliminating of some equations. The R^2 analysis

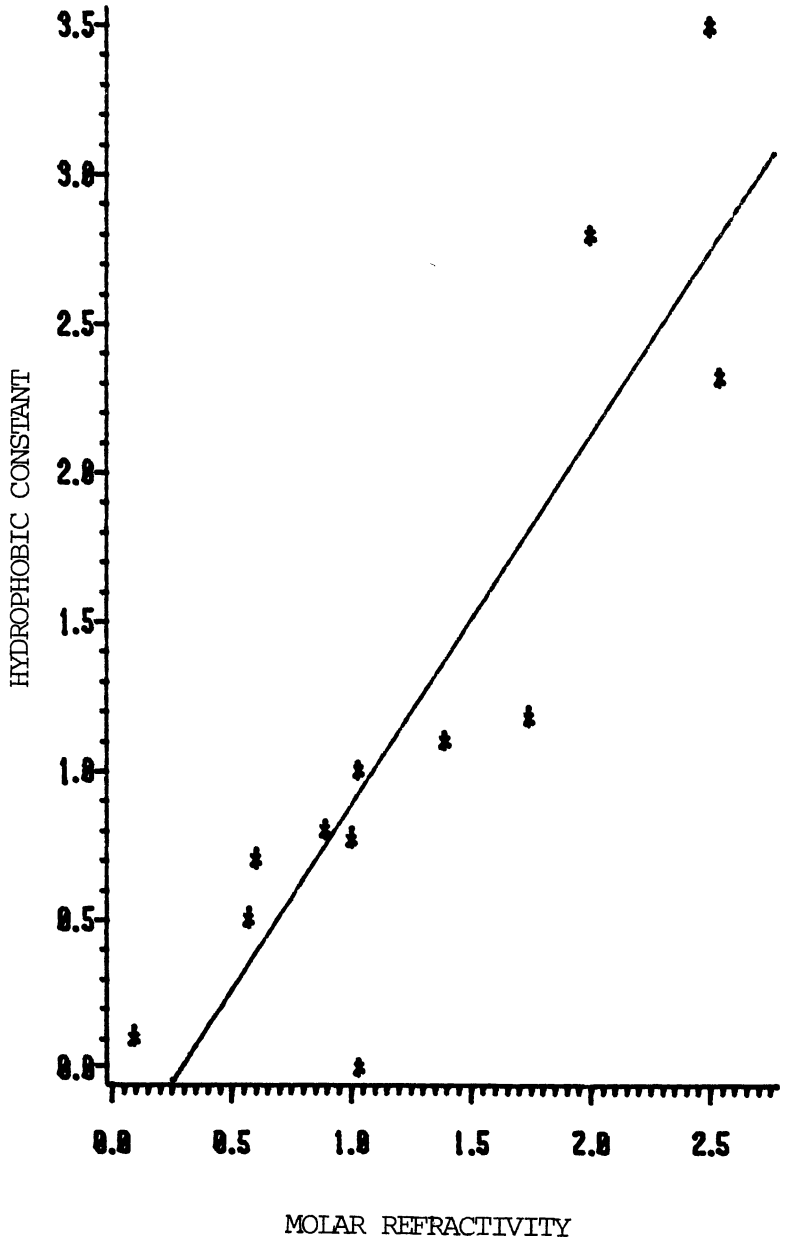

Figure 4. Scatter plot and fitted regression line of the relationship between hydrophobic constant and molar refractivity

TABLE II

R-SQUARE STATISTICS FOR ALL-POSSIBLE COMBINATIONS OF INDEPENDENT VARIABLES

N= 12 Regression Models for Dependent Variable Y

Number in Model	R-Square	Variables in Model
1	0.62400036	π^2
1	0.63551929	MR^2
1	0.66083486	MR
1	0.75724121	π
2	0.66240083	MR MR^2
2	0.67480847	MR^2 π^2
2	0.70857310	MR π^2
2	0.75796103	MR^2 π
2	0.76602637	MR π
2	0.79033396	π π^2
3	0.71653892	MR MR^2 π^2
3	0.77590834	MR MR^2 π^2
3	0.79082001	MR^2 π π^2
3	0.79231614	MR π π^2
4	0.79355448	MR MR^2 π π^2

TABLE III

CORRELATION MATRIX FOR INDEPENDENT VARIABLES

	MR	π	MR^2	π^2
MR	1.00000	0.88378	0.96855	0.81659
	0.0000	0.0001	0.0001	0.0012
π		1.00000	0.90285	0.96363
		0.0000	0.0001	0.0001
MR^2			1.00000	0.86699
			0.0000	0.0003
π^2				1.00000
				0.0000

shows that the equation containing π and π^2 provided a high correlation with the biological response, log 10 (1/LD50) comparable to equations containing three or four variables. The addition of one or two more variables did not represent a substantial increase in the R^2 values when compared with the equation containing π and π^2 terms.

By using the criteria outlined above, the equations in Table IV were selected as being appropriate for the group of chemicals analyzed. Equation 1 represented the relationship between toxicity and molar refractivity. The R^2 value was relatively high and indicated that about 66% of the variation in the observed biological response could be explained by molar refractivity. The equation was also significant at the 0.05 level of significance (Appendix D).

TABLE IV

SELECTED REGRESSION EQUATIONS

Equation	R^2	F	Sample Size (N)	S
1. log 1/LD50 = -3.698 + 0.323 MR	0.661	19.48*	12	0.134
2. log 1/LD50 = -3.619 + 0.177π	0.757	31.19*	12	0.114
3. log 1/LD50 = -3.683 + 0.310π - 0.039π^2	0.790	16.96*	12	0.111

* = significant at α = 0.05

The equation derived using π provided a higher R^2 value (R^2 = 0.757) (Appendix E) when compared with Equation 1, thereby demonstrating the superiority of π over MR in predicting toxicity. Equation 2 in

Table IV shows that toxicity can be predicted from π at 0.05 level of significance (Appendix E).

Equation 3 in Table IV provided the best fit for the regression analysis. The equation indicated a quadratic relationship between the dependent and independent variables (Appendix F). This implied that an optimal hydrophobicity existed to observe a maximum biological response for the group of chemicals analyzed. Although the equation was significant at 0.05, due to the high correlation between the two variables, it was not possible to identify the contribution of the square term in the equation. The high correlation was expected since one was the square of the other. However, the equation would have limited application providing that no extrapolations were attempted beyond the range of values analyzed. The three equations have low standard error of estimate or standard deviation (S). The lowest S value of 0.111 was obtained in Equation 3.

The predicted and observed values of the actual biological response (log 10 1/LD50) versus the two independent variables MR and π were within the 95% confidence limit (Figures 5 and 6).

A quadratic relationship between the dependent variable, log 10 1/LD50, and the independent variables π and π^2 provide a better fit than one containing only (Figure 7).

Figure 8 is the computer generated quadratic relationship between the biological response and hydrophobic constant using Equation 3 in Table IV. The graph shows that there was an increase of the log 10 (LD50) values with increasing π and π^2 values. However, an optimum value of hydrophobic constant existed for which no further increase in the biological response occurred.

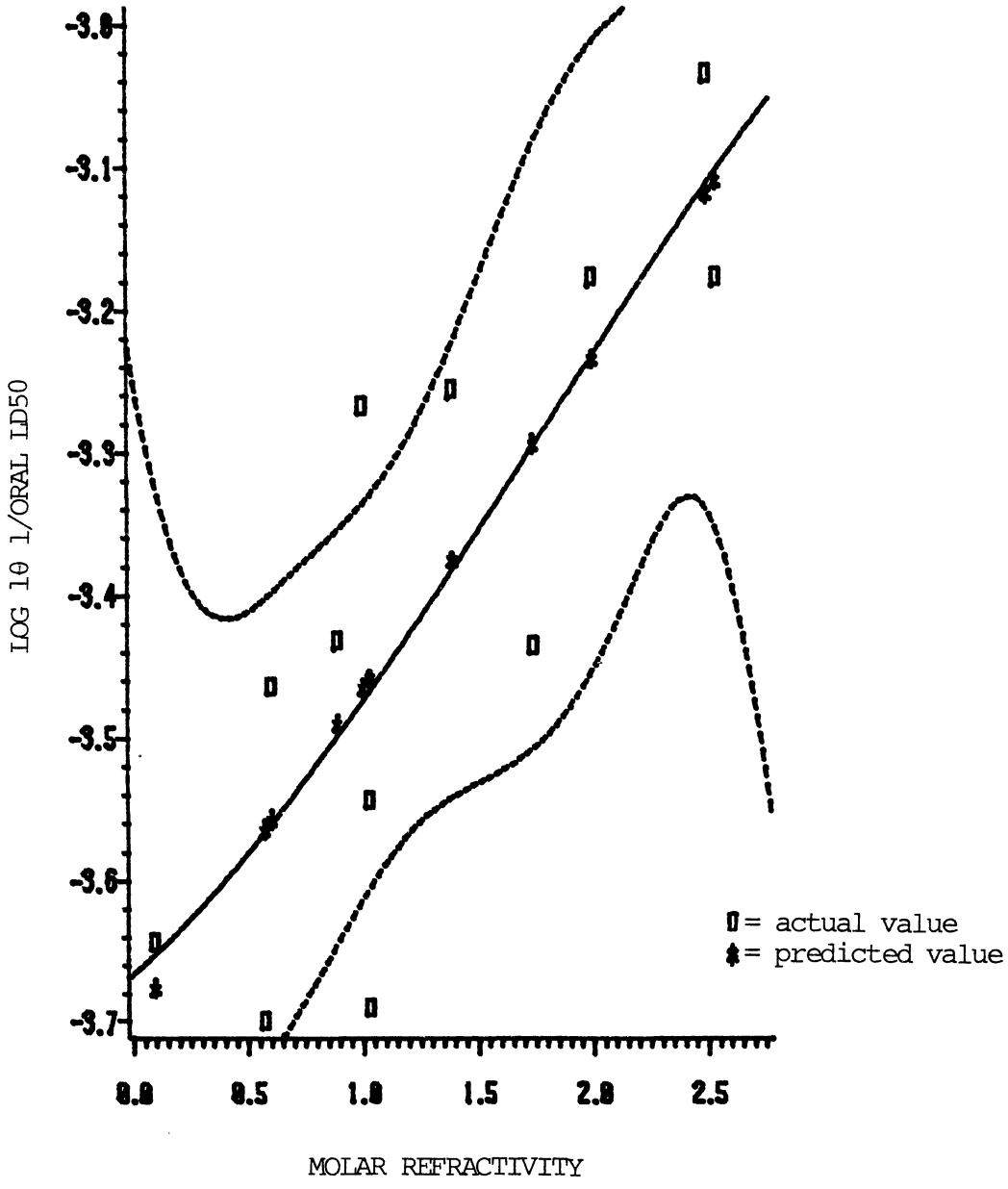

Figure 5. Actual and predicted values versus molar refractivity with 95% confidence intervals

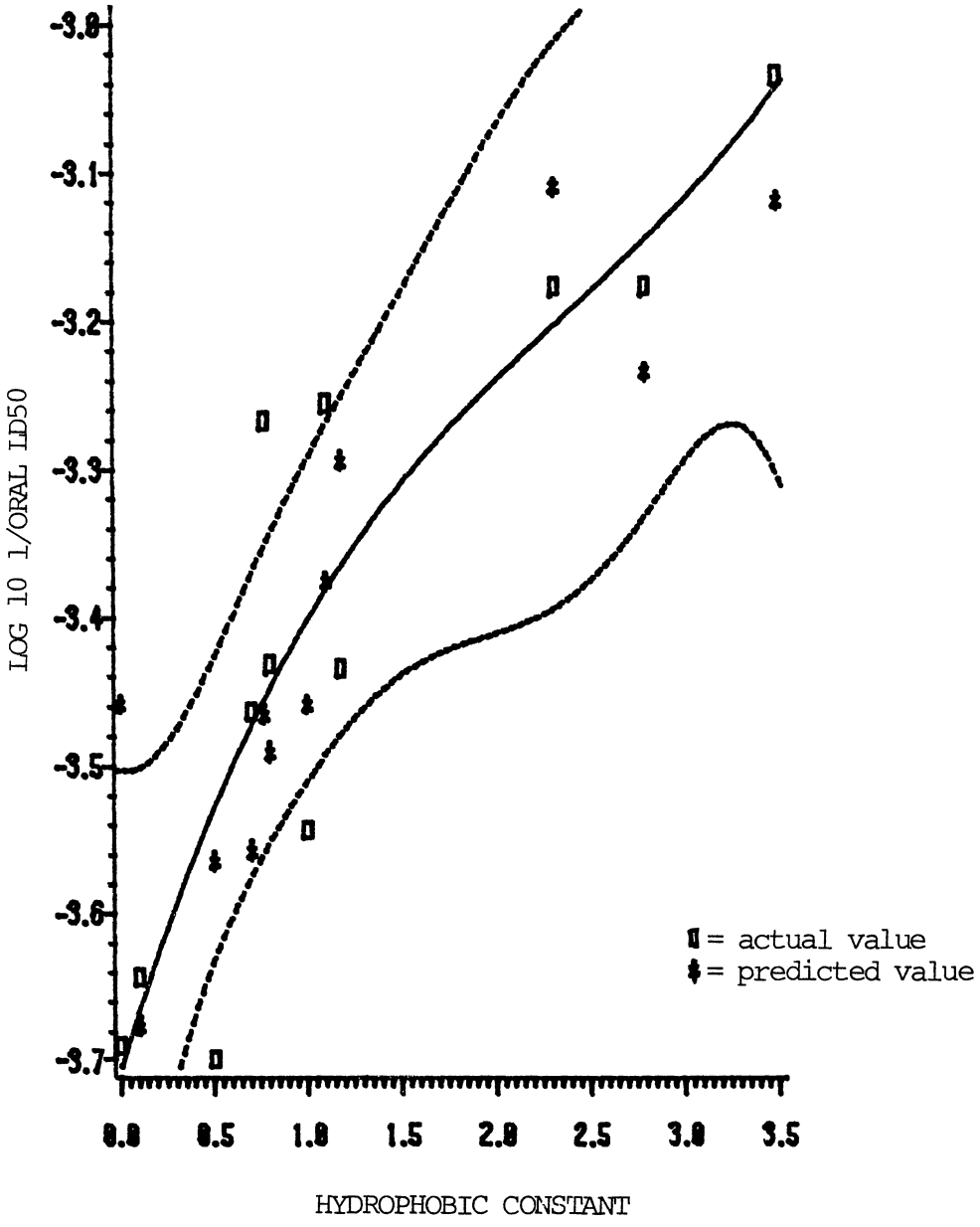

Figure 6. Actual and predicted values versus hydrophobic constant with 95% confidence intervals

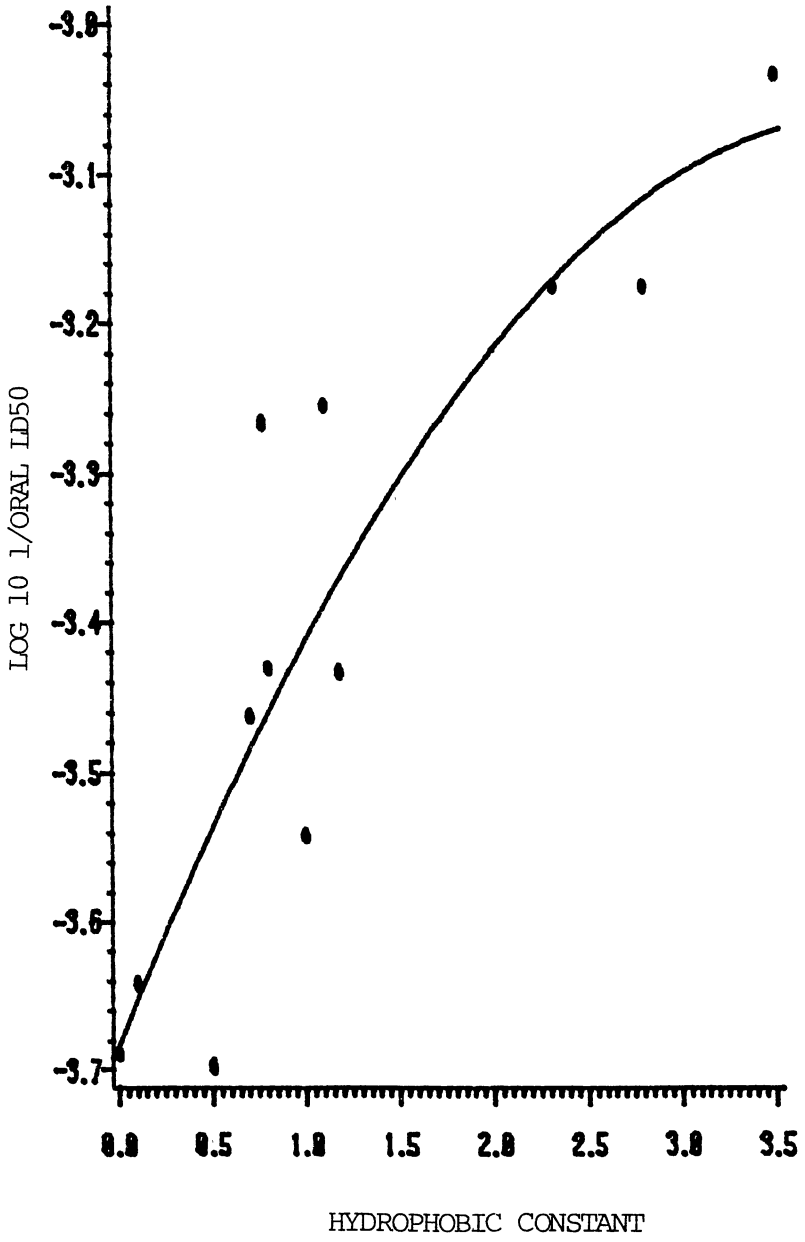

Figure 7. Quadratic relationship between the log of the inverse of oral LD50 (rat) and hydrophobic constant

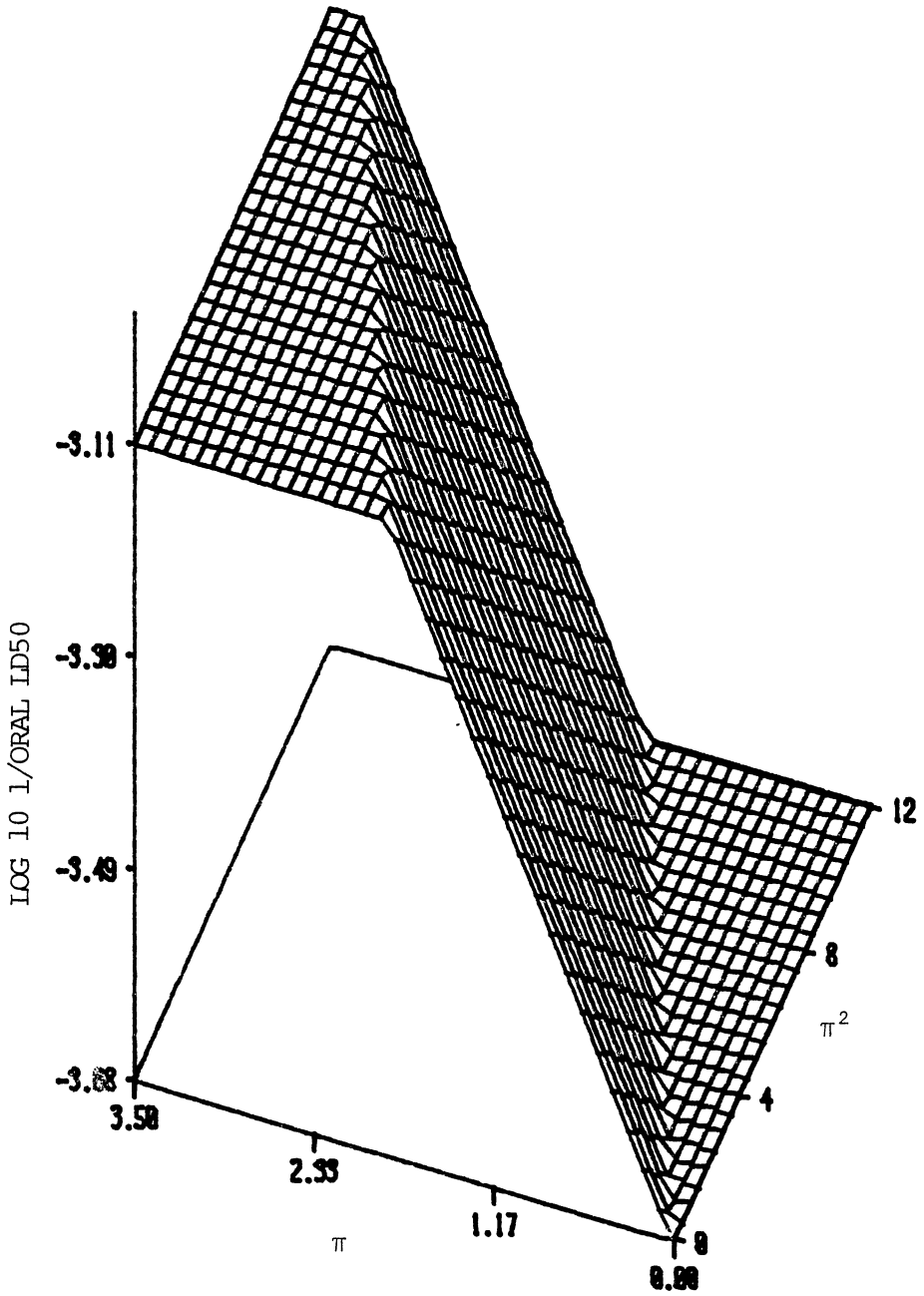

Figure 8. Three dimensional computer-generated plot of response function using π and π^2

Residual values obtained by substracting the predicted from actual or observed values were used in evaluating the assumption of homoscedasticity and the aptness of the simple and quadratic linear equations selected. Scatter plots of the residual values versus MR and π were random and did not show any systematic deviation (Figures 9 and 10). This indicated that the assumption of homoscedasticity was not violated and that linear regressions provide good fits for the data set. Figure 11 shows the plot of the residuals versus predicted values for the multiple regression analysis using hydrophobic constant. The plot also showed the error variance was independent.

The variation between predicted and observed values about the regression line was more when MR was used when compared with the variation when π was used (Figures 12 and 13). This was consistent with an earlier observation which showed that π provided a better estimate of the biological response than MR.

Finally, the observed, predicted, and residual values using the selected equation in Table IV were calculated in Tables V, VI, and VII. The chemicals initially withheld from the analysis were reintroduced in order to evaluate the regression equation. In Table V, the maximum residual value obtained was -0.4136 for trimethylbenzene. In Table VI, $\alpha\alpha$-dichloro-p-xylene had the highest residual value of 0.3690 while Table VII showed trimethylbenzene as the chemical with the highest difference between predicted and observed residual value. As a whole, the equations provide good estimates of biological response with the R^2 values ranging from 0.66 to 0.79.

The results of the regression analysis indicated that a high correlation existed between MR and toxicity and between π and toxicity. Thus proving the hypothesis that toxicity can be predicted from

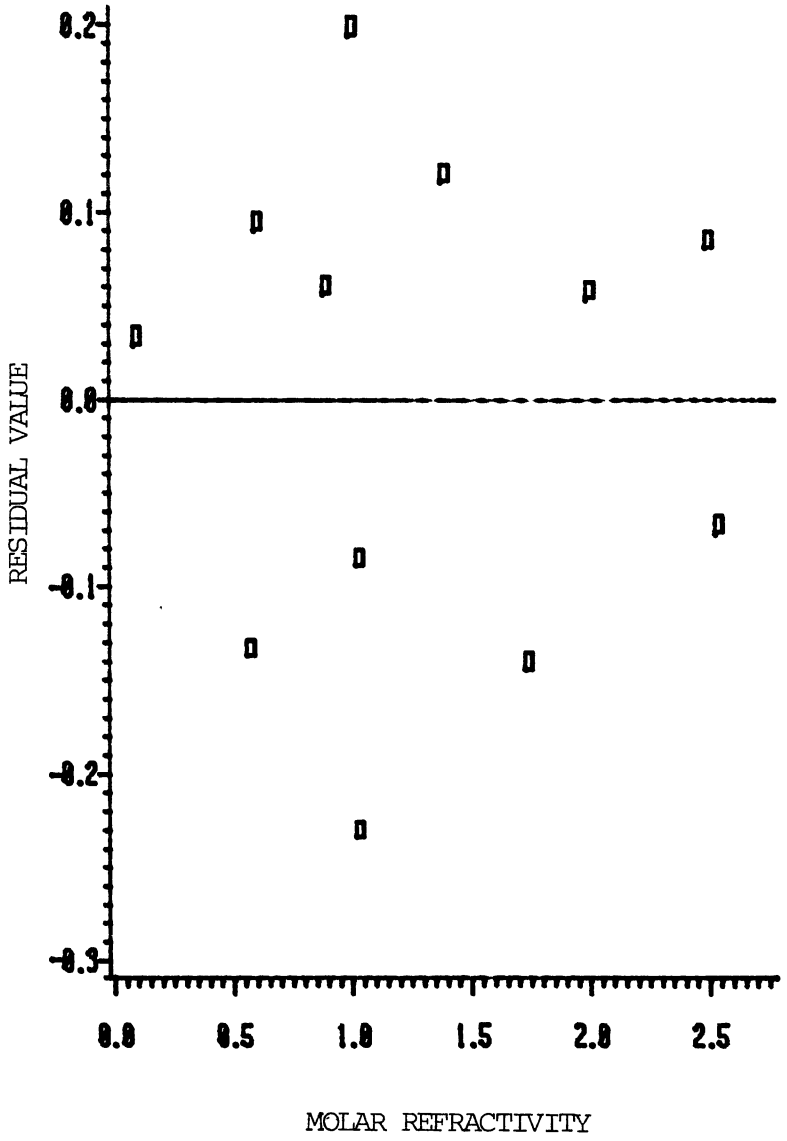

Figure 9. Scatter plot of the residual values versus molar refractivity

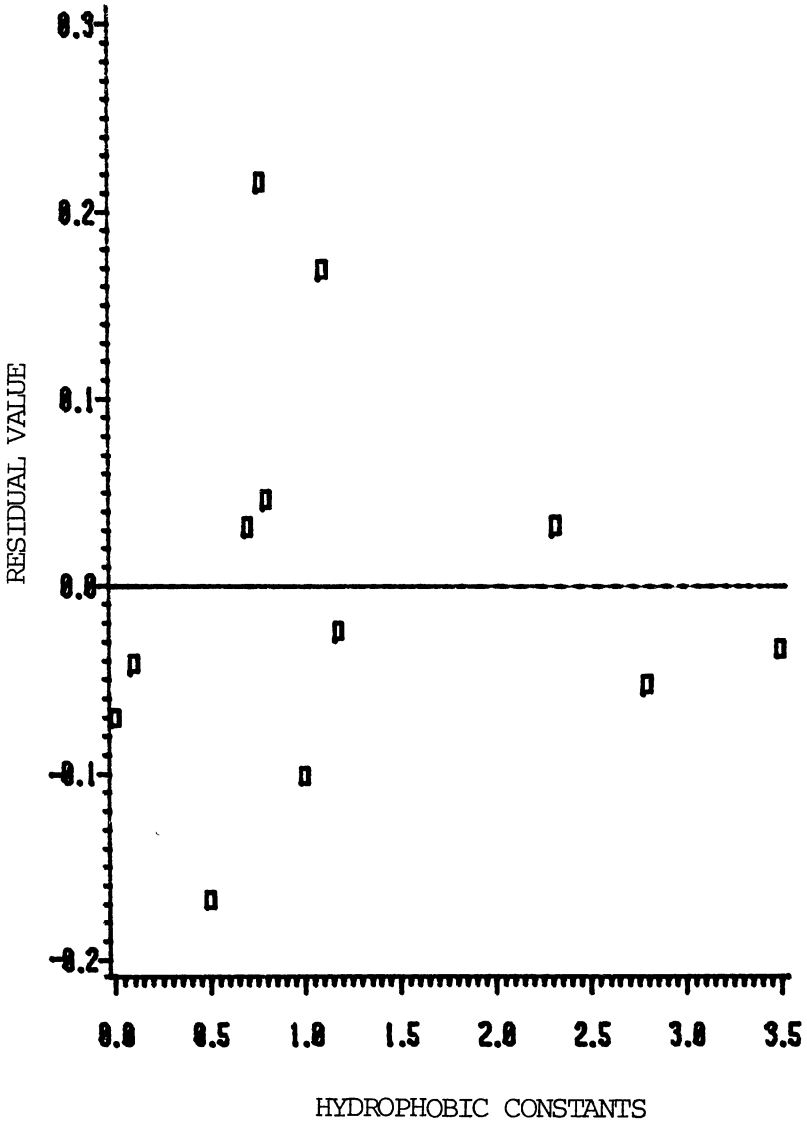

Figure 10. Scatter plot of the residual values versus hydrophobic constant

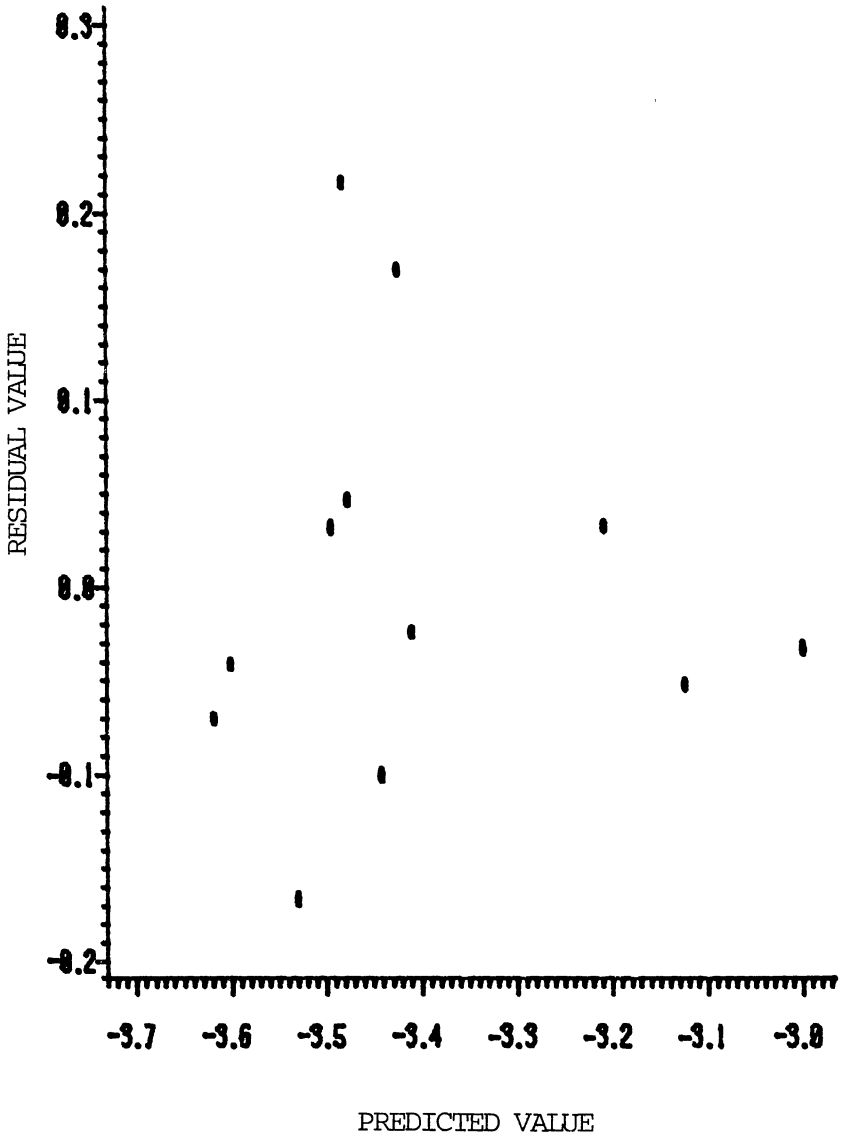

Figure 11. Residual versus predicted values using π and π^2

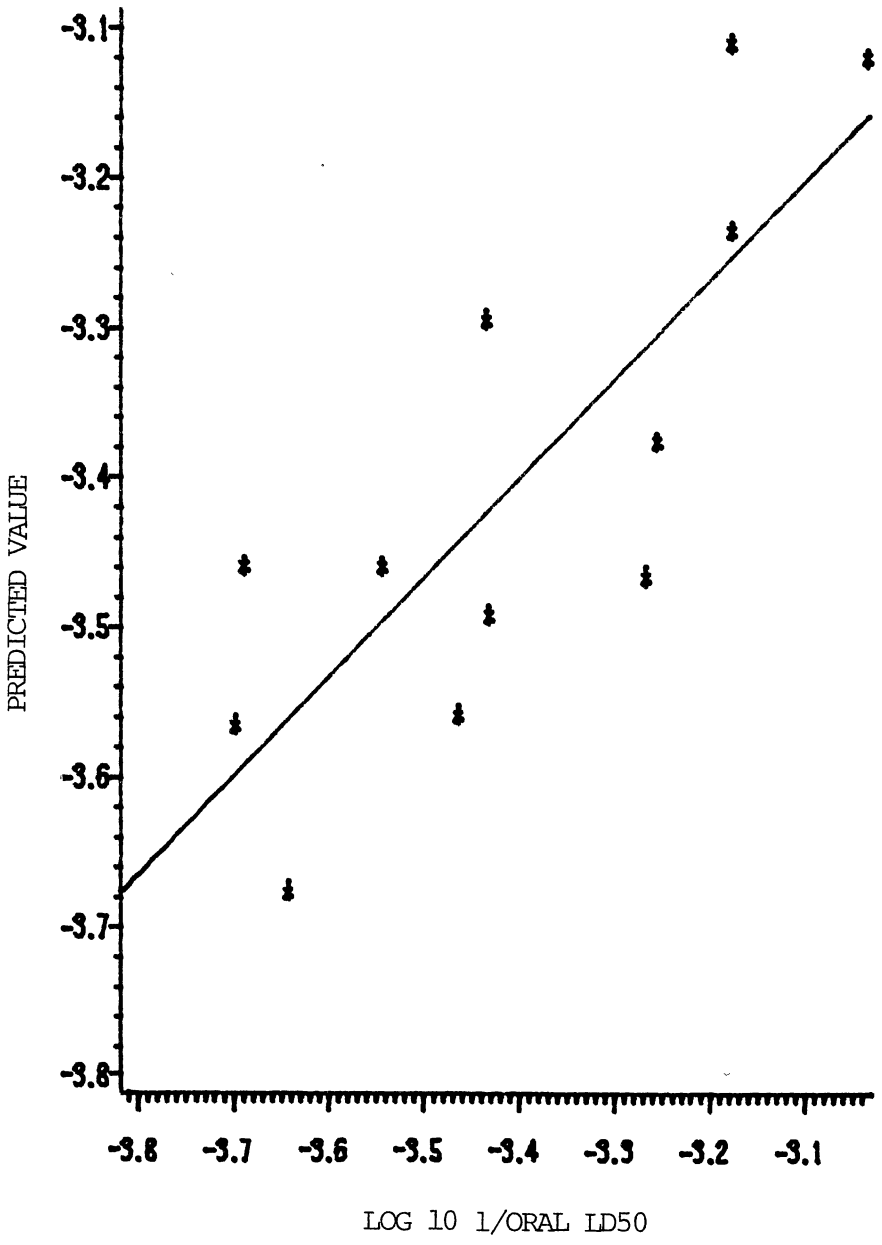

Figure 12. Predicted versus actual values using molar refractivity

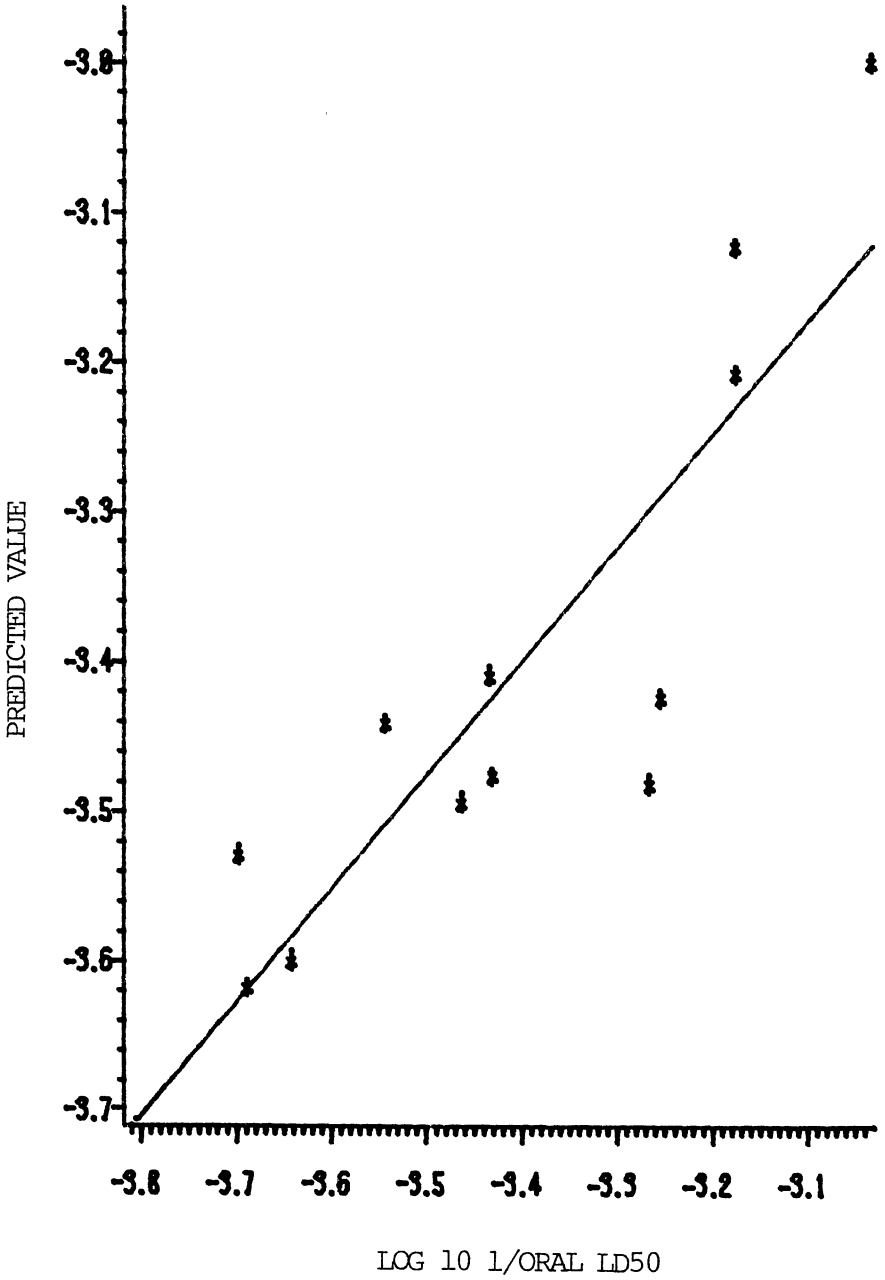

Figure 13. Predicted versus actual values using hydrophobic constant

TABLE V

ORAL LD50 VALUE FOR RATS USING EQUATION OBTAINED FROM π AND π^2

Compound	Log 1/LD50 Observed	Log 1/LD50 Predicted	Residuals
Benzene	-3.6897	-3.6833	-0.0064
Bromobenzene	-3.4312	-3.4596	0.0284
Chlorobenzene	-3.4639	-3.4849	0.0210
Ethylbenzene	-3.5441	-3.4115	-0.1326
Fluorobenzene	-3.6434	-3.6526	0.0092
Iodobenzene	-3.2550	-3.3885	0.1335
Pentachlorobenzene	-3.0334	-3.0698	0.0364
Toluene (methylbenzene)	-3.6990	-3.5377	-0.1613
Xylene (dimethylbenzene)	-3.6990	-3.3885	-0.3105
Propylbenzene	-3.6839	-3.3045	-0.3794
Tetrachlorobenzene	-3.1761	-3.1168	-0.0593
Trimethylbenzene	-3.6990	-3.2854	-0.4136
O-bromo-ααα-trifluorotoluene	-3.4346	-3.3707	-0.0639
P-tert-butyltoluene	-3.1761	-3.1709	-0.0052
1-fluoro-2-bromotoluene	-3.2672	-3.4671	-0.1999
αα dichloro p-xylene	-3.2504	-3.2345	-0.0159

TABLE VI

ORAL LD50 VALUE FOR RATS USING THE EQUATION
OBTAINED FROM MOLAR REFRACTIVITY

Compound	Log 1/LD50 Observed	Log 1/LD50 Predicted	Residuals
Benzene	-3.6897	-3.4594	-0.2303
Bromobenzene	-3.4312	-3.4919	0.0607
Chlorobenzene	-3.4639	-3.5591	0.0952
Ethylbenzene	-3.5441	-3.4594	-0.0847
Fluorobenzene	-3.6434	-3.6773	0.0339
Iodobenzene	-3.2550	-3.3760	0.1210
Pentachlorobenzene	-3.0334	-3.1188	0.0854
Toluene (methylbenzene)	-3.6990	-3.5661	-0.1329
Xylene (dimethylbenzene)	-3.6990	-3.4849	-0.3141
Propylbenzene	-3.6839	-3.3505	-0.3334
Tetrachlorobenzene	-3.1761	-3.2347	-0.0586
Trimethylbenzene	-3.6990	-3.6773	-0.0217
O-bromo-ααα-trifluorotoluene	-3.4346	-3.2949	-0.1397
P-tert-butyltoluene	-3.1761	-3.1095	-0.0666
1-fluoro-2-bromotoluene	-3.2672	-3.4664	-0.1992
ααdichloro p-xylene	-3.2504	-3.6194	-0.3690

TABLE VII

ORAL LD50 VALUE FOR RATS USING THE EQUATION OBTAINED FROM π

Compound	Log 1/LD50 Observed	Log 1/LD50 Predicted	Residuals
Benzene	-3.6897	-3.6190	-0.0707
Bromobenzene	-3.4312	-3.4774	0.0462
Chlorobenzene	-3.4639	-3.4951	0.0312
Ethylbenzene	-3.5441	-3.4420	-0.1021
Fluorobenzene	-3.6434	-3.6013	0.0421
Iodobenzene	-3.2550	-3.4243	0.1692
Pentachlorobenzene	-3.0334	-3.9995	0.0339
Toluene (methylbenzene)	-3.6990	-3.5305	-0.1385
Xylene (dimethylbenzene)	-3.6990	-3.4243	-0.2747
Propylbenzene	-3.6839	-3.3535	-0.3304
Tetrachlorobenzene	-3.1761	-3.1234	-0.0527
Trimethylbenzene	-3.6990	-3.3358	-0.3632
O-bromo-$\alpha\alpha\alpha$-trifluorotoluene	-3.4346	-3.4101	-0.0245
P-tert-butyltoluene	-3.1761	-3.1761	-0.0322
1-fluoro-2-bromotoluene	-3.2672	-3.4827	-0.2155
$\alpha\alpha$ dichloro p-xylene	-3.2504	-3.2845	-0.0341

individual parameters. It was, however, not possible to predict toxicity from an equation containing the combined parameters (π or MR) because of the high correlation between them.

The results obtained in this study can be compared with earlier attempts at predicting toxicity from physical and chemical parameters (Appendix B). While using the log of octanol/water partition coefficient (log P) as an estimate of toxicity, Konemann (1981) obtained R^2 values that ranged from 0.962 to 0.974. The use of molecule connectivity index resulted in R^2 values ranging from 0.314 to 0.64 (Konemann and Musch, 1981). R^2 value of 0.962 was obtained when toxicity was estimated by log P and pKa. Schultz et al. (1980) obtained a R^2 value of 0.962. R^2 vlaue of 0.991 was obtained for only three azarenes using molecular weight (Southworth et al., 1978b). An estimate of toxicity using substructural approach attempted by Craig and Enslein (1978) gave low R^2 values when compared with the results from Hansch type approach to QSAR. The substructural approach is, however, applicable to a wider range of chemicals.

Based upon a comparison with published literature, it would appear that the use of log P of a whole molecule provides a better estimate of toxicity than the use of the substituent, π, evaluated in this study. However, using π resulted in the elimination of the need to experimentally measure the partition coefficient of individual chemicals. Even though more rapid procedures have been developed to estimate hydrophobicity by high pressure liquid chromatographic analysis (Konemann, 1981), a considerable amount of time and costly equipment would be required to experimentally estimate hydrophobicity for all chemicals. Therefore, the use of substituent effects (π) may be justified in some

cases where log P data is not available and could not be easily measured. Substituent effects data could be quickly and easily calculated for organic chemicals and used to make preliminary predictions of relative toxic effects.

CHAPTER V

SUMMARY AND CONCLUSION

The Toxic Substances Control Act (1970) requires that new chemicals be tested for their health and environmental effects. The act also provides that authorization should not unduly impede or create an unnecessary economic barrier to technological innovations. With an estimated 400,000 chemicals synthesized yearly, the cost of individual testing is rapidly becoming prohibitive. The use of Quantitative Structure-Activity Relationship (QSAR) studies can substantially reduce the amount of toxicological testing required for new chemicals. This can be done by making use of the existing data base of physical, chemical, and biological parameters. QSAR methods assume that an observed biological response is a function of the structure of the chemical inducing it. As such, it should be possible to predict the toxicity of a chemical from a knowledge of its structure.

QSAR methods are relatively inexpensive and the required statistical analysis can be performed at most computer installations. The methods can, therefore, provide for time and cost effective means of screening industrial chemicals. With this approach, QSAR methods supplement rather than replace other forms of biological testing. A preliminary quick screen of biological activity of chemicals through the use of QSAR should proceed detailed biological or ecotoxicological testing.

In this study, the use of substituent effects in quantifying a biological activity of selected organic compounds was attempted. Specifically, the contribution of a substituent's size or hydrophobicity to the toxicity of the chemicals was determined. Chemicals were selected from the Registry of Toxic Substances (1983). The selected compounds have the same parent compound, namely benzene, the same mode of action, and contain relatively nonpolar substituents. The substituents' hydrophobicity was estimated by the use of hydrophobic constant, π , and the size by its molar refractivity, MR. Attempts were made to derive regression equations which will provide good estimates of the observed toxicity. Statistical analysis allowed the evaluation of the possible equations derived from the data set which contained statistically significant equations.

The results indicated that toxicity, as measured by log (1/LD50) for rats, can be predicted from either molar refractivity or hydrophobic constant. Hydrophobic constant, however, provides a better estimate of toxicity than molar refractivity. An estimation of the combined effects of both molar refractivity and hydrophobic constant was not possible due to high correlation between the two parameters. The observed high correlation indicated that the two parameters estimated essentially the same thing and can be used interchangeably.

The best estimate of toxicity was obtained when a square term was introduced into an equation containing π. This indicated that an optimum biological response existed for the chemicals analyzed when hydrophobic constant was used.

A test of the regression equation with four chemicals not included in the analysis showed that all chemicals can be predicted with less than 0.4136 log units deviation between observed and calculated LD50.

By using substituent effects of chemicals in estimating their toxicity, a considerable amount of money and time was saved when compared with experimental estimation.

Further research efforts should be directed towards:

1. Estimating the contribution of π and MR at specific positions on the molecules by factoring π and MR by position in order to understand the three dimensional contribution of the shape of a chemical in estimating toxicity,

2. Evaluating the use of other parameters in toxicity estimation,

3. Detailed examination of the biological activities of deviant molecules whose activities did not conform with other members of the same group, and

4. Incorporating obtained statistical models and large data base of useful parameters and biological data in a more sophisticated program for easy retrieval and utilization.

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APPENDIXES

APPENDIX A

FINAL LIST OF COMPOUNDS USED IN THE QSAR ANALYSIS

OBS	Compound	Subst	LD50	MR	π	Log(1/LD50)	MR^2	π^2
1	Benzene	H	4894	1.03	0.00	-3.6897	1.0609	0.0000
2	Bromobenzene	BR	2699	0.89	0.80	-3.4312	0.7921	0.6400
3	Chlorobenzene	CL	2910	0.60	0.70	-3.4639	0.3600	0.4900
4	Ethylbenzene	C2H5	3500	1.03	1.00	-3.5441	1.0609	1.0000
5	Fluorobenzene	FL	4399	0.09	0.10	-3.6434	0.0081	0.0100
6	Iodobenzene	I	1799	1.39	1.10	-3.2550	1.9321	1.2100
7	Pentachlorobenzene	5CL	1080	2.50	3.50	-3.0334	6.2500	12.2500
8	Toluene (Methylbenzene)	CH3	5000	0.57	0.50	-3.6990	0.3249	0.2500
9	Tetrachlorobenzene	4CL	1500	2.00	2.80	-3.1761	4.0000	7.8400
10	O-bromo-ααα trifluoro toluene	CH3 BR	2720	1.74	1.18	-3.4346	3.0276	1.3924
11	P-tert-butyl toluene	C(CH3)3CH3	1500	2.54	2.32	-3.1761	6.4516	5.3824
12	1-floro-2-bromo benzene	F BR	1850	1.00	0.77	-3.2672	1.0000	0.5929

APPENDIX B

REGRESSION EQUATIONS FOR PREDICTING TOXICITY OF SELECTED ORGANIC COMPOUNDS

Source	Type of Chemicals	Equation	R^2	N	S
Konemann, H. 1981	Benzene and chlorobenzene	log 1/LC50 = 0.845 log Poct - 4.63	0.974	12	0.133
	Nondissociating organic chemicals	log 1/LC50 = 0.907 log Poct - 4.94	0.976	21	0.201
		log 1/LC50 = 0.871 log Poct - 4.87	0.976	50	0.237
		log 1/LC50 = 0.698 log S + 0.09	0.941	27	0.214
		log 1/LC50 = 0.799 $^{O}X^{V}$ - 7.05	0.640	50	0.929
		log 1/LC50 = 0.269 $^{O}X^{V}$ - 6.034	0.314	14	0.538
		log 1/LC50 = 0.626 $^{O}X^{V}$ - 5.62	0.881	36	0.318
Konemann, H. and Musch, A. 1981	Chlorophenols	log 1/LC50 = 1.12 log Poct + 0.43 Pka - 8.35 at pH - 7.8	0.962	11	0.102
Schultz, T.W. et al., 1980	Nitrogen-containing heterocyclic compounds	log LC100 = -1.042 log P + 2.900	0.962	11	-----
Southworth, G.R. et al., 1978b	Azaarenes	log LC50 = -0.0219(MW) + 4.047	0.991	3	-----

APPENDIX C

STATISTICAL ANALYSIS OF ORIGINAL LIST OF CHEMICALS

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Model	1	0.00644883	0.00644883	0.06	0.8163
Error	21	2.44696144	0.11652197		
Corrected Total	22	2.45341026			

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Model	1	0.00069271	0.00069271	0.01	0.9437
Error	21	2.84435255	0.13544536		
Corrected Total	22	2.84504525			

Observation	Observed Value	Predicted Value	Residual	Lower 95% CL for Mean	Upper 95% CL for Mean
1	-3.68966397	-3.39830424	-0.29135973	-3.56034265	-3.23626582
2	-3.43120288	-3.40199325	-0.02920964	-3.57981279	-3.22417371
3	-3.69897000	-3.37379867	-0.32517134	-3.58503034	-3.16256690
4	-3.46389299	-3.40963477	-0.05425822	-3.63211018	-3.18715936
5	-2.69897000	-3.39909474	0.70012474	-3.54609986	-3.23408962
6	-3.69897000	-3.37669717	-0.32227283	-3.57048328	-3.18291107
7	-3.54406804	-3.39830424	-0.14576381	-3.56034265	-3.23626582
8	-3.64335396	-3.42307331	-0.22028065	-3.74411550	-3.10203112
9	-3.25503116	-3.38881821	0.13378705	-3.53791922	-3.23971720
10	-3.03342376	-3.35956962	0.32614587	-3.67317679	-3.04596246
11	-3.69897000	-3.41042527	-0.28854473	-3.63816495	-3.18268560
12	-3.09025805	-3.39777724	0.30751918	-3.55797672	-3.23757775
13	-3.69897000	-3.40120275	-0.29776726	-3.57524715	-3.22715834
14	-3.68394713	-3.38591970	-0.29802743	-3.54021225	-3.23162715
15	-3.80672250	-3.37669717	-0.43002533	-3.57048328	-3.18291107
16	-3.17609126	-3.37274466	0.19665340	-3.59702122	-3.15476811
17	-3.84410423	-3.37669717	-0.46740706	-3.57048328	-3.18291107
18	-2.87852180	-3.38591870	0.50739791	-3.54021225	-3.23162715
19	-3.43456890	-3.37959568	-0.05497322	-3.55792193	-3.20126944
20	-3.17609126	-3.35851562	0.18242436	-3.68036689	-3.03666435
21	-2.82607480	-3.39909474	0.57301994	-3.56409986	-3.23408962
22	-3.26717173	-3.39909474	0.13192301	-3.56409986	-3.23408962
23	-3.25042000	-3.41648579	0.16606579	-3.68718908	-3.14578250

Observation	Observed Value	Predicted Value	Residual	Lower 95% CL for Mean	Upper 95% CL for Mean
1	-3.68966397	-3.52703587	-0.16262809	-3.81155896	-3.24251279
2	-3.43120288	-3.53113280	0.09992992	-3.72866988	-3.33359573
3	-3.69897000	-3.53779032	-0.16117969	-3.71506040	-3.36052023
4	-3.46389299	-3.53062069	0.06672770	-3.73728478	-3.32395660
5	-2.69897000	-3.53420550	0.83523550	-3.69607112	-3.37233989
6	-3.69897000	-3.53727820	-0.16169180	-3.70858999	-3.36596641
7	-3.54406804	-3.53215704	-0.01191101	-3.71374239	-3.35057169
8	-3.64335396	-3.52754799	-0.11580597	-3.79987025	-3.25522573
9	-4.00000000	-3.54854476	-0.45145524	-3.96985217	-3.12723735
10	-3.25503116	-3.53266915	0.27763799	-3.70763981	-3.35769850
11	-3.03342376	-3.54495995	0.51153619	-3.87218221	-3.21773768
12	-3.69897000	-3.52959646	-0.16937355	-3.75635400	-3.30283891
13	-3.09025805	-3.52754799	0.43728994	-3.79987025	-3.25522573
14	-3.69897000	-3.53266915	-0.16630085	-3.70763981	-3.35769850
15	-3.69897000	-3.53512731	-0.16384269	-3.69471443	-3.37554019
16	-4.17609126	-3.53113280	-0.64495845	-3.72866988	-3.33359573
17	-3.68394713	-3.53471762	-0.14922951	-3.69476809	-3.37466715
18	-3.80672250	-3.53830243	-0.26842007	-3.72254462	-3.35406025
19	-3.17609126	-3.54137513	0.36528387	-3.78300017	-3.29975009
20	-3.84410423	-3.53830243	-0.30580180	-3.72254462	-3.35406025
21	-2.87852180	-3.53779032	0.65926852	-3.71506040	-3.36052023
22	-3.69897000	-3.54239936	-0.15657064	-3.80711970	-3.27767902
23	-3.69897000	-3.53522974	-0.16374027	-3.69483981	-3.37561966

APPENDIX D

REGRESSION ANALYSIS USING MOLAR REFRACTIVITY

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Model	1	0.35209838	0.35209838	19.48	0.0013
Error	10	0.18071004	0.01807100		
Corrected Total	11	0.53280842			

APPENDIX E

REGRESSION ANALYSIS USING HYDROPHOBIC CONSTANT

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Model	1	0.40346449	0.40346449	31.19	0.0002
Error	10	0.12934393	0.01293439		
Corrected Total	11	0.53280842			

APPENDIX F

REGRESSION ANALYSIS USING π AND π^2

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	PR > F
Model	2	0.42109659	0.21054830	16.96	0.0009
Error	9	0.11171183	0.01241243		
Corrected Total	11	0.53280842			

Observation	Observed Value	Predicted Value	Residual	Lower 95% CL for Mean	Upper 95% CL for Mean
1	-3.68966397	-3.68327220	-0.00639176	-3.84956740	-3.51697701
2	-3.43120288	-3.45964261	0.02843972	-3.54533327	-3.37395194
3	-3.46389299	-3.48489323	0.02100024	-3.56890139	-3.40088508
4	-3.54406804	-3.41145828	-0.13260977	-3.50586827	-3.31704828
5	-3.64335396	-3.65261543	0.00926147	-3.79784829	-3.50738257
6	-3.25503116	-3.38852457	0.13349341	-3.48844788	-3.28860126
7	-3.03342376	-3.06980774	0.03638399	-3.29004847	-3.84956702
8	-3.69897000	-3.53771140	-0.16125860	-3.62779956	-3.44762324
9	-3.17609126	-3.11681456	-0.05927670	-3.24945541	-3.98417370
10	-3.43456890	-3.37073367	-0.06383524	-3.47511755	-3.26634978
11	-3.17609126	-3.17091953	-0.00517173	-3.29816074	-3.04367832
12	-3.26717173	-3.46713670	0.19996497	-3.55206498	-3.38220843

VITA 2

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