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Authors
Tanji, Kenneth K
Sullivan, Jonathan J

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QSAR ANALYSIS OF THE CHEMICAL HYDROLYSIS OF ORGANOPHOSPHORUS PESTICIDES IN NATURAL WATERS.

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Kenneth K. Tanji
Principal Investigator
and
Jonathan J. Sullivan
Graduate Research Assistant
Department of Land, Air and Water Resources
University of California, Davis

Technical Completion Report
Project Number W-843
August, 1995

University of California Water Resource Center

The research leading to this report was supported by the University of California Water Resource Center as part of Water Resource Center Project W-843.

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Abstract

Statistical techniques for screening experimental or literature chemical databases for compounds exhibiting potential environmental activity are becoming increasingly utilized in environmental analysis as pragmatic and economical complementary tools to enhance or augment costly traditional analytical procedures. Utilizing the predictive modeling approach, it is often argued, implicitly permits an unlimited number of chemicals to be screened for specific behavioral or physicochemical characteristics in a variety of environmental and biological matrices, consequentially conserving the financial resources for exhaustive testing, yet providing a methodology that helps to insure that questionable compounds are more thoroughly tested. Moreover, such techniques provide a database of exhaustive test results from which investigators and regulators can extract relevant information for further research or decision-making.

To assess the efficiency of statistical modeling methods for predicting chemical processes in the environment, a one-year exploratory study utilizing Quantitative Structure-Activity Relationship (QSAR) methodology to obtain linear model equations for estimating the rates of chemical hydrolysis of several organophosphorus (OP) pesticides in natural river waters has been conducted. This modeling effort specifically considers the effects of chemical structure on reactivity and utilizes connectivity parameters from graph theory as quantitative structural descriptors. Derived model equations were examined to establish whether quantitative correlations between fundamental molecular characteristics and observed hydrolytic properties were possible. Inconclusive results for a training set of six OP pesticides indicate that there are inherent weaknesses in molecular connectivity theory when applied to complex reaction parameters that require further exploration. The inherent complexity of most chemical reaction mechanisms and the indistinct influence of both adjoining and distant atoms in the molecular environment makes it difficult for a single descriptor, even one as widely successful as connectivity indices, to adequately account for definitive structural characteristics of molecules. It is apparent from results of this study that molecular connectivity indices alone are often not discriminating enough descriptors for procuring comprehensive structure-property relationships beyond a rather restricted range of structural variation, at least when characterizing chemical reaction parameters.

Technical Completion Report

PROJECT NUMBER W-843 START: July 1, 1994

TITLE: **QSAR ANALYSIS OF THE CHEMICAL HYDROLYSIS OF ORGANO-PHOSPHORUS PESTICIDES IN NATURAL WATERS.**

INVESTIGATORS: Kenneth K. Tanji and Jonathan J. Sullivan

KEY WORDS: Model Studies, Pesticides, Organic Compounds, Risk Analysis And Management, Water Chemistry, Toxic Substances, Water Pollution.

PROBLEM AND RESEARCH OBJECTIVES

Since 1978, the California State Water Resources Control Board in association with the California Environmental Protection Agency has conducted a comprehensive statewide survey (Toxic Substances Monitoring Program) of hundreds of streams, rivers, lakes, and estuaries suspected of having water quality problems. This annual assessment encompasses nine regions and as many as 150 monitoring stations representing over 130 water bodies. In 1990, sixty-five percent of the stations exceeded both state and federal EPA criteria for metals, organics, and fish from 95 of the 145 stations sampled exceeded at least one metal or organic chemical comparative criterion (TSMP, June,1991). Such reports suggest that the extensive use and misapplication of agrochemicals on California's vast and productive agricultural lands is a primary factor affecting surface water quality. As a result, the potential exists for elevated levels of agricultural and industrial chemicals to affect both local ecosystems and human health, especially if downward migration of groundwater containing high concentrations of contaminants reach regional aquifers used as drinking water sources.

These concerns emphasize that the appropriate utilization and management of California's limited freshwater resources strongly relies on the ability to accurately assess the potential health and environmental impacts of xenobiotics in aquatic ecosystems and on our understanding of the physicochemical pathways and mechanisms by which they react and are distributed under natural conditions. Scientists and regulators, in turn, require thorough and reliable information about the

fate, behavior, and toxicity of chemicals of actual or potential environmental concern in order to develop defensible risk assessments and sensible risk management strategies for contaminants. However, the experimental determination of the necessary environmental parameters is often immoderately resource-consuming. Bioconcentration tests, for example, have been estimated to cost $6,000-$10,000 for each chemical, and acute toxicity tests cost $2,000-$3,000 for each analysis. Furthermore, the cost of testing derivatives of these compounds—which potentially number in the hundreds of thousands— has been estimated to be about $2500-$4500 for acute dermal toxicity, $20,000-$25,000 for 14 day inhalation studies, $300,000-$400,000 for two-year dietary studies, and $1,000,000-$1,500,000 for two-year inhalation studies. Because about 1000-1600 new chemicals are evaluated each year, regulatory decisions involving such testing must have a firm technical basis and justification. A recent report of the National Research Council found that in only 20% of all cases can health hazard assessments be satisfactorily performed (Nirmalakhandan and Speece, 1988). Clearly, it is not pragmatic in terms of human and material resources to perform detailed experimental analysis on all known or uncharacterized compounds and their derivatives. It is evident that the ability to quantitatively estimate such properties would be extremely useful assessment tool.

Developing a reliable method to estimate degradative processes under environmental conditions would be a practicable tool for screening large numbers of compounds without having to rely exclusively on costly and time-consuming analytical procedures to establish their characteristic behavior in the environment. Utilizing the QSAR modeling approach, compounds may be screened for specific physicochemical characteristics in a variety of environmental and biological matrices, conserving the financial resources for exhaustive testing, yet providing a methodology that helps to insure that questionable compounds are more thoroughly tested. Moreover, QSAR models provide a data base of exhaustive test results from which investigators and regulators can extract pertinent information for further research or decision-making (Karcher and Devillers, 1990). Hydrolysis is usually the initial and often the primary degradative pathway undertaken by agrochemicals in aqueous media, therefore we have focused our attention on

developing QSAR models for estimating the rates of hydrolytic degradation of organophosphorus pesticides in natural waters.

Objectives:

(1) Explore the utility of statistical models to evaluate the rates of chemical hydrolysis of organophosphorus pesticides in environmental waters based on literature-derived reaction rates and graph invariant connectivity indices.

(2) Validate derived models using experimentally acquired hydrolysis rate data. Ascertain the efficacy of model equations for predicting chemical degradation processes in environmental waters.

INTRODUCTION

The use of screening models for estimating the behavior and physicochemical properties of environmental contaminants has increased steadily in recent years as more practical and economical analytical methods for attaining comprehensive data for assessment and regulatory purposes are vigorously pursued. Because the costly and time-consuming nature of analytical testing and the enormous number of possible environmental contaminants makes the rigorous assessment of most chemicals infeasible, it has become increasingly necessary to methodically rank contaminants by some practicable means according to their inherent behavior or risk and analyze them selectively on such a basis. Deciding which chemicals warrant exhaustive testing and which do not, however, is not a trivial matter, and much effort has been invested into developing accurate and reliable predictive screening models for systematically evaluating a contaminants potential for harmful activity. Ideally, once this judgment is made, those chemicals whose risk appears high may then be justifiably analyzed in detail, otherwise they need not be, conserving both the financial and human resources.

With respect to this developing need, Quantitative Structure-Activity Relationship (QSAR) methodology is becoming a significant source of statistical models specifically designed to selectively screen environmental data and flag potential problem chemicals. QSAR's are regression equations which attempt to correlate a particular chemical's observed behavior or properties with one or more of its inherent molecular or physicochemical characteristics. Such relationships often

produce linear models having powerful predictive capabilities and as a result QSAR has evolved into one of the more routinely utilized property estimation and screening techniques. This project has focused primarily on investigating, via QSAR, the influence of molecular form and organization on the chemical degradation of organophosphorus (OP) pesticides in natural waters by implementing a comprehensive search for quantitative relationships between the structural (topological) characteristics of a series of OP esters and their experimentally determined rates of hydrolysis in natural river waters. Structural information for the compounds of interest have been derived from Graph Theory algorithms and pertinent programs for calculating various graph invariant indices (descriptors) have been designed and validated. Our objective is to explore the utility of this approach for deriving practical chemical property estimation models for use as predictive tools in environmental applications.

THEORETICAL BACKGROUND

QSAR Methodology.

QSAR is essentially the search for a model which correlates activity to independent variables. For this reason, analysis in QSAR is statistical in nature. (Kier and Hall, 1986). Implicitly, the QSAR approach is based on the assumption that the structure of an entire molecule must contain the features responsible for its physical, chemical, and biological properties and that it is possible to represent a molecule by numerical descriptors. Hence, our principal objective was to utilize connectivity indices to delineate these features and employ them as molecular descriptors to develop comprehensive semi-empirical QSAR models that capably estimate the rates of hydrolytic degradation of a broad range of organophosphorus pesticides in natural waters. Upon completing necessary molecular descriptor calculations and obtaining the relevant training set data, regression analysis is performed, the results plotted and tested statistically, and a model equation derived. Such equations are regression models which are utilized to seek quantitative correlations between given properties of a set of chemicals and their structure in terms of the molecular, topological, or electronic descriptors that are determined to best define their molecular framework. The ensuing

mathematical model can be either linear or nonlinear, but is generally a simple or multiple linear regression equation of the form

$$y = b_0 + b_1 x_1 + b_2 x_2 + \ldots b_n x_n \qquad (1)$$

where the independent variable is a descriptor derived from molecular structure (topological indices) or some easily measurable or calculable physicochemical property (log p, molar refractivity, solubility) and the dependent variable is a chemical or physical property of interest (toxicity, degradation rates, transport or partitioning characteristics, bioaccumulation, etc.). The constants b_0, b_1, etc., are determined statistically (Kier and Hall, 1986). This study has utilized molecular connectivity indexes as molecular descriptors because they are based on sound chemical and mathematical principles and have shown to consistently provide excellent correlation with many physiochemical properties (Seybold, May, and Bagel, 1987). Figure 1 summarizes the general design for a QSAR study and the selection of an appropriate training set .

Table 1 and Figure 2 illustrate the fundamental QSAR approach. The rates of hydrolysis of eleven phosphate, phosphorothioate and phosphorothiolate pesticides (Methyl Parathion, Dimethoate, Diazinon, Dichlorvos, Carbophenothion, Fenthion, Phosphamidon-α, Oxydemeton-Methyl, Demeton-S-Methyl, Trichlorfon, Phenkapton), was evaluated with respect to first-order connectivity indices and was found to yield a model equation having a correlation coefficient of 0.947 (Eto, 1974). Hence, results indicate that close to 95% of the variation in rates may be predicted given the structural parameters and initial rate data. It is often possible to derive better relationships, however, by utilizing more than one structural descriptor. Bulk molecular properties, for instance, such as molar volume, bond lengths, molar refractivity, etc., have shown promise as structural descriptors when used in combination with topological indices in multivariable analysis, frequently leading to noticeable improvements in statistical relationships over single-variable models. Often, the inclusion of additional molecular descriptors can greatly extend the predictive capability of the original model equation. Much of the effort expended in the derivation of useful QSAR models, in fact, involves diligently testing various combinations of descriptors until the most statistically significant models are found.

Molecular Connectivity Theory

Molecular connectivity indices (MCI) are derived from Graph Theory, a branch of mathematics which considers the quantitative manner in which objects are connected. Molecules can be represented graphically when the only property considered is the existence or not of a chemical bond. This property is known as *molecular topology*. Graphs corresponding to molecules are called chemical graphs and provide a pictorial representation of the topological structure (connectivity) of a molecule. Graph vertices correspond to atoms and graph edges depict bonds between pairs of atoms. Figure 3 illustrates how a typical pesticide is represented graphically for the purpose of determining molecular topological properties for QSAR applications.

Molecular connectivity indexes are the most successful of the topological (structural, geometrical) indices available to the QSAR practitioner at present. Their success has been attributed to their sound mathematical, chemical, and structural grounds and because they were developed specifically to parallel important physicochemical properties such as boiling points, molecular surface area, enthalpies of formation, and so on (Kier and Hall, 1983). Developed by Randic in the mid-seventies, MCI's were originally developed and utilized to rank alkanes according to the notion of branching (Randic,M., 1976) Kier and Hall later extended Randic's original approach to include valence indices, which allowed inclusion of heteroatoms, rings, and more complex structural forms (Kier and Hall, 1976). They have since been shown to be abundant in structural information related to topological, geometric and spatial attributes of molecules. A typical molecular connectivity index is represented by ${}^{n}\chi^{v}_{m}$where

- The Greek letter chi (χ) represents the index itself.
- Two superscripts and one subscript are used to specify the particular index:
    - The left-side superscript (zero or positive integers) is used to designate the order of the index.
    - The right-side superscript (letter v) differentiates between valence and non-valence type indices.

- The right-side subscript (p, c, pc, or ch) specifies the subgraph or suborder of the molecular connectivity index in which may be path, cluster, path /cluster or chain type index. If no subscript is indicated it is assumed to be a path type index.

The path and cluster structural forms are designated ${}^{n}\chi_{p}$ and ${}^{n}\chi_{c}$, respectively. The order of path subgraphs run from a minimum of zero to usually a maximum of seven (orders higher than seven are possible but their merit and significance has not been established). Cluster subgraphs have orders of three (e.g., a tertiary carbon or heteroatom) and four (e.g., a quatenary carbon or heteroatom). The path/cluster (minimum fourth order) subunit is designated ${}^{n}\chi_{pc}$ and the chain or cycle (minimum third order, e.g., cyclopropane) by ${}^{n}\chi_{cy}$. Fundamental examples of each subgraph are illustrated in Figure 4.

Information used in the calculation of molecular connectivity indices are the number and type of atoms and bonds as well as the numbers of all valence electrons. For molecules containing heteroatoms the valence chi, χ^{v}, is used. All molecular connectivity indices are calculated from the non-hydrogen part of the molecule, i.e., information is extracted from hydrogen-suppressed chemical graphs. Each non-hydrogen atom is described by its atomic δ or δ^{v} value, which is equal to the number of adjacent non-hydrogen atoms:

$$ {}^{n}\chi = \sum (\delta_i * \delta_j)^{-1/2} \qquad (2) $$

$$ {}^{n}\chi^{v} = \sum (\delta^{v}_i * \delta^{v}_j)^{-1/2} \qquad (3) $$

where i and j correspond to the pairs of adjacent non-hydrogen atoms and the summation is over all bonds between non-hydrogen atoms. Valence values of atoms are assigned according to

$$ \delta_i^{v} = Z^{v} - h_i \qquad (4) $$

where Z^{v} is the number of valence electrons of atom i and h_i is the number of hydrogen's bonded to it. Valence connectivity indices χ^{v} are then calculated from

$$\chi^{v} = \sum(\delta_i^v \delta_j^v)^{-1/2} \qquad (5)$$

In the case of unsaturated carbon compounds the assignment of the delta values is based on the explicit counting of each bond to an adjacent atom, irrespective of its type (suppressing hydrogen as usual) or by using the equation $\chi^V = Z^V$ - h. Both methods work for alkanes and alkenes.

The heteroatom treatment is based upon the explicit count of adjacent bonded atom (excluding hydrogen) plus a count of all pi and lone pair electrons. Alternatively, to take account of both valence and core electrons, the valence delta value can be written as a fraction:

$$\delta^v = \frac{(Z^v - h)}{(Z - Z^v - 1)} \qquad (6)$$

so that, for any molecule,

$$^{n}\chi_m^v = \sum_{i=1}^{n}\left(\delta_i^v \delta_j^v \ldots \delta_n^v\right)^{-1/2}$$

$$= \sum_{i=1}^{n}\left[\left(\frac{Z_i^v - h_i}{Z_i - Z_i^v - 1}\right)\left(\frac{Z_j^v - h_j}{Z_j - Z_j^v - 1}\right)\cdots\left(\frac{Z_n^v - h_n}{Z_n - Z_n^v - 1}\right)\right]^{-1/2} \qquad (7)$$

where

χ = *Chi = molecular connectivity index.*

n = Index order (0,1,2.....n).

v = Valence number (number of valence electrons).

m = Index suborder (path, cluster, path/cluster, etc.).

δ^v = *Valence delta (fragment term).*

Z^v = *the number of valence electrons.*

Z = the total number of electrons (atomic number).

h = the number of hydrogen atoms bonded to the atom of interest.

In order to utilize MCI's, a given molecule must be broken up into fragments according to the order and suborder of interest. Each fragment is then analyzed mathematically according to

equation (7). The reciprocal square root of the sum of the ensuing fragment values is the derived *nth* order and *mth* suborder MCI for that particular molecule. Beyond second order indices, the suborder (path, cluster, path-cluster, etc.) must be designated and calculations become inherently more difficult. Hence, a computer algorithm must be written or otherwise employed in order to calculate the indices for more complex molecules (Sabljic, A., 1990). A program for calculating connectivity indices (MCIndex) was developed and written specifically for this study. Required input values include the number of valence electrons, the total number of valence and sub-shell electrons (atomic number), and the number of bound hydrogens at each atom a. In addition, the number of *nth* order fragments characteristic of the molecule of interest is requisite.

To illustrate the computation process utilizing the MCIndex program, consider the organophosphorus pesticide chloropyrifos (Figure 5). For calculation purposes, the following steps are taken:

(1) Decide on an order and suborder of interest. In this example, we have chosen to calculate the fourth order path/cluster index (${}^{4}\chi_{pc}{}^{v}$).

(2) Determine the number of fourth order path/cluster fragments in the molecule. Chloropyrifos has fourteen fourth order path/cluster fragments (Figure 5).

(3) For all atoms (excluding hydrogen) in each of the fourteen fragments, determine the number of valence electrons, the atomic number, and the total number of bound hydrogens at each atom.

(4) Enter the collected data into the program and calculate the fourth order path/cluster connectivity index. An abridged MCIndex output file demonstrating the preceding calculation procedure is shown in Figure 6.

Organophosphorus Pesticides

Because of their versatility and breadth of activity, the organophosphorus class of pesticides are probably the most widely used among current insect control agents. Organophosphorus esters, in particular, are useful as broad-spectrum insecticides, plant and animal

systematics, soil insecticides, fumigants, aquatic larvicides, household and stored-product insecticides, and nematocides. Of the top 30 pesticides utilized for home and agricultural applications in California, 11 are organophosphorus compounds. Unfortunately, the majority of OP compounds are also extremely toxic to aquatic organisms and often do significant damage to aquatic ecosystems before they can be degraded (Eto, 1974). Accounts of wildlife and livestock poisonings are not uncommon and human illness due to exposure to OP compounds ("Farmers Flu") have been reported (Shepherd, C., 1988). Although a great deal is known about the chemical properties of OP pesticides, their behavior in the environment is not well understood. The ultimate bioavailability of these compounds in natural waters, soils and sediments appears to be predominately influenced by the extent and velocity of transformation that occurs. Esters of pentavalent phosphorus acids are the most important class of organophosphorus compounds. They are derivatives of the following three parent acids (Figure 7):

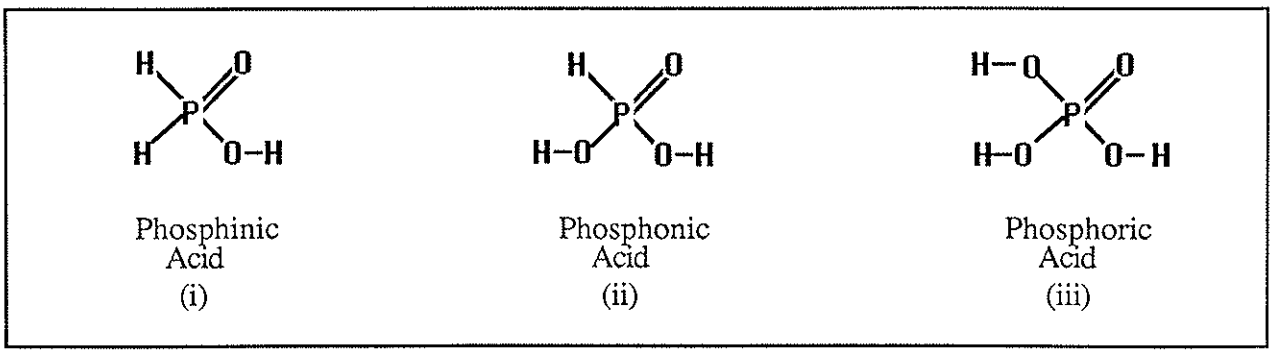


Figure 7. *Parent acids of most common organophosphorus pesticides.*

The majority of phosphorus pesticides are neutral esters or amides of phosphoric acid (iii), its anhydride, or sulfur analogs and may be classified into six fundamental types (Figure 8). Contrasting with naturally occurring, biologically active ester acids, all biocidal phosphorus compounds are neutral esters, with few exceptions. The phosphorus atom in neutral esters is generally electron deficient and reactive as an electrophile because of the high polarity of the phosphoryl bond. However, in partial esters this property is greatly diminished by dissociation and subsequent delocalization of the pi electrons.

Ester Hydrolysis: Hydrolytic transformation is an extremely important pathway for dissipation of organophosphorus pesticides in the environment, because organophosphorus esters are very susceptible to hydrolysis. In fact, their mechanism of toxicity results from this property. Thus, from an environmental viewpoint the phosphate ester bond can be considered a weak point in the molecule that is prone to cleavage resulting in detoxification. The hydrolysis of organophosphorus compounds generally follows several mechanistic patterns, depending upon the type of ester, the solvent, the pH range or upon the presence of catalytic agents. As with other esters, the rate of hydrolysis is a function of the nature of the acid and alcohol moieties, pH, and temperature. With most organophosphorus compounds, hydrolysis occurs at the leaving group and increases with higher pH, although acid-catalyzed hydrolysis occurs in mineral acids at very low pH. Hydrolysis rates increase with temperature and are often enhanced by photolysis (Eto, 1974).

Most natural waters fall within a pH range of 5 to 8 and accordingly neutral and basic hydrolysis mechanisms generally prevail under environmental conditions. Alkaline hydrolysis rates are much faster than the corresponding rates under neutral conditions since the hydroxyl group is a better nucleophile than water (with phosphorus by a factor of 10^8 and with saturated carbon by a factor of 10^4). The reaction is initiated by the nucleophilic attack of the water or hydroxide ion at the phosphorus atom. The reaction depends on the electron deficiency of the phosphorus atom, which may be affected by the electronic properties of substituents on phosphorus. Thus, the hydrolyzability of the esters is increased by the presence of electron-withdrawing groups and is decreased by the presence of electron-releasing groups.

The majority of organophosphorus pesticides are phosphorothionate esters, which are generally 2 to 20 times more stable than the corresponding phosphate esters. Sulfur is around 1.4 times less electronegative than oxygen, and therefore the phosphorus atom of phosphorothionate esters is less electrophilic and consequently less reactive with either water or hydroxide ion. In contrast, phosphorothiolate, phosphoroamidate, enol phosphate and cyclic phosphate pesticides are generally much more reactive toward nucleophiles than phosphate esters, due to a variety of innate

properties such as increased polarizability, mesomeric effects of pi bonds, inductive effects, and so on (Carey and Sundberg, 1974).

Kinetics: In general, the reaction between a phosphorus ester (A) and water, base, or acid (B) obeys second order kinetics:

$$A + B \longrightarrow C + D$$

$$\text{rate} = k[A][B] \tag{7}$$

$$\text{rate} = dx/dt = k\,[a-x]\,[b-x] \tag{8}$$

where a and b are the initial concentrations of A and B and x is the decrease in concentration after time t. In aqueous media, water (reactant B) is in large excess and its concentration can be considered to be constant; hence, the reaction may be considered pseudo first order and equation (8) reduces to

$$\text{rate} = dx/dt = k\,(a-x) \tag{9}$$

The derivation of the first order rate equation from equation (9) is shown in Figure 9. Pseudo order is usually involved in catalyzed reactions, such as the enzymatic hydrolysis of pesticides mediated by microorganisms found in natural waters. Because our initial interest is in chemical hydrolysis, waters utilized in the experimental determination of rate constants for this study have been filtered (0.45 μm) specifically to remove microorganisms which may contribute to overall hydrolytic degradation. As a result, derived rate constants have not adhered as closely to pseudo first order kinetics as expected. Nonetheless, derived rates exhibit enough first order characteristics to allow meaningful correlations with structural parameters to hold.

Besides the hydrolysis rate constants, the rate is often shown by the half-life of the ester, i.e., the time in which 50% of ester originally present has hydrolyzed. For first order processes, the half-life is given by

$$t_{1/2} = (1/k)\ln 2 = 0.693/k \tag{10}$$

The rate of hydrolysis for organophosphorus esters is independent of pH in the acidic range (pH 1-5) and they tend to be very stable under such conditions. Conversely, they are much more unstable under alkaline conditions. The hydrolysis rate increases steeply at pH higher than 7 to 8. Since the hydrolysis is primarily catalyzed by hydroxide ion under alkaline conditions, the hydrolysis rate increases around tenfold with each additional pH unit. Rates of hydrolysis are also affected by temperature. In general, a 10º C rise in temperature increases the rate of hydrolysis by four times (Drossman, Johnson, and Mill, 1988).

EXPERIMENTAL DETERMINATION OF RATES

General Methodology and Conditions.

The rates of hydrolysis for 3 organophosphorus pesticides (EPN, methidathion, and phorate) were determined in two environmental surface waters and DDI water over a period of two weeks and the rates of three less reactive compounds (methyl parathion, parathion, and guthion) will be determined under the exact experimental conditions over a six month period concluding in November, 1995. Both the temperature and the pH of each water type were noted initially but not monitored. Sacramento and Navarro River water samples were collected, characterized in terms of pH, Eh, and EC (Table 2), filtered (45 μm) and allowed to adjust to ambient room temperature of about 25-30°C before the experiment was initiated. Samples of pesticides were prepared from 1000 PPM stock solutions in pure methanol and 2.5 ml of each stock solution was transferred to separate 100 ml volumetrics and diluted to the mark with each water type to yield 100 ml solutions having concentrations of 25 PPM (three for each pesticide). In addition, controls were prepared using the same method as above but lacking the pesticide solutions. Aliquots of 4-5 ml were periodically taken from each sample, vigorously mixed, solid phase extracted with methanol and analyzed by HPLC using a UV detector.

Materials. Stock solutions were prepared from pesticide standards provided by ChemService (West Chester, Pa.) and had a purity level of at least 95+%. Methanol was HPLC grade and was purchased from Aldrich Chemical Co., Inc (Milwaukee, Wi). Glassware, Supor-450 menbrane filters (0.45μm), and polyethylene bottles were supplied by Fisher Scientific (Pittsburgh, Pa.).

Micromate 5 cc glass syringes, Econosil C-18 reverse-phase HPLC column , Econosil C-18 guard columns, and Maxi-Clean C-18 solid phase cartridges, 1.5 ml amber sample vials, lids, and septa were acquired from Alltech Associates, Inc. (Deerfield, Il).

Water	pH	EC (μΩ)	Eh (mV)	Temperature (°C)
Sacramento River	7.039	184.9	297.2	ambient (≈ 25°C)
Navarro River	7.258	107.8	337.8	ambient (≈ 25°C)
DDI Water	7.737	18.2	245.4	ambient (≈ 25°C)

Table 2. *Inherent conditions of waters used in experimental determination of OP hydrolysis rates.*

Extraction Procedure. Samples of 4 - 5 ml of water were drawn from each pesticide sample at various times by syringe, mixed and labeled. Before sample application, each solid phase cartridge was activated with HPLC grade methanol and rinsed with DDI water. Then, 2.0 ml of sample was applied, extracted and eluted with 2.0 ml pure HPLC grade methanol at a flow rate of 1-2 ml/min into graduated 10 ml tapered bottom vials. Eluent was collected, amalgamated on a Brownwill vortex mixer (VWR Scientific, Brisbane, Ca.), and 1.0 ml was transferred to 1.5 ml amber sampling vials, labeled, and analyzed by HPLC within a few hours of extraction. This exact procedure was followed for each timed run.

HPLC Analysis. A Shimadzu LC-6A HPLC equipped with a Shimadzu SCL-6B System Controller and a Shimadzu SPD-6AV UV detector was used under the following conditions: flow rate (1.2 ml/min, 200 kgf/cm^3 pressure max), wavelength settings (254 nm, 0.16 abs) , analysis parameters (width: 5, method: # 2041, slope: 400, minimum area: 10), injection volume (phorate/phosmet: 10μl; all other pesticides: 6μl). Data was processed by a Shimadzu CR 601 Chromatopac integrator.

Kinetics Experiments. Kinetic runs were initiated at ambient temperature and indigenous pH (control over incipient conditions was minimized because it was decided to leave some parameters

as characteristic as possible in order to reflect a more natural state). Before the experiment began, a number of 250 ml amber borosilicate bottles were acid washed and rinsed with DDI water and allowed to dry. Upon drying, 100 ml of 25 PPM pesticide solution was prepared and transferred to the bottles, mixed and allowed to stand. Aliquots were removed by syringe at various times, including at t=0 (initial concentration), solid phase extracted in methanol, and analyzed within a few hours. The determination of rates is based directly on the disappearance of the parent OP compound over time. Once concentrations were determined, they were plotted against time according to the first order rate law to obtain both initial and final rates.

RESULTS AND DISCUSSION

Trial Models. Several preparatory studies utilizing literature sources indicate that satisfactory correlations with kinetic data can be obtained utilizing structural indices which characterize intramolecular bonding (Molecular Connectivity Indices) and, to a lesser degree, direct atomic adjacency and distance calculations from topological matrices. Aside from trial runs utilizing organophosphorus compounds, test models were also examined for alkyl halides, alkyl chlorides, amides, carboxylic acid esters, and ethyl alkanoates (Figures 10 and 11). To test the ability of MCI-based QSAR models to estimate kinetic properties for organophosphorus compounds, the model equation derived from the literature (see Figure 2) was utilized to examine a series of compounds taken from the same data set, i.e., analyzed under the same conditions, and not used in calibrating the acquired model. The ensuing results, shown in Table 3, are not encouraging.

It has become apparent over the course of this study that connectivity indices have somewhat of an ambiguous character in relation to reaction characteristics. They tend to perform extremely well at the model development level, consistently yielding model equations which are capable of explaining at least 85% and up to 98% of the variation in kinetic data. However, we have found that model equations only infrequently have been derived that are capable of executing at levels of accuracy which reflect their initial statistical characteristics and predictive potential. The data in Table 3 is indicative of this intrinsic characteristic. Both model calibration data and model validation data were drawn from the same source and half-lives for both data sets were obtained

under the same conditions (Eto, 1974). The model equation utilized to estimate the kinetic parameters shown in Table 3 has an excellent correlation coefficient of close to 95%, yet the predictive capability of the model is unsatisfactory. It appears that an inherent weakness exists in the ability of molecular conncetivity indices to adequately describe the essential structural features of a given molecule which presumably constrains, directs or in some fundamental way influences its observed physicochemical properties and behavior.

Experimental-Based Models. Although the inherent limits of the molecular connectivity approach as applied to complex reaction parameters was evident before experimental work was initiated, the goal of this exploratory study has remained devoted to investigating their ability to estimate kinetic characteristics from structural features and hydrolysis half-lives obtained experimentally from environmental waters. To this end, an ongoing kinetics study has provided experimental rate data on several organophosphorus pesticides which have been utilized as a small validation set for a model derived from comprehensive environmental kinetic data obtained from the literature (Lartigues and Garrigues, 1995). Half-lives of eight organophosphorus pesticides that were obtained experimentally in river water at pH 7.3 and at three different temperatures (6°C, 22°C, and exposed to sunlight) were used as dependent variables in the derivation of the model. Table 4 shows the kinetic values investigators found during the course of their study. For each organophosphus pesticide listed the first order connectivity index was calculated and regression analysis performed on the resulting values. Correlations are shown in Figure 12. Discouragingly, relationships derived between variables were not much more than chance correlations. In effect, no correlations exist between first order connectivity indices and kinetic data at this early research stage of model development. A multiple variable approach was also tried, using first order connectivity indices and molar refractivity (an indicator of the ease of orbital distortion on a molecular scale) as independent variables, but only slight improvement was seen in model correlation coefficients and therefore the data has not been presented. Because the derived regression equation was not a sound statistical model, it was not possible to utilize our experimentally derived rates as a validation set at this time (Table 5). Instead, we look at these

results as a call for further research. The inherent complexity of reaction mechanisms and the ambiguous influence of both adjoining and distant atoms in the molecular environment makes it difficult for a single descriptor, even one as widely successful as connectivity indices, to adequately account for definitive structural characteristics of molecules. Hence, a new approach is required in order to find or derive a more reliable and accurate method for mathematically describing structural properties for modeling purposes.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Although based on sound and well-established theory, the notion that such fundamental parameters as interatomic distance, adjacency and connectivity between atoms in a molecule are a determinant and driving force directing its observed physicochemical behavior is probably somewhat overstated in many cases. There is little doubt that the arrangement of atoms in a particular molecule, the length of the bonds between atoms, the kind and proximity of neighboring atoms, and so on, are all very important features which contribute enormously to observed molecular attributes, but the degree to which these features are useful for establishing relationships between known properties and structure is equivocal at least.

One of the primary goals of this exploratory study was to determine, if possible, just how persuasive the relationships between structure and reactivity are. As mentioned earlier, it appears at this early stage in this research that it is much easier to calibrate a creditable statistical model than it is to validate it. The primary limit of the molecular connectivity approach is that it is a inclusive technique which considers the entire molecule, including regions that are entirely ineffectual in terms of their influence on physicochemical parameters. For example, consider the general organophosphorus pesticide shown in Figure 13. Hydrolysis is a substitution (S_N2) reaction and, in the case of phosphorus esters, is localized at the electron deficient phosphoryl group. The influence of, say, a methylene carbon or a secondary nitrogen on one of the R groups attaced to the phosphorus atom is minimal or nonexistent. Yet, the connectivity algorithm explicitly includes such irrelevant constituents as efficacious contributors affecting behavior at the site of reaction no matter how distant or improbable its contribution. As a consequence, molecular connectivity

indices often contain a significant amount of redundant and/or meaningless information which appears to muddle structure-property correlations in relation to chemical reactivity. In particular, such information tends to limit an otherwise statistically sound models' predictive range, as the data in Table 3 clearly demonstrates.

On a larger scale, we have endeavored to transform the primary MCI algorithm into a more comprehensive tool by earnestly exploring both conventional and novel ways of numerically describing molecules from principles of MCI theory. However, this effort is still in the research and development stage and we have not obtained enough results to draw significant conclusions. We are searching for a descriptor or combination of descriptors by which fundamental molecular characteristics responsible for substitution-type (hydrolysis) reactions can be linked and modeled through QSAR methodology. We have outlined a general strategy for further research which endeavors to avoid or minimize some of the more conspicuous limitations of the molecular connectivity approach that have been discovered during the course of this study:

1. Extend the MCI algorithm to include more definitive strucutral characteristics, such as bond lengths between connected atoms, atomic volumes, and electronegativity. Some of these algorithms have been developed and coded but are as yet unvalidated.

2. Develop a descriptor to complement to complement the connectivity approach whichaccounts only for structural/electronic characteristics at and near the site of reaction, thereby minimizing redundant or inconsequential information inherent to MCI's.

3. Explore the use of more quantitative methods for defining structure, e.g., electron density calculations, molecular orbital calculations, Slater orbitals, etc., and develop descriptors based on these parameters.

It is apparent from results of this exloratory study that molecular connectivity indices alone are often not discriminating enough descriptors for procuring comprehensive structure-property relationships beyond a rather restricted range of structural variation, at least when characterizing chemical reaction parameters. It is anticipated that a renewed effort and renovated research strategy will lead to more satisfying and pertinent results.

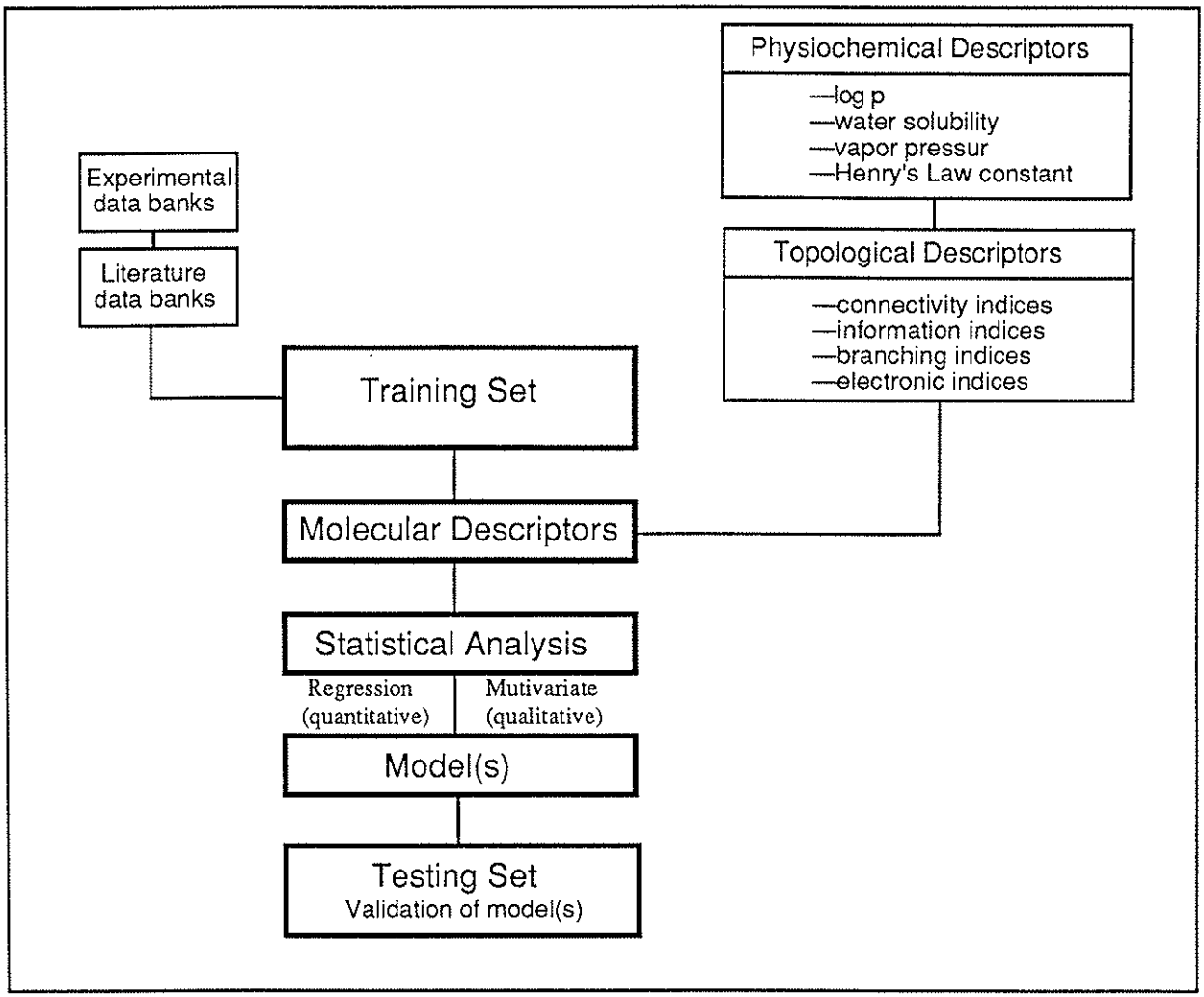


Figure 1. *Essential Features Of QSAR Modeling Methodology.*

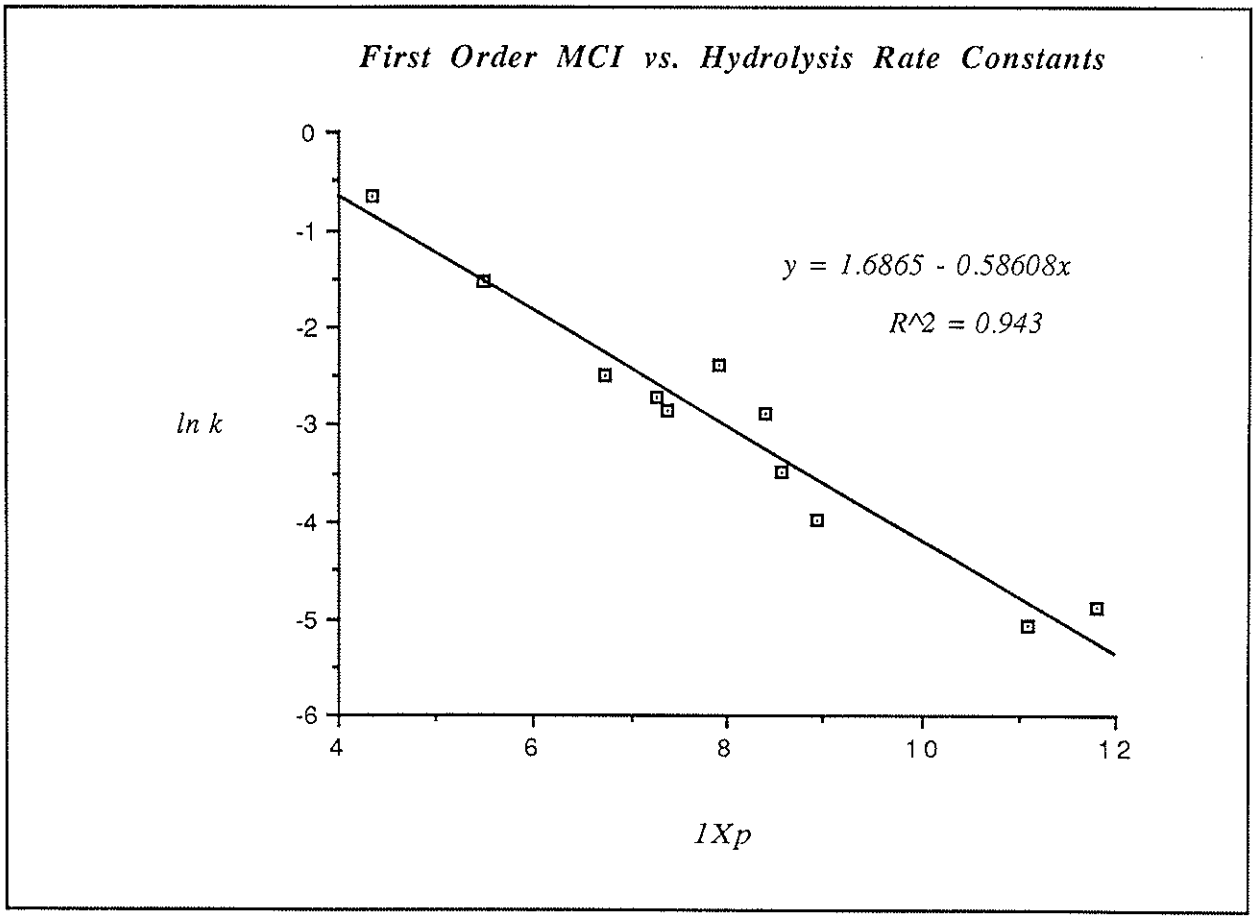


Fig 2. *Regression plot for ln hydrolysis rate vs.* $^{1}\chi^{v}_{p}$ *among organophosphorus pesticides.*

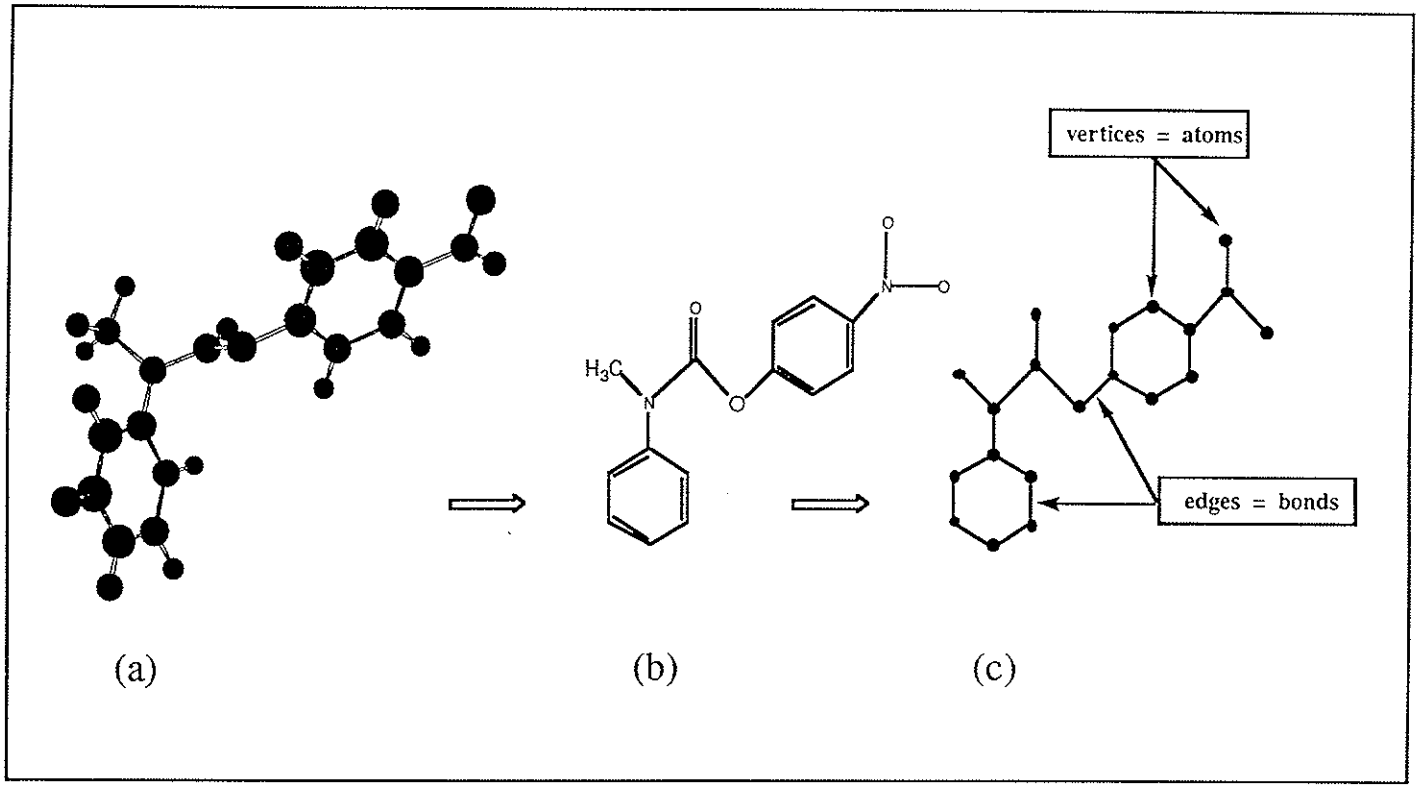


Figure 3. *4-nitrophenyl N-methyl-N-phenyl carbamate exhibited as (a) a three-dimentional molecular model, (b) a typical line-segment model and (c) a graphical model. In chemical graph theory, edges are defined as sigma bonds and vertices as any atom exclusive of hydrogen in the molecular framework.*

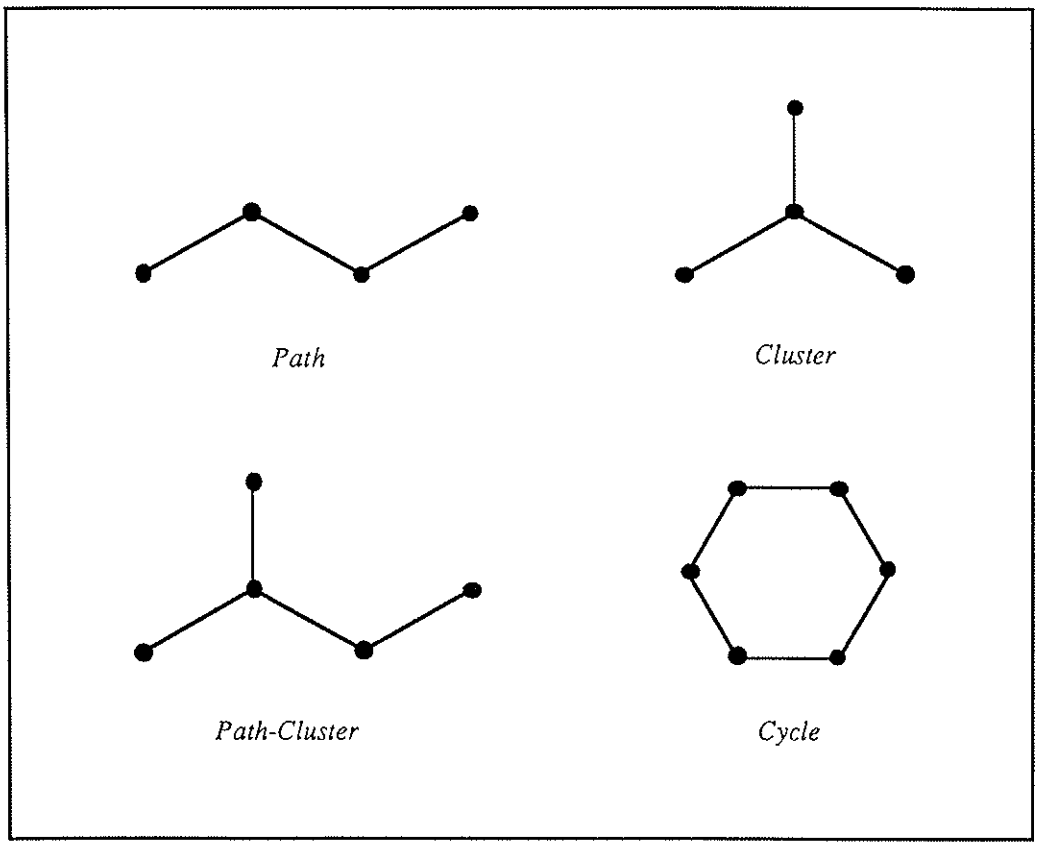
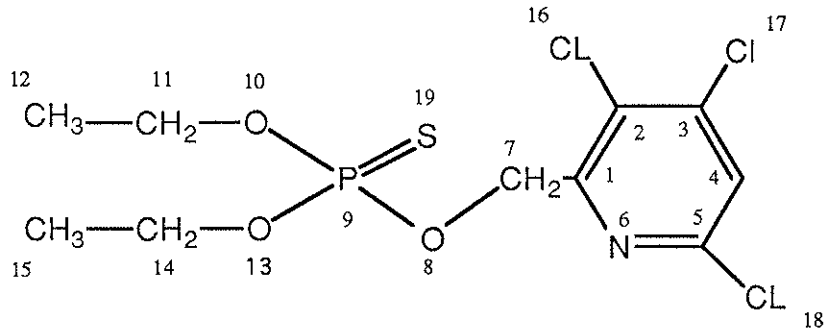


Figure 4. *Molecular connectivity index suborders.*



Chlorpyrifos

[Phosphorothioic acid O,O-diethyl O-(3,5,6-trichloro-21-pyridinyl) ester]

4th order path/cluster ⟶ 14 $^{4}\chi^{v}_{pc}$ fragments

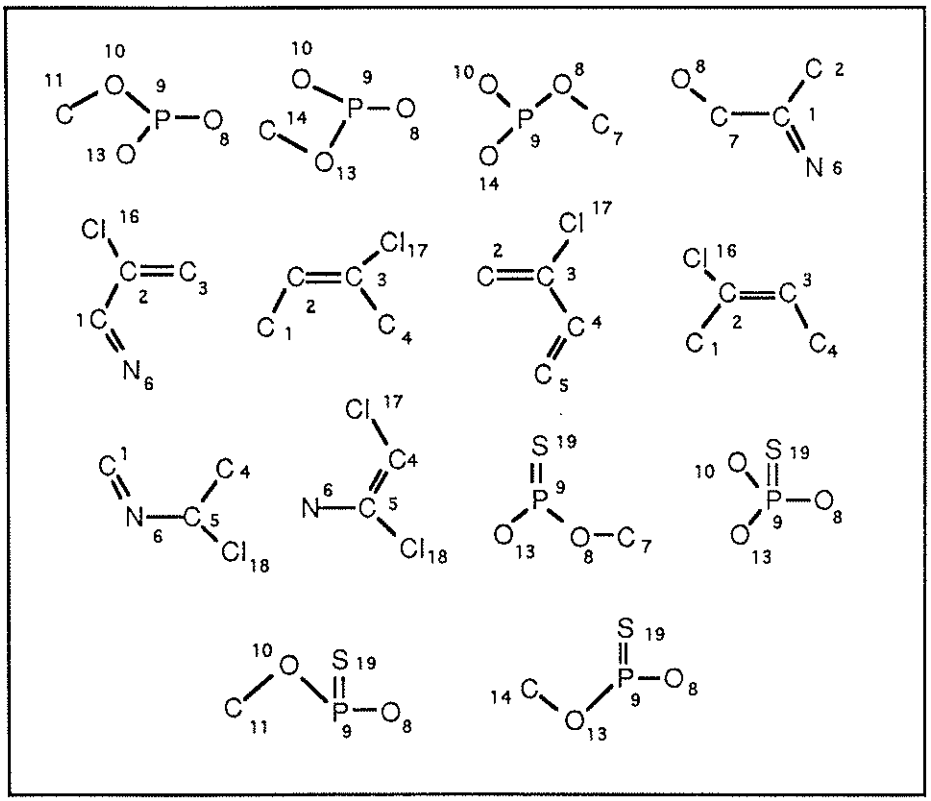


Figure 5. The OP pesticide Chlorpyrifos and its fourteen fourth order path/cluster fragments.

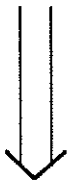
******** MCIndex ********

FILENAME : Fourth P/C

**** The number of fourth order fragments: 14 ****

Atom	Fragment	Valence Electrons	Number of H"s	Atomic Number	Valence Deltas	Fragment Value
1	1	4	3	6	1.000	
2		6	0	8	6.000	
3		5	0	15	0.556	
4		6	0	8	6.000	
5		6	0	8	6.000	0.09129

*** FRAGMENT 1 HAS A VALUE OF 0.09129 ***



... twelve more fragments

Atom	Fragment	Valence Electrons	Number of H"s	Atomic Number	Valence Deltas	Fragment Value
1	14	4	2	6	1.000	
2		6	0	8	6.000	
3		5	0	15	0.556	
4		6	0	16	6.000	
5		6	0	8	6.000	0.19365

*** FRAGMENT 14 HAS A VALUE OF 0.19365 ***

◊◊ THE FOURTH ORDER MOLECULAR CONNECTIVITY INDEX IS 1.4029 ◊◊

Figure 6. *Abridged MCIndex output showing calculated 4th order path/cluster index for chlorpyrifos.*

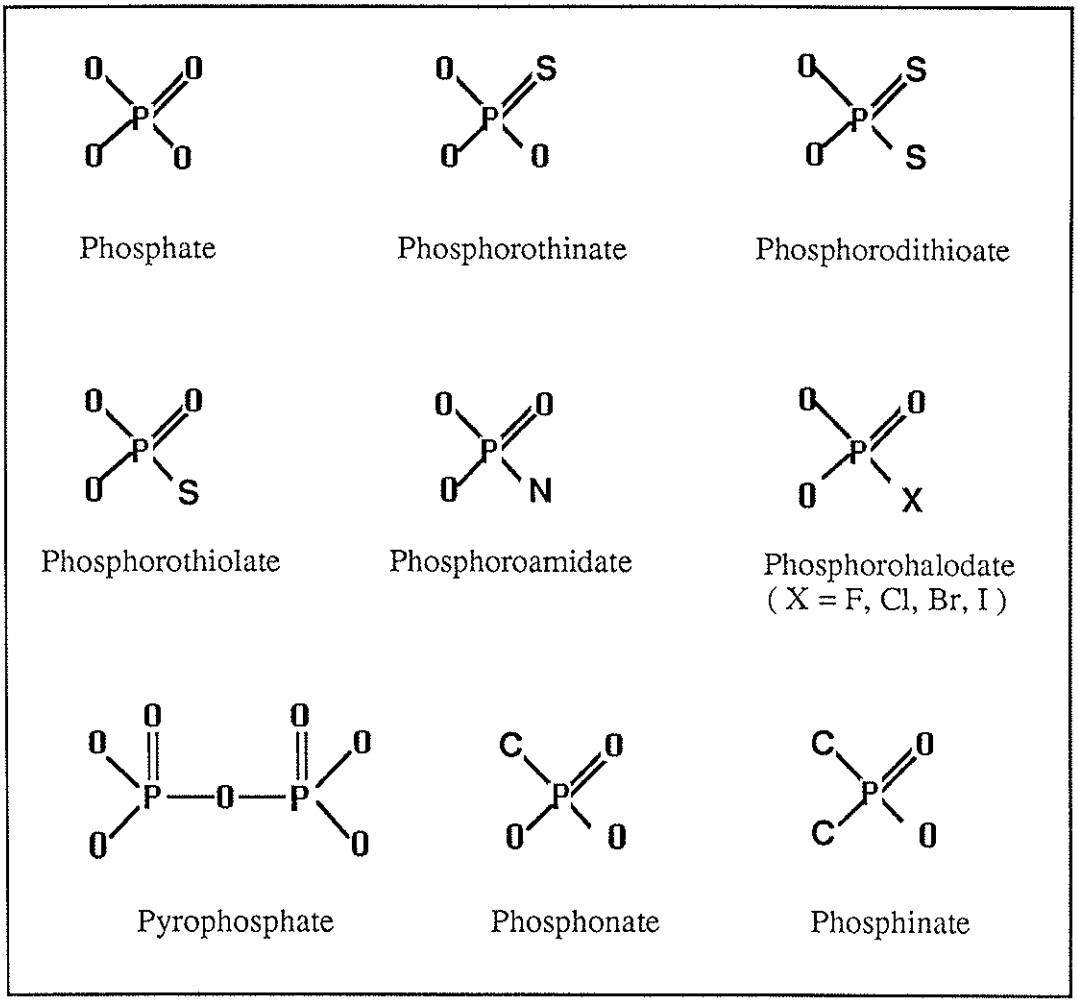


Figure 8. *Six fundamental neutral esters or amides of phosphoric acid (iii), its anhydride, or sulfur analogs.*

$$OP\ Ester + H_2O \longrightarrow Hydrolysis\ Products$$

$$Rate = -d\frac{[OP\ Ester][H_2O]}{dt} = k[Parathion][H_2O]$$

$[H_2O] >> [OP\ Ester]$ *so that*

$$Rate = -d\frac{[OP\ Ester]}{dt} = k[OP\ Ester] = pseudo\ first\ order$$

1. *Separate variables:* $$-d\frac{[OP\ Ester]}{[OP\ Ester]} = kdt = \frac{1}{[OP\ Ester]}d[OP\ Ester] = -kdt$$

2. *Integrate:* $$\int_0^t \frac{1}{[OP\ Ester]}d[OP\ Ester] = \int_0^t -kdt$$

Which yields: $$ln[OP\ Ester]_t - ln[OP\ Ester]_0 = ln\left(\frac{[OP\ Ester]_t}{[OP\ Ester]_0}\right) = -k\int_0^t dt$$

3. *Multiply by - 1:* $$ln\left(\frac{[OP\ Ester]_0}{[OP\ Ester]_t}\right) = kt$$

4. *Plot of left side vs t gives k:* $$ln\left(\frac{[OP\ Ester]_0}{[OP\ Ester]_t}\right) = kt$$

5. *Alternatively, to calculate k directly:* $$\frac{1}{t}ln\left(\frac{[OP\ Ester]_0}{[OP\ Ester]_t}\right) = k$$

Figure 9. *Derivation of first order rate equation from initial differential form.*

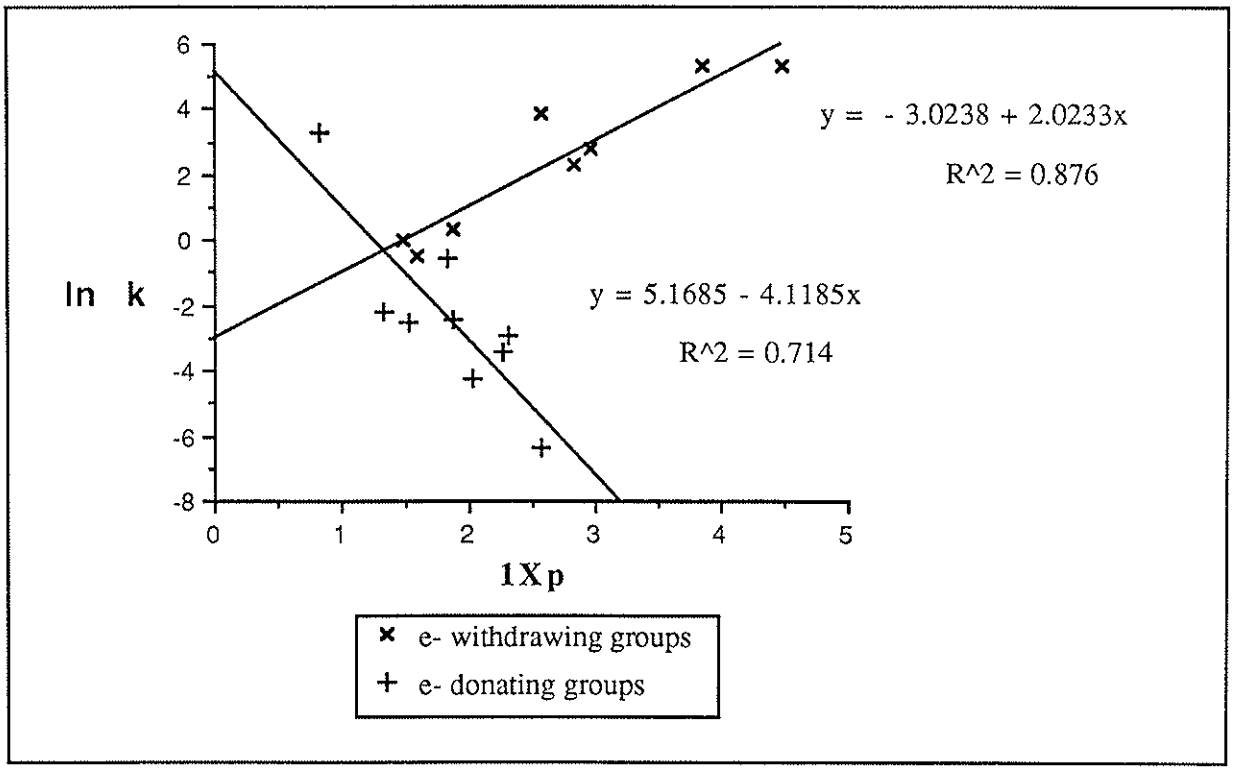


Figure 10. *First order MCI's vs. ln k for a series of ethyl alkanoates. Compounds were separated according to the presence of electron withdrawing or donating groups before analysis was performed.*

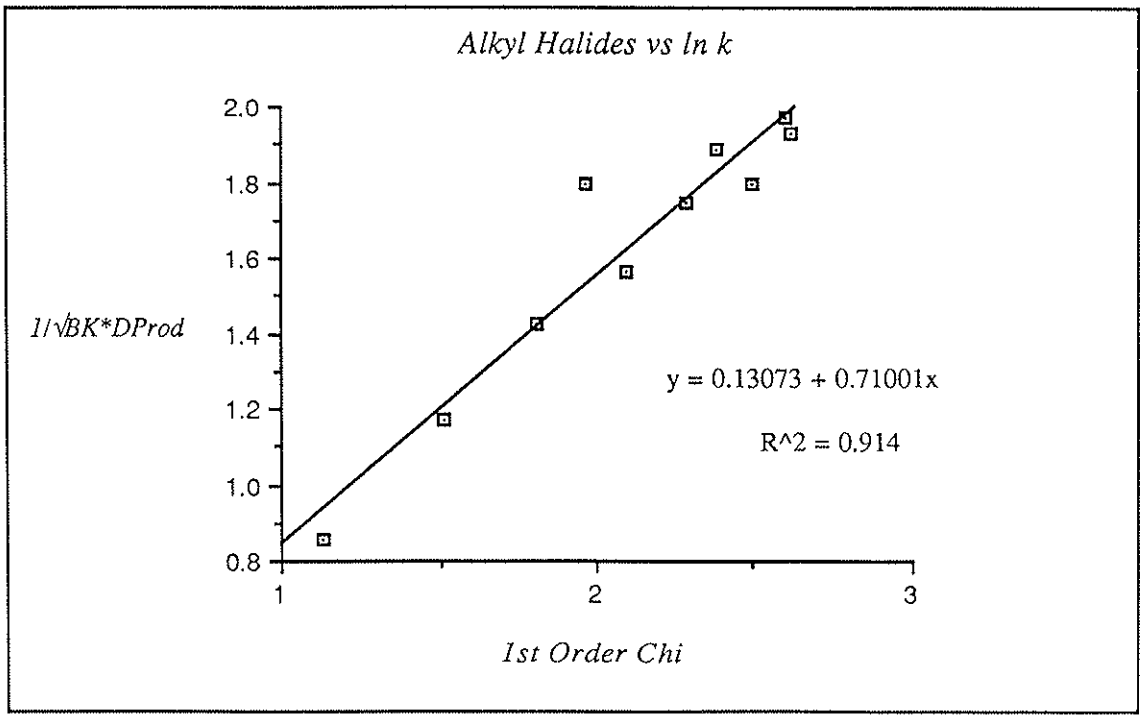


Figure 11. *First order MCI's vs. ln k for a series of alkyl halides.*

Compound	$^1\chi p$	Experimental *Rate Constant* *k (s-1)*	Experimental *Half life* *(Hrs)*	Predicted *Rate Constant* *k (s-1)*	Predicted *Half life* *(Hrs)*	*Percent* *High/Low*
Methyl Parathion	7.294	0.0825	8.4018	0.0642	10.7967	-28.50%
Dimethoate	7.372	0.0578	11.9922	0.0614	11.2890	5.86%
Diazinon	8.931	0.0187	37.0667	0.0250	27.7259	25.20%
Dichlorvos	4.345	0.5133	1.3504	0.3523	1.96750	-45.70%
Carbophenothion	11.106	0.0063	110.023	0.0071	97.6264	11.27%
Fenthion	8.557	0.0309	22.4319	0.0310	22.3596	0.32%
Phosphamidon-α	7.252	0.0660	10.5022	0.0658	10.5342	-0.30%
Oxydemeton-Methyl	8.408	0.0559	12.3998	0.0338	20.5073	-65.38%
Demeton-S-Methyl	7.908	0.0912	7.6003	0.0451	15.3691	-102.2%
Trichlorfon	5.482	0.2166	3.2001	0.1828	3.79180	-18.49%
Chlorfenvinphos	8.331	0.0075	92.4196	0.0353	19.6359	78.75%
Phenkapton	11.793	0.0075	92.4196	0.0048	144.4057	-56.25%

Table 1. *Statistical relationship between some commonly used organophosphorus pesticides and calculated first order path molecular connectivity indices,* $^1\chi p$. *Given rates were calculated from half-lives obtained from the literature (Eto, 1974).*

Compound	1st order MCI	Experimental Half Lives (Hrs)	Predicted Half Lives (Hrs)	Predicted vs. Experimental: % high/low
Parathion	7.8921	43.0000	12.6839	-70.50%
Me Parathion	6.7171	8.4000	6.3075	-24.91%
Thionazin	7.3604	29.2000	9.2462	-68.33%
Demeton-S	7.9876	18.0000	13.4249	-25.42%
Malathion	8.6655	7.8000	20.0888	157.55%
Disulfoton	7.2529	32.0000	8.6737	-72.89%

Table 3. *Estimated half-lives for six organophosphorus esters derived from model equation shown in Figure 2.*

OP Compound	Half Life (day) (6℃)	Half Life (day) (22℃)	Half Life (day) (Sun)	Molar Refractivity	$^{1}\chi^{v}_{p}$
azinphos-ethyl	171	65	9	90.5970	10.6843
azinphos-methyl	278	42	8	76.5560	9.5092
coumaphos	165	59	5	-	9.6494
diazinon	181	80	43	71.6070	8.9310
dimethoate	171	43	29	43.6640	7.3720
fenitrothion	103	31	4	67.0560	7.1337
fenthion	149	42	2	73.0360	8.5575
malathion	55	8	8	64.0610	8.6655
ethyl parathion	120	86	8	38.5050	7.8921
methyl parathion	95	23	11	44.5560	7.2944

Table 4. *Half-lives and first-order MCI's for model calibration data set.*

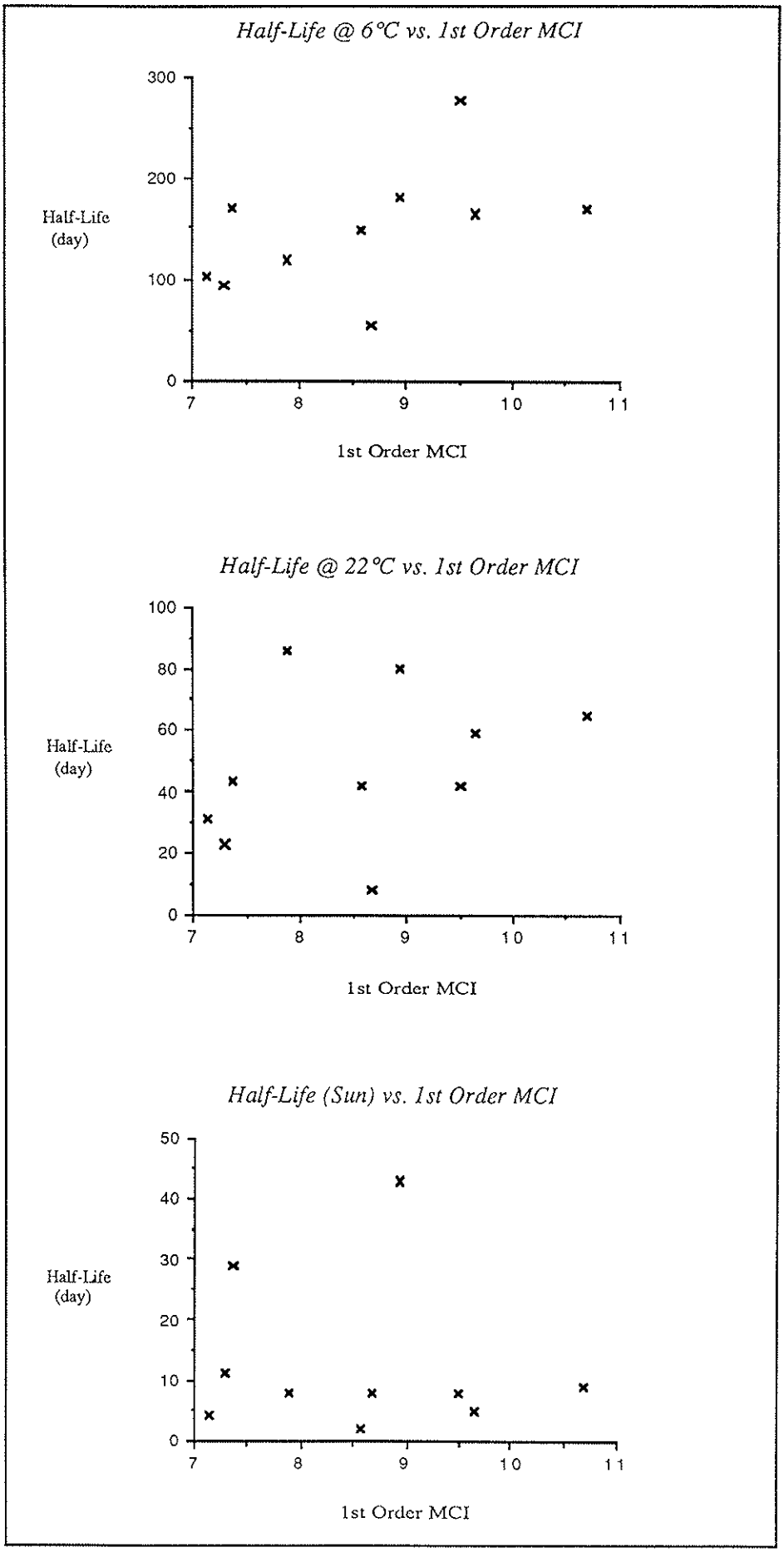


Figure 12. *Correlations between environmental half-lives and 1st order MCI's.*

Compound	Half Life (s)	Half Life (hr)	Half Life (day)	Rate (s^{-1})	Rate (hr^{-1})	Rate (day^{-1})
Methidathion	1.823×10^{6}	5.064×10^{2}	2.110×10^{1}	3.847×10^{-11}	1.385×10^{-7}	3.324×10^{-6}
Phorate	2.478×10^{5}	6.939×10^{1}	2.891×10^{0}	2.783×10^{-10}	1.002×10^{-6}	2.405×10^{-5}
EPN	8.897×10^{5}	2.471×10^{2}	1.030×10^{1}	7.001×10^{-11}	2.521×10^{-7}	6.049×10^{-6}
*Parathion**	9.486×10^{5}	2.635×10^{2}	1.098×10^{1}	7.573×10^{-11}	2.726×10^{-7}	6.543×10^{-6}
*Me Parathion**	2.131×10^{6}	5.921×10^{2}	2.467×10^{1}	3.884×10^{-11}	1.398×10^{-7}	3.356×10^{-6}
*Azinphos-Me**	2.911×10^{6}	8.086×10^{2}	3.369×10^{1}	2.656×10^{-11}	9.561×10^{-6}	2.295×10^{-6}

* *Incomplete data after only 144 hours. These compounds require longer time frame for the determination of half-lives.*

Table 5. *Experimental kinetic data for validation set compounds, Sacramento River.*

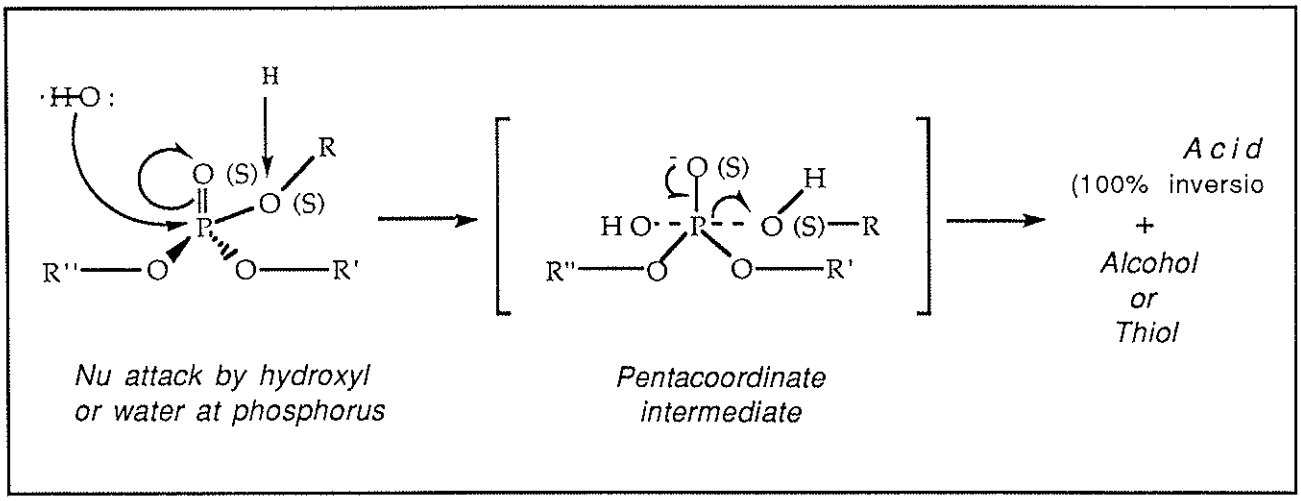


Figure 13. *Simplified hydrolysis mechanism for the reaction of the constituents of water at the electron deficient phosphoryl group of a typical organophosphous compound.*

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