DETERMINATION OF BIOTRANSFORMATION AND BIODEGRADATION RATE CONSTANTS FOR NAPHTHALENE, LINDANE, AND PHENOL

Thesis

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Biotransformation and biodegradation rate constants were determined for naphthalene, lindane, and phenol in water samples from three different sources. Rate constants produced from monitoring disappearance of the parent chemical (biotransformation) were compared to those obtained from mineralization of the chemical (ultimate biodegradation) by $^{14}CO_2$ evolution as well as acidification of the residual ^{14}C-labeled compound (primary biodegradation). Rate constants were statistically different for the three chemicals. The water source affected the rate constants. When biomass measurements of the waters were considered and second-order rate constants were derived, there was no statistical evidence that this parameter gave a reliable rate constant statistic that could be useful in predicting the fate of any of naphthalene, lindane, and phenol in these waters.

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CHAPTER I

INTRODUCTION

Microbial degradation (biodegradation) and transformation (biotransformation) are major processes for the removal of many chemicals in the environment. Few organic chemicals remain completely conservative, (nonreactive) and the majority undergo a variety of changes in structure and toxicity, depending on environmental properties. Since such properties (pH, biomass, temperature, and other characteristics) may cause variable behavior, the kinetics approach to quantification of chemical degradation has not been applied to natural waters or to native mixed populations.

This study investigates both biodegradation and biotransformation processes of three chemicals (naphthalene, lindane, and phenol) with the purpose of determining rate constants which will be of value in the estimation of environmental concentration. Of special significance is that the inherent mixed microbial populations of the waters under investigation were used, instead of selected laboratory-cultured single species. Also of importance is the procedure of using environmentally relevant concentration levels of the chemical. The approach for this

study has been to implement a laboratory system to determine biodegradation and biotransformation rate constants while maintaining pertinent environmental conditions.

Many biotransformation and biodegradation studies have been qualitative in nature, such as the determination of chemical pathways as they are changed under a variety of conditions or in differing ecosystems (13,23). Other studies have used high concentration levels which may not reflect that chemical's biodegradation in a natural system (5,19). When single substrates are tested, the possibility of co-metabolism (microbial alteration of a substance in which no energy is derived to support growth of those organisms) may be overlooked. The use of a single species precludes the possibility of detecting pertinent interaction by other species.

While the use of these procedures can be helpful and informative, there is a need to assess the fate of chemicals in the environment so that the biodegradation and biotransformation rates can be predicted as accurately as possible.

Environmental Systems and Xenobiotics

The Principle of Le Chatelier is the first rule of ecology (15). It states that when a stress is applied to a

system, the system responds in such a manner as to relieve that stress.

The principle does not include a time frame or a degree-of-stress limitation; thus it is essential to understand the long- and short-term relationships of the natural and man-inspired processes within the environment. There will be a system reaction to any stress, large or small, and it may be reacted to immediately, far in the future, or at a time in between.

Environmental systems are composed of many compartments, each of which may interact with the other by complex and dynamic means. In the utilization of the environmental systems, man has changed the nature and concentration levels of materials released into the air, soil, and water.

A chemical discharged into the environment, intentionally or by accident, will either degrade or remain unaltered, and it (or its metabolites) may remain at the point of release or be transported to other locations. Upon entering the atmosphere, a chemical may be carried long distances by winds, may be deposited on soil or water, or may be changed from the original form to another (including ultimate degradation to carbon dioxide, water, and minerals). A chemical in soil can migrate to ground or surface water, be sorbed to the soil and remain there, or be

eroded to aquatic systems, be bioaccumulated by plants, evaporate into the atmosphere, or be degraded. A chemical in water can stay in solution, sorb to suspended particles or sediments, bioaccumulate into plants and animals, volatilize into the atmosphere, or be degraded.

The Environmental Protection Agency (EPA) inventoried potential commercial chemicals and suggested that there may be as many as 30,000 chemicals produced and used in commercial quantities (17). Eight hundred million pounds of pesticides are applied annually in the United States and over 700 synthetic organics have been identified in drinking water supplies, many of which have been found to be toxic or carcinogenic in animals (23). It is very important to recognize potential danger in uncontrolled or misunderstood discharges to the environment. Government legislation (such as the Toxic Substances Control Act, Public Law 94-469), increased research efforts, industrial and municipal process changes, and growing public concern are indications of an awareness that chemical releases need to be evaluated and monitored in such a way that the fate of a material will be predictable.

While some release of chemicals to the environment can be made without having deleterious effects, intelligent choices are best made when one can reliably estimate the outcome of a particular course of action. Reliable

estimates, however, are obtained only after thoughtful investigation. It is important to remember that nothing we do has a zero risk (10); and hence, the more we know concerning environmental processes that control the fate of chemicals, the better our chances are of dealing sucessfully with them.

Definitions

The following is a list of terms and their definitions which often are used when referring to aquatic environments. They will be used throughout this thesis.

1. Microorganisms---bacteria, fungi, algae, and protozoa in aquatic systems; they may be planktonic or grow attached to substrates.
2. Biodegradation---biologically mediated reduction in complexity of a particular chemical by splitting off one or more constituent groups or components; it may include both primary biodegradation and ultimate biodegradation.
3. Primary biodegradation---biodegradation to the minimum extent necessary to change the identity of the compound.
4. Ultimate biodegradation---biodegradation to (a) water, (b) carbon dioxide and (c) inorganic compounds (if elements other than carbon, hydrogen, and oxygen are present).

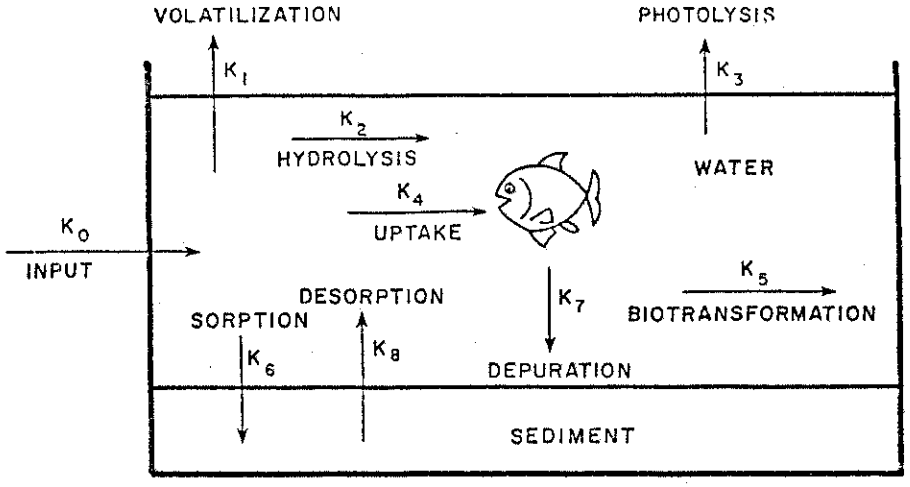
5. Biotransformation---biologically mediated change in the composition or structure of a particular chemical. This is an encompassing term including additions to the parent chemical or biodegradation.
6. Bioconcentration---the increase in concentration of a particular chemical in (or on) an organism by direct contact with the chemical in water; in the microbial literature, this term has been used interchangeably with the term "bioaccumulation".
7. Uptake, sorption, partitioning---the process by which the microorganism-associated chemical concentration is increased.
8. Depuration, desorption---the process by which the microorganism-associated chemical concentration is decreased.
9. Adsorption---adhesion of chemicals from water to exposed surfaces of microorganisms. It may be followed by internal diffusion.
10. Absorption---active uptake or transport of adsorbed chemicals into microorganisms.
11. Metabolism---all biochemical reactions in microorganisms, both anabolic (biosynthetic) and catabolic (biodegradative).
12. Co-metabolism---the process in which microorganisms oxidize a compound without utilizing the energy

derived from this oxidation to support growth (the presence or absence of growth substrate during oxidation is not inferred).

The Aquatic Environment

Water covers most of the earth's surface. It is the medium, catalyst, and participant in nearly all of the chemical reactions occurring in our environment, including those of the life processes. Water is the vehicle for many of the natural cycles that transform energy and carry chemical materials from place to place. It is also the principle carrier and depository of man-made pollution (15).

In the aquatic environment there are a variety of reactions, enzymatic and nonenzymatic, by which a chemical can be assimilated, such as hydrolysis, photolysis, sorption, bioconcentration, volatilization, and biodegradation and biotransformation. Figure 1-1 illustrates a mass balance equation used to model and predict the fate of a chemical released into a body of water (7). The importance of each parameter is dependent on the physical-chemical characteristics of the chemical itself, as well as the properties of the environment. However, nonenzymatic reactions usually do not lead to appreciable changes in chemical structure, and it is the biodegradative sequences that bring about major changes (2).



MATERIAL BALANCE EQUATION

$$V\frac{dCw}{dt} = K_0 - K_1 ACw - K_2 VCw - K_3 ACw - K_4 FCw - K_5 BCw - K_6 SCw + K_7 FC_f + K_8 SCw$$

INPUT; VOLATILIZATION; HYDROLYSIS; PHOTOLYSIS; FISH UPTAKE; BIOTRANSFORMATION; SORPTION; FISH DEPURATION; DESORPTION

where V = Volume of water , ℓ
A = Surface area , m^2
F = Fish mass , kg
S = Sediment mass , kg
B = Microbial biomass , kg
Cw = Concentration of chemical in water , mg/ℓ
K = Rate constant
C_f = Concentration of chemical in fish , mg/kg
C_s = Concentration of chemical in sediment , mg/kg

FIGURE 1-1. SCHEMATIC DIAGRAM OF A POND-MODEL ILLUSTRATING THE KEY ENVIRONMENTAL PROCESSES AND THE MATERIAL BALANCE EQUATION (7).

Although degradation may conceivably be accomplished in part by any living organism, microorganisms by far play the dominant role. This is attributed to their ubiquitous presence, their high catabolic versatility, their species diversity, and their metabolic rate per unit weight (17). Because of this wide range of degradative ability, some bacteria and fungi have been utilized by industry in a variety of processes such as the production of antibiotics, steroids, vitamins, solvents, and foods, as well as in municipal and industrial sewage and waste-water treatments (1).

Microorganisms are chemically similar to higher animal cells, and they can perform many of the same biochemical reactions (1). In addition, many microorganisms can utilize sunlight or inorganic salts in meeting requirements for growth. Another phenomenon, co-metabolism, exists and occurs when chemicals are subject to microbial-transforming action where the organisms apparently are not utilizing that material but presumably are growing on another substrate (2, 16).

In order to grow and multiply, microorganisms require (a) a source of energy, (b) a source of water, (c) a source of carbon, (d) a source of nitrogen, and (e) a source of minerals.

The most important compound in energy transformations in

cells is adenosine triphosphate (ATP). The energy for ATP to be formed comes from the sun, oxidation of inorganic salts, or the oxidation of organic compounds. Since ATP is associated with living cells only, it is used as an estimate of viable biomass. The ratio of ATP to biomass varies somewhat from species to species, but appears to be constant enough to permit reliable biomass estimates from ATP measurements (23).

Water is essential to living things and it dominates the chemical composition of all organisms. Water's unique physical and chemical properties are the reason for the central role it plays in biochemical metabolism. Aquatic organisms reside within a water medium and may be affected by its solvent and thermal-density properties, high specific heat, and liquid-solid characteristics. The organism's osmotic control keeps a compatible narrow range of internal solute concentration in spite of a wider and possibly fluctuating external range of solute concentration.

The carbon needs of a cell usually are supplied with the energy source. Chemotrophic and photosynthetic bacteria use carbon dioxide. It has been found that facultative organisms incorporate about ten percent of substrate carbon in cell material when metabolizing anaerobically and with full aerobic metabolism, 50 to 55 percent of substrate carbon may be converted to cell

materials (1). Therefore, the presence or absence of oxygen may make a difference in the degradation of a chemical. One method to measure assimilated carbon from a specific source is by using a radioactive-labeled chemical in the test system as a substrate and then monitoring over time the accumulation of the labeled carbon.

Many microorganisms can use more than one kind of nitrogen source, with preferences often being shown when a choice is available. For instance, amino acids are broken down before ammonia, and ammonia before nitrate (8). In aquatic environments, nitrogen content generally is low and is in the form of nitrate and ammonia. Nitrogen makes up approximately ten percent of the dry weight of most organisms (1).

Other elements required by microorganisms in addition to carbon and nitrogen are calcium, potassium, sulphur, and sodium as well as the trace metals iron, cobalt, copper, and zinc. These usually are present in the substrate but, if minuscule or lacking, may limit the organism's growth. Phosphorus and magnesium are particularly important since they are concerned in all energy-transfer reactions involving ATP.

Microorganisms and microbial communities have the ability to incorporate environmental information. Their status in a system at any time is related to the

availability of their required materials. If their basic needs are adequate and there are no factors (toxicants, abnormal physical conditions, or excessive predation) impinging on their situation, the system should be expected to be productive and support relatively high population levels. Knowledge of the organism's requirements, environmental concentrations, and other pertinent factors can be important in the prediction and identification of impacts in the system. Therefore, the present and potential microbial biomass level in an aquatic system may be relevant to the overall productivity or trophic status of the system.

Environmental Parameters

Some of the environmental factors that could affect the biodegradation of a chemical are (a) temperature, (b) dissolved oxygen, (c) pH, (d) light, (e) chemical concentration, (f) salinity, and (g) mixing.

Within a physiological range, rates of biological reactions tend to be proportional to temperature. The proportion is lost when temperature exceeds a maximum level and denaturation of cellular components takes place or when a minimum level is reached so that the enzyme control systems are inhibited. Although biodegradation rates are affected significantly by temperature, it is possible to predict rate constants at different temperatures within the

maximum and minimum levels (18,22).

While the level of dissolved oxygen is very important to aerobic microorganisms, it is not likely to be a crucial variable in the majority of biodegradation studies unless the level drops below one milligram per liter (mg/L) (18).

All microorganisms have their optimum pH for growth and enzyme reactions. For most microorganisms, the optimum pH for growth is near neutral (pH 7). However, some organisms can grow in acidic conditions and some in a basic environment as the substrate is metabolized.

Light may be an important parameter because of its effect on the growth of algae. These photosynthetic organisms are thought to contribute significantly to biotransformation and biodegradation processes (11).

It has been suggested that laboratory tests involving chemical concentrations greater than those in nature may not correctly assess the biodegradation rate in natural ecosystems and that low substrate concentration may be important in limiting biodegradation (5). A chemical may persist because of an inability of the microorganisms to metabolize biodegradable molecules at low concentrations.

These factors and others may affect the enzymatic conversion of chemical compounds in numerous and interrelated ways. Some of them (pH, temperature, mixing, salinity) are probably predictable but further research is

needed so that reliable assessments can be made with the inclusion of these influencing parameters. Table 1-1 lists some controlling parameters affecting the fate of organic chemicals (24).

It is a complex problem to establish the metabolic fate of a chemical in the environment. Identification of various degradation pathways and specific enzymatic reactions has been the qualitative approach to degradative studies.

Of the biological, chemical, and physical factors influencing the transformation of a chemical, the least predictable is caused by microorganisms (6). When a microbial species encounters a substrate it may (a) use it for growth and energy, (b) transform the chemical by co-metabolism, (c) combine it with naturally occurring compounds, or (d) accumulate the substrate within organisms.

Microbial Kinetics

Often when considering biodegradation in a qualitative or quantitative sense, it is necessary to extrapolate from laboratory data to predict the fate of a chemical. An environmental system model consists of representations of many simultaneous and serial processes and can help make evaluations of such complex situations possible. However, the results must still be examined for accuracy and pertinance to the real system.

TABLE 1-1

SYNOPSIS OF PROCESSES AFFECTING THE FATE OF ORGANICS (24)

Process	Controlling Parameters	Compartment
Photolysis	Chemical Structure, Ultraviolet Intensity	Soil-Atmosphere-Water
Evaporation	Activity Coefficients, Partial Vapor Pressure Diffusion Rates, Desorption Rates, Temperature	Soil-Atmosphere-Water
Leaching	Hydrodynamic Diffusion and Dispersion, Adsorption-Desorption, Degradation Rates, Evaporation	Soil
Adsorption-Desorption	Heat of Adsorption, Functional Groups, Surface Area, Water Solubility of Organic, Organic Content of Soil, pH, and Temperature	Soil-Atmosphere-Water
Dispersion	Advection, Diffusion, Aerosol Properties, Chemical Reactions, Meteorological Factors, Topographical Features	Atmosphere
Bacterial-Degradation	Chemical Nature of Organics, Diffusion, Adsorption, Temperature, Microbial Dynamics	Soil, Water
Chemical Decay	Chemical Structure, Catalyzers, pH, Temperature	Soil-Atmosphere-Water
Cultural Input	Agricultural, Industrial, and Political Practices	Soil-Atmosphere-Water

Microbial kinetics is concerned with the rates of production of cells and products, as well as the rates of biotransformation and ultimate biodegradation of a substrate. It is generally agreed that quantitative assessment in the form of rate expressions and rate constants is needed to more accurately describe chemical reactions. Only recently have kinetic studies begun with chemical concentrations for natural waters lower than those associated with waste treatment problems. More effort and agreement is needed in developing rate equations to describe biodegradation or biotransformation in environmental systems.

The biodegradation rate is a function of microbial biomass and chemical concentration at higher concentrations. It is possible that there may be a threshold at low concentrations below which no significant degradation occurs (5). When microorganisms utilize chemical substrates as sources of carbon and energy, there is an increase in biomass. Specific microbial growth and specific rates of chemical degradation are not linear with respect to the chemical concentrations. Frequently there is a lag between exposure of the chemical to the microbes and the beginning of biodegradation or biotransformation. This lag may be due to the microorganism's need for acclimation such as an enzyme induction or an increase in

relevant populations. In biodegradation reactions, rate equations may contain first-order, mixed-order, and zero-order regions depending on the compound used and the character of the microbial population (3,18).

Research has been done under conditions where the test material is rate-limiting for biodegradation and the assay system is optimized to measure the biodegradation rate (18). In this approach, first-order rate constants can be determined for a variety of compounds. Variables such as temperature, light, oxygen, and pH can be incorporated to observe rate expressions under a variety of conditions.

Another application of kinetics is the use of a second-order equation when considering the context of multiple carbon sources (there is no rate-limiting substrate) (3). It incorporates the effects of organic substrate concentration, microbial concentration, and sorption by suspended sediment.

Microbial kinetics have often relied on the empirical equation based on one first suggested by Monod (21), which is

$$\frac{d[B]}{dt}\left(\frac{1}{[B]}\right) = u = \frac{u_m[S]}{K_s+[S]} \qquad (1.)$$

where $[B]$ is microbial population or activity per unit volume, and $[S]$ is substrate concentration, u_m and u are maximum specific and specific growth rates, respectively,

and $K_s = [S]$ when $u = 1/2\ u_m$.

This equation has often been modified by addition of a yield coefficient ($Y = d[B]/d[S]$) which describes the efficiency with which substrate is converted to biomass because it is the substrate transformation that is of interest rather than the biomass growth rate (3).

$$\frac{d[B]}{dt}\left(\frac{d[S]}{d[B]}\right) = \frac{-d[S]}{dt} = \frac{u_m[B][S]}{Y(K_s+[S])} \qquad (2.)$$

Under conditions where very low-level concentrations of substrate are entering an aquatic system the value of $[S]$ may be much lower than K_s and can be written YK_s instead of $Y(K_s + [S])$. Paris, et al. demonstrated that u_m/YK_s and k are approximately equivalent in single carbon-source systems where second-order kinetics were observed (23). This simplifies equation 2. to the following form.

$$\frac{-d[S]}{dt} = \frac{u_m}{YK_s}[B][S] = k[B][S] \qquad (3.)$$

While rates are functions of conditions used in the kinetic determination, rate constants presumably are independent of environmental factors and may be reproducible from site to site (4). Therefore, in the mathematical process to describe biotransformation and biodegradation a rate equation should be used from which a rate constant can be derived. Presently rate constant data

are available for only a limited number of chemicals and sites, thus, more study is needed to (a) increase the number of known rate constants, (b) examine and assess their similarity or differences, and (c) readjust the formulation of rate constants as necessary.

Purpose of This Investigation

This study is one part of a larger environmental investigation examining fate processes. The purpose of this research was to determine, in the laboratory, biotransformation and ultimate biodegradation rate kinetics in three aquatic systems for three organic chemicals ranging from fairly labile to recalcitrant. The chemicals used were (a) phenol, (b) naphthalene, and (c) lindane.

Phenol is composed of a benzene ring with a bonded C-OH group and occurs widely in nature in plants and animals. Alternate names for phenol include carbolic acid, hydroxy benzene, phenyl hydroxide, phenic acid, and phenyl hydrate (32). Natural phenolics occur from aquatic and terrestrial vegetation and are released by the pulp and paper industry. It also has many industrial uses as disinfectants, photographic developers, synthetic resins and plastics, antioxidants, flavors, and perfumes, to name a few.

The toxicity of phenol varies with the type, position, and number of substitutions of the parent molecule as well

as with certain environmental factors such as photolytic action, microbial degradation, pH, water hardness, and temperature (9). Under the EPA Guidelines to protect freshwater organisms, the maximum concentration of phenol should be 600 ug/L as a 24 hour average and the concentration should not exceed 3,400 ug/L at any time. This limit also applies for human health (26).

Biodegradation seems to be very important in the fate of phenol in water. One in situ investigation in river water reported a degradation rate of 30 ug/L per hour in contrast to less than 1 ug/L per hour in sterilized controls from an initial concentration of 125 ug/L (29). While biodegradation of phenol in surface waters tends to proceed relatively rapidly, there may be problems of persistance in groundwater contamination (12).

The dominance of any particular pathway depends on the environmental conditions, however, the degradation products are similar for all phenol fate pathways (32).

Naphthalene is an aromatic hydrocarbon consisting of two connected benzene rings. Other names for naphthalene include mothballs, tar camphor, and naphthene; and it is used in pellet form as a moth-proofing agent and is an important intermediate for dyes (32). It may make its way into the environment through natural seepage of petroleum and accidental oil spillage.

Because of insufficient data, there are no EPA Guidelines or criteria for naphthalene for freshwater or marine life. For the protection of human health from naphthalene, the ambient water criterion is 143 ug/L (28).

An important aquatic fate process for naphthalene seems to be adsorption onto suspended particulates and biota (32). Naphthalene is probably the most easily biodegraded of the polycyclic hydrocarbons, being degraded or transformed relatively rapidly into metabolites which are quickly eliminated, especially in systems chronically affected by contamination (32). A degradation half-life of 5 hours for naphthalene was determined for petroleum-contaminated sediment while degradation rates in pristine sediment were much slower, indicating the importance of microbial adaptation and acclimation to this process (14).

Lindane is a chlorinated hydrocarbon insecticide (γ-1,2,3,4,5,6,-hexachlorocyclohexane), also called gamma-BHC, gammahexane, benzenehexachloride, and jacutin. It is a persistent chemical, for it can take up to three years to complete 75 to 100 percent disappearance in soils (26).

The fate of lindane in aquatic systems appears to be controlled by the availability of biotransformation processes which are favored in biologically rich, anaerobic environments (30). While sorption is not as important a

factor in transportation as it is with naphthalene, it does provide for the ultimate placement of lindane in the anaerobic sediments where transformation may occur (30).

It has been suggested that lindane may be a chemical carcinogen due to electrophilic reactions as it is metabolized. Lindane, therefore, may be capable of impairing molecular functions such as loss of growth controls in cells (20).

In the investigation of these chemicals and their rates of biotransformation and biodegradation, the following three hypotheses were examined:

1. H_o: There is no difference between the microbially-mediated biotransformation rate constants and the biodegradation rate constants derived from the three water sources for naphthalene, lindane, and phenol.
2. H_o: There is no difference in the biotransformation and biodegradation rate constants of the chemicals due to the water source and its intrinsic water quality.
3. H_o: There is no difference in the biotransformation and biodegradation rate constants of the chemicals due to the

microbial biomass.

The three different waters used in the study were (1.) An oligotrophic source represented by Indiana Limestone Quarry. This is located in south-central Indiana near Bedford. (2.) A mesotrophic or medium-productive lake, Cross Lake, which is a reservoir in northwest Louisiana near Shreveport. (3.) Roselawn Cemetery Pond, which is a small eutrophic system located in Denton, Texas. Large water samples were delivered to the North Texas State University Aquatic Station from Indiana Quarry and Cross Lake and stored in metal barrels for the duration of the experimentation. A water sample was drawn from Roselawn Cemetery Pond for each experiment.

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CHAPTER II

MATERIALS AND METHODS

Materials and methods for this investigation will be discussed in four sections: biomass determination, water quality, biodegradation, and biotransformation.

Biomass Determination

An estimate of the amount of living material present was made by several methods: plate counts, for an estimate of heterotrophic bacteria; ATP (adenosine triphosphate), for an estimate of the viable biomass; and chlorophyll-a, for an estimate of the photosynthic organisms.

Plate counts were obtained by using Difco Bacto standard plate count agar in 100 x 15 millimeter (mm) disposable sterile plastic petri dishes. Every sample, dilution, or control was done in triplicate. Dilution water, dilutions, and the plating were prepared and used according to Standard Methods for the Examination of Water and Wastewater (5).

Agar and dilution water were checked for contamination at each use. Unless indicated differently, 0.5 milliliters

(ml) were pipetted to each plate. A bent glass rod ("hockey stick"), kept in alcohol and briefly flamed between water samples, was used to spread the sample over the agar plate as it revolved on a Lab-Line rotary auto-plater.

The plates normally were not inverted but were placed on shelves at 27 ± 1 C for 48 hours in a Lab-Room Controlled Environmental Room incubator by Lab-Line Instruments, Incorporated.

After 48 hours of incubation, unless otherwise indicated, the colonies were counted with a Darkfield Quebec Colony Counter.

Chlorophyll-a determinations were made on the water samples at the beginning and end of each biotransformation experiment in order to obtain an estimate of the algal biomass. This analysis was done according to Standard Methods (5), as was the analysis of pheophytin-a (a degradation product of chlorophyll-a). Water samples of 100 ml were filtered (0.45-micron pore-size membrane filters), the filter was macerated in 2 or 3 ml 90 percent (v/v) spec-grade acetone with a tissue grinder, brought up to 5 ml with acetone in a test tube, covered securely with parafilm, and stored at -20 ± 3 C until a later date when spectrophotometric analysis could be done. The extraction of chlorophyll-a took place at the same time as the other

biomass determinations.

A Leland Sklar Compressor Unit was used to filter water samples through 47-mm-diameter 0.45-micron (u) pore size Millipore membrane filters. The vacuum pressure never exceeded 20 inches mercury. Also used were the laboratory-supplied vacuum lines in the room.

Stored samples were thawed and centrifuged (tabletop International Model HN) at full speed, which when measured by a Strobotec strobe tachometer showed 2000 ± 100 revolutions per minute (rpm). After twenty minutes of centrifugation the cleared extract was poured into a one-centimeter cuvette for optical density determination.

Estimation of biomass was also done by Standard Method's (5) ATP method. ATP extraction was done routinely at the same time as chlorophyll-a extraction and microbial inoculation onto agar plates, at the beginning and end of each experiment.

ATP was extracted by the boiling tris buffer (trishydroxymethylaminomethane) process. Reagent grade Trizma Base by Sigma Chemical Company was used. The water sample (usually 100 ml, 50 ml, or 25 ml) was filtered through a Millipore filter apparatus and a 47-mm-diameter, 0.45-u pore size Millipore membrane filter with the aid of the Leland Sklar Compression unit and the laboratory vacuum lines.

After the filter was boiled in tris and the extract was collected in glass tubes, they were capped tightly with parafilm and stored in the dark at -20 ± 3 C until time permitted analysis.

Analysis was accomplished with an SAI Model 2000 ATP photometer. A range of standards, 20 nanogram (ng) to 0.25 ng ATP, was prepared each time from a stock solution of ATP. The stock ATP was obtained from the Sigma Chemical Company and prepared and frozen in 1-ml-aliquots; 1 ml of the stock ATP being equivalent to 100 ng or 10 ug ATP. From this a standard curve could be made and the extracted sample's ATP content estimated.

ATP extraction efficiency, Table 2-1, was determined by comparing ATP photometer results of the following samples: (a) 100 ml aquarium water, (b) 100 ml aquarium water with 1 ml stock ATP added, and (c) 1 ml stock ATP added to 10 ml boiling tris. There were three replicates of each and they were processed as described for the ATP method. The 10-ml extracts were analyzed within a week with the ATP photometer.

Water Quality

The quality of the water samples was evaluated by the parameters and methods listed in Table 2-2. Water quality was analyzed two times during the study; the second time both filtered (#30 glass fiber filters burned at 500 C for

TABLE 2-1

ATP EXTRACTION EFFICIENCY

Sample	ATP Added (ng)	ATP Required (ng)	Extraction Efficiency (%)
Water Sample - 100 ml	none	23.35	-
ATP Stock Solution - 1 ml	10.00	9.20	92
Water Plus ATP Stock Solution	10.00	33.65	99

$$\frac{33.47}{33.65} = 0.99 \text{ or } 99\% \text{ recovery}$$

To determine extraction efficiency, ATP was was extracted and analyzed from the following: 100 ml aliquots of aquarium water, from 1 ml ATP stock solution which had been diluted to 10 ng ATP/ml, and from 100 ml aquarium water aliquots plus 1 ml ATP stock solution.

Table 2-2

WATER QUALITY PARAMETERS AND METHODOLOGIES

Parameter	Units	Method	Standard Methods Page (5)
Acidity	mg/L as $CaCO_3$	pH-titration	273
Alkalinity	mg/L as $CaCO_3$	pH titration	278
Chloride	mg/L	Specific Ion Meter	*
Phosphorus	mg/L P	Ascorbic Acid	481
Dissolved Solids	mg/L	Gravimetric	89
Suspended Solids	mg/L	Gravimetric	89
Sulfate	mg/L SO_4	Turbidimetric	493
Dissolved Organic	mg/L Carbon	Carbon Analyzer	532
Turbidity	NTU	Nephelometric	131
Apparent Color	Color Units	Visual Comparison	64
True Color	Color Units	Visual Comparison	64
Nitrate	mg/L NO_3-N	Specific Ion Meter	*

*This parameter was analyzed with a Corning pH/ion Meter 135.

60 minutes) and unfiltered water samples were used.

Nitrogen (mg/L N) and chloride (mg/L Cl) content were analyzed with an ion meter equipped with specific ion probes for those functions. This meter, by Science Products, Corning Glass Works, Medfield, Maryland, also was used to measure pH.

Biodegradation

The biodegradation experiment objective was to measure the formation of ^{14}C-labeled carbon dioxide ($^{14}CO_2$), which is produced when a uniformly ^{14}C-labeled chemical (naphthalene, lindane, and phenol) is broken down by microbial action.

In order to affect and monitor biodegradation in the laboratory, a one-way enclosed shake-flask carbon dioxide evolution system was used (2). The main component was a one-liter Kimax Erlenmeyer glass flask fitted with a rubber stopper which allowed an air source entry below the water level, an air source exit above the water level, and another stoppered entry through which water samples could be taken during the experimental process.

Amber latex tubing (0.25 inches in diameter) connected the entry to a filtered air supply through which it flowed from an air pump. The pump was turned on so that air

would be bubbled through the water sample for about fifteen minutes before the collection of water and sodium hydroxide (NaOH) samples.

Three vials were connected in a series by the tubing to the flask's air exit. Each of the first two contained 3.5 ml NaOH, into which the air was bubbled, while the third held activated charcoal and served as a final trap. After the naphthalene experimentation was completed, the second NaOH vial was eliminated because no ^{14}C had been detected there.

This apparatus was set up in a Hythermco incubator with a temperature of 26 ± 1 degree C. The flasks were shaken at fifty revolutions per minute on an Eberbach shaker and dissolved oxygen values were stable at approximately 4 mg DO/L.

The biodegradation and biotransformation experiments for each chemical investigation usually were done at the same time and always in the same incubator with the same shaker operating. The sampling sequences were the same for both biotransformation and biodegradation.

The exact experimental schedule varied with each of the chemicals. Beginning with naphthalene, two trial runs were made in June, 1980. After necessary adjustments were made, the naphthalene experiment, using Roselawn pond water, was begun (day zero) on June 30, 1980.

The biodegradation flasks contained 300 ml water. There were four experimental flasks and six controls. Controls A (three replicates) consisted of autoclaved Roselawn pond water, while Controls B (also three replicates) were filtered (0.45-u pore-size membrane filters) and autoclaved. Degradation occurring in Control A flasks would have been due to processes other than microbial metabolism, such as hydrolysis, volatilization, or sorption. In the Control B flasks the situation would have been the same except that no sorption to particulate matter within the water would occur.

One to two days before the experimental process, the lake water was collected, stock solutions were prepared, lake water for the controls was filtered and/or autoclaved, and all equipment, glassware, and reagents were rechecked for cleanliness, working order, and availability. The 300-ml aliquots of pond water were distributed into labeled flasks and, as soon as the 1 ml ^{14}C-naphthalene dose was added on day zero, $^{14}CO_2$ monitoring was initiated. The naphthalene was from Amersham Corporation (CFA.36, batch 26-naphthalene-UL-^{14}C) and was diluted to 3 microCurie (uCi) per ml, making a concentration of approximately 25 ug/L in each flask, with a specific activity of 6.6×10^6 dpm/300 ml.

The naphthalene degradation (and transformation)

schedule ran for eight days, with samples taken on day zero, one, two, five, and eight. Biodegradation sampling consisted of forcing air to flow through the apparatus for approximately fifteen minutes. The air bubbled through the water, forcing $^{14}CO_2$ into the NaOH traps. When the air flow was stopped the NaOH vials were collected and fresh NaOH vials (3.5 mls in each) were put in their place. Two ml of the water sample were put into each of two vials. One was acidified with a drop of 1N hydrochloric acid (HCl).

With the acidification of the water sample by HCl to approximately pH 2, the total inorganic ^{14}carbon evolved could be accounted for in waters with differing pH. At the lower pH, $^{14}CO_2$ remaining in the water would be driven off. The unacidified water sample, after analysis and comparison with the acidified sample, would show the amount of $^{14}CO_2$ released after acidification.

The $^{14}CO_2$ recovered in the NaOH vials represents the amount liberated since the last collection, so that the summed total over the experiment should indicate an estimate of $^{14}CO_2$ evolved over the eight-day period. The unacidified water sample should reflect a measure of the labeled chemical (and degradation forms) remaining in the water.

On day eight, after the final sampling, water samples were filtered to determine the radioactivity in the

particulate fraction. Generally, 25 ml from each experimental flask and 100 ml from each control were filtered. Each filter was put into a vial, and so were 1.5-ml aliquots of each filtrate.

Fifteen ml scintillation solution (Aquasol II) was added to each of the biodegradation sample vials. Radioactivity of the samples was analyzed on a Beckman LS-100 liquid scintillation system. Data were corrected by internal standard techniques, which consisted of six separate standards containing 3.5 ml NaOH, 15 ml Aquasol II, and 500 or 250 ul aliquots of ^{14}C-toluene (NES-006, 4.1 x 10^5 dpm/ml). This counting efficiency is shown in Table 2-3.

The lindane and phenol ultimate biodegradation experiments followed essentially the same procedures. The second NaOH trap was eliminated from both investigations. All three lake waters were tested simultaneously when lindane was being used. The lake waters were tested separately when naphthalene was used. Each flask had one ml of ^{14}C-lindane added at 1 uCi/ml (California Bionuclear Corporation, CBN 484-Lindane-UL-^{14}C). Lindane concentration was approximately 48 ug/L and each flask contained about 2.2x10^6 dpm. Samples were removed on days zero, twenty, forty, and sixty.

TABLE 2-3

BIODEGRADATION SCINTILLATION COUNTING EFFICIENCY

500ul ^{14}C-Toluene Standard 205,000 dpm	250ul ^{14}C-Toluene Standard 102,500 dpm	Machine Standard 45,500 dpm
172,714	92,588	42,898
171,719	91,691	
171,986	91,846	
170,911	92,391	
170,975	92,385	
$\bar{X}$=171,661	$\bar{X}$=92,180	$\bar{X}$=42,898
83.74%	89.93%	94.28%

^{14}C-labeled standards were prepared using ^{14}C-toluene from New England Nuclear (lot number 697-266; activity at 4.1x10^5 dpm/ml). All scintillation vials contained 15 ml Aquasol liquid scintillation counter cocktail and 3.5 ml 0.5M NaOH. Into five vials 500 ul ^{14}C-toluene was added and in another five vials 250 ul ^{14}C-toluene was added. These vials and the machine standard were analyzed and the counts compared with the total dpm number for each.

In the phenol experiment, 1 ml of ^{14}C-phenol (California Bionuclear Corporation, CBN 620-Phenol-UL-^{14}C) at 2.5 uCi/ml was added to each flask. Phenol concentration was approximately 38 ug/L, with about $5.5x10^6$ dpm per flask. The three waters were done at the same time, with samples removed on days zero, one, two, three, six, seven, eight, nine, ten, and fifteen.

Biotransformation

The primary objective in the biotransformation investigation was to measure the disappearance of the introduced chemical from the experimental flasks.

The flasks contained 500 ml of water from the same sources used as the biodegradation study---Roselawn Pond, Indiana Quarry,and Cross Lake. The chemicals were a. naphthalene (Baker 1-2718, reagent grade), b. lindane (Sigma H-4500-99%), and c. phenol (Baker 1-2858, reagent grade). According to Boethling (1), low-level chemical concentrations should be used to portray more realistic and valid results when attempting to simulate natural biotransformations. The chemical concentrations used for naphthalene and lindane were 100 ug/L, 200 ug/L, and 500 ug/L. Phenol was observed at 500 ug/L and 1 mg/L.

Two types of controls were used and are the same as described in the ultimate biodegradation experiments. However, due to the difficulty of maintaining sterility in

the control flasks, the phenol control flasks also had 2 mg/L mercuric chloride ($HgCl_2$) added to stop microbial growth. The biotransformation flasks were incubated with the biodegradation flasks, utilizing the same shaker.

Naphthalene experimentation used destructive sampling techniques, with samples taken on days zero, one, two, five, and eight. At each sampling period the entire contents of three replicate flasks from each concentration of naphthalene and one each of Controls A and B at each concentration were transferred to one-liter volumetric flasks modified with a screw-top cap.

The cap contained a rubber septum through which a syringe needle could be used to withdraw a sample without removal of the whole cap, thus limiting sample exposure to the air. The samples then were layered with 5 ml Baker Resi-Analyzed pentane and stirred vigorously (producing a vortex) for approximately 10 minutes with a 6-cm magnetic stir bar and stir plate (Corning P-353).

Naphthalene analysis was made from 5-ul aliquots drawn from the pentane layer with a Hamilton syringe and injected into a Hewlet Packard 5710A gas chromatograph equipped with a flame ionization detector. The chromatograph used helium as the carrier gas (flow rate of forty cc/min), passing through a 2.44-meter column with an inside diameter of 0.5 mm and packed with SP-2100. The program used an oven

temperature of 90 C for two minutes, which was then increased at a rate of 8 degrees per minute to 160 C. Attenuation used was four and range was ten. Extraction efficiency of naphthalene in pentane was approximately 98%, as shown in Table 2-4.

Experimental naphthalene chromatogram peak heights were compared to freshly prepared standards each time samples were analyzed.

Lindane experimentation proceeded on the same time schedule as that for biodegradation. The flasks were dosed on day zero and repeatedly sampled on the same schedule as for ultimate biodegradation. Non-destructive sampling was used for lindane. Samples were taken from each of four replicates at each concentration in the following amounts (a.) 3 ml were taken from the 100-ug/L and 200-ug/L concentrations, (b.) 3 ml were taken from the 500-ug/L flasks. All were put in clean 5-ml capped vials with 1 ml of Baker Resi-analyzed hexane for extraction. The differing sample amounts were necessary to obtain satisfactory chromatogram results for all concentrations without having to change chromatograph attenuation.

The vials of water and hexane were agitated for one minute on a Sybron Thermolyne Maxi-Mix. After mixing, 5-ul samples were drawn from the hexane layer with a Hamilton syringe and injected into a Tracor 560 gas chromatograph

TABLE 2-4

NAPHTHALENE EXTRACTION EFFICIENCY IN PENTANE
BY GAS CHROMATOGRAPHY WITH A
FLAME IONIZATION DETECTOR.

Naphthalene Concentration	Pentane $\bar{X}$*	Milli-Q Water $\bar{X}$*	Roselawn Pond $\bar{X}$*
100 ug/L	1.53 = 100%	1.31 = 86%	1.50 = 98%
200 ug/L	3.05 = 100%	2.77 = 91%	2.97 = 97%
500 ug/L	8.76 = 100%	8.61 = 98%	8.66 = 99%

*Mean peak height in millimeters.

Naphthalene concentrations of 100, 200, and 500 ug/L were evaluated by comparing chromatogram peak heights from pentane samples and water samples with a 100:1 concentration factor (naphthalene in 500 ml water was extracted into a 5-ml pentane layer). The pond water extraction efficiency is 98±1%.

equipped with an electron capture detector. The oven temperature was 210 C, the detector temperature was 350 C, and the injection port temperature was 200 C. During the experiment the attenuation was set at 200 or 500. The carrier gas (argon-methane) flow rate was 10 cc/min through a 180-cm glass column with a 2-mm internal diameter packed with 10 percent SP-2100 on Supelco port 100/200. Extraction efficiency of lindane in hexane was approximately 83 percent, as shown in Table 2-5.

The phenol biotransformation flasks were prepared in the same manner as naphthalene and lindane except for the concentrations used and the absence in these experiments of Controls B (filtered and autoclaved). The previous experiments showed no appreciable difference between Controls A and B.

Roselawn pond water aliquots (500 ml) were non-destructively sampled on days zero, one, two, three, and six. Indiana Quarry and Cross Lake aliquots (500 ml) were sampled on days zero, one, two, three, six, seven, eight, nine, and ten.

Ten-ml samples were removed from the flasks and filtered through a glass fiber filter in preparation for direct analysis. A Waters Associates liquid chromatograph with a 200-ul sample loop and a Schoeffel Instrument Corporation fluorometer were used. The carrier solvent was

TABLE 2-5

LINDANE EXTRACTION EFFICIENCY IN HEXANE
BY GAS CHROMATOGRAPHY WITH AN
ELECTRON CAPTURE DETECTOR.

Lindane Concentration	Hexane $\bar{X}$*	Milli-Q Water $\bar{X}$*	Roselawn Pond $\bar{X}$*
100 ug/L	1.67 = 100%	1.38 = 82%	1.33 = 80%
200 ug/L	3.20 = 100%	2.75 = 86%	2.71 = 84%
500 ug/L	8.50 = 100%	9.03 = 106%	7.34 = 86%

*Mean peak height in millimeters.

Lindane concentrations of 100, 200, and 500 ug/L were evaluated by comparing chromatogram peak heights from hexane samples and water samples. Chromatograph attenuation was set at 500. Lindane/hexane concentrations of 100 and 200 ug/L were diluted 2:1. The 500 ug/L concentration was diluted 3:1 in hexane so that the chromatograph attenuation would not have to be changed. Pond water efficiency is 83$\pm$3%.

30% methanol in HPLC water (Baker) and had a flow rate of 2 ml/min. The stainless steel column was packed with M-Bonda Pak Phenyl. As with the other experiments, peak heights from the chromatograms were measured and compared to standards to determine concentrations.

Statistical Analyses

The biotransformation and biodegradation data were analyzed with a National Advance Systems AS 5000 Computer and the Statistical Analysis System (SAS) (4). First-order rate constants were determined by regression of the natural log of the test chemical concentration versus time, and are expressed as the rate constant value per day. Analysis of covariance was used to determine if differences exist among biotransformation and biodegradation rate constants between chemicals and lakes.

Analysis of covariance may be used for comparing two or more slopes, with the null hypothesis being $slope_1 = slope_2 = slope_3 = slope_n$. The alternate hypothesis is that the k (rate constant) regression lines are not derived from samples of populations with equal slopes. It must be assumed that the k residual mean squares are homogeneous. However, this test is robust and can operate well even with considerable heterogeneity of variance as long as all sample sizes are equal or nearly equal. Analysis of covariance is also strong with respect to the

assumption of normality in populations, with the validity being affected only slightly by deviations (6).

Second-order rate constants were determined by dividing the first-order rate constant by the corresponding biomass estimate and are expressed as the rate constant value per organism per day (from plate count data) or rate constant value per ug per day (when using ATP data).

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CHAPTER III

RESULTS AND DISCUSSION

The first hypothesis asks if there is no difference between the biotransformation rate constants and the biodegradation rate constants of naphthalene, lindane, and phenol. The alternate hypothesis is that the rate constants are different for biotransformation and biodegradation.

This hypothesis can be divided into several parts: 1. Is there a difference in the biotransformation rate constants for the three chemicals? 2. Is there a difference in the biodegradation rate constants for the three chemicals? 3. Is there a difference between the biotransformation and the biodegradation rate constants for the three chemicals?

The procedures for monitoring chemical disappearance and deriving the rate constants have been described in the materials and methods section, Chapter II. Tables 3-1, 3-2, and 3-3 illustrate the first-order rate constants of the three chemicals determined in this study for each water source.

TABLE 3-1

BIOTRANSFORMATION AND BIODEGRADATION FIRST-ORDER RATE CONSTANTS

FOR ROSELAWN CEMETERY POND

Units are Day^{-1}

Sample	Naphthalene	Lindane	Phenol
K_{100}	0.2525 ± 0.0176[a]	0.0360 ± 0.0046	*
K_{200}	0.1790 ± 0.0140	0.0299 ± 0.0034	*
K_{500}	0.1826 ± 0.0246	0.0271 ± 0.0018	1.1513 ± 0.1698
K_{1000}	*	*	1.1800 ± 0.1422
K_d	0.2179 ± 0.0086	0.0168 ± 0.0024	0.0824 ± 0.0094
K_u	0.0461 ± 0.0045	0.0075 ± 0.0006	0.0264 ± 0.0013

K_{100} = Biotransformation rate constant for 100 ug/L initial concentration of chemical.

K_{200} = Biotransformation rate constant for 200 ug/L initial concentration of chemical.

K_{500} = Biotransformation rate constant for 500 ug/L initial concentration of chemical.

K_{1000} = Biotransformation rate constant for 1000 ug/L initial concentration of chemical.

K_d = Biodegradation rate constant derived from ^{14}C-tagged chemical after acidification.

K_u = Biodegradation rate constant derived from $^{14}CO_2$ evolved.

[a] ±one standard deviation.

*This initial concentration level was not used.

TABLE 3-2

BIOTRANSFORMATION AND BIODEGRADATION FIRST-ORDER

RATE CONSTANTS FOR INDIANA QUARRY

Units are Day^{-1}

Sample	Naphthalene	Lindane	Phenol
K_{100}	0.1443 ± 0.0091[a]	0.0097 ± 0.0008	*
K_{200}	0.1024 ± 0.0088	0.0056 ± 0.0003	*
K_{500}	0.1177 ± 0.0117	0.0100 ± 0.0007	0.0044 ± 0.0004
K_{1000}	*	*	0.0050 ± 0.0002
K_d	0.1514 ± 0.0080	0.0030 ± 0.0001	0.0749 ± 0.0119
K_u	0.0317 ± 0.0023	0.0069 ± 0.0010	0.0131 ± 0.0010

K_{100} = Biotransformation rate constant for 100 ug/L initial concentration of chemical.

K_{200} = Biotransformation rate constant for 200 ug/L initial concentration of chemical.

K_{500} = Biotransformation rate constant for 500 ug/L initial concentration of chemical.

K_{1000} = Biotransformation rate constant for 1000 ug/L initial concentration of chemical.

K_d = Biodegradation rate constant derived from ^{14}C-tagged chemical after acidification.

K_u = Biodegradation rate constant derived from $^{14}CO_2$ evolved.

[a] ±one standard deviation.

*This initial concentration level was not used.

TABLE 3-3

BIOTRANSFORMATION AND BIODEGRADATION FIRST-ORDER

RATE CONSTANTS FOR CROSS LAKE

Units are Day^{-1}

Sample	Naphthalene	Lindane	Phenol
K_{100}	0.1705 ± 0.0085[a]	0.0073 ± 0.0005	*
K_{200}	0.1159 ± 0.0078	0.0071 ± 0.0006	*
K_{500}	0.1270 ± 0.0120	0.0129 ± 0.0014	0.0087 ± 0.0006
K_{1000}	*	*	0.0027 ± 0.0002
K_d	0.0522 ± 0.1092	0.0079 ± 0.0007	0.1264 ± 0.0115
K_u	0.0317 ± 0.0012	0.0033 ± 0.0001	0.0245 ± 0.0014

K_{100} = Biotransformation rate constant for 100 ug/L initial concentration of chemical.

K_{200} = Biotransformation rate constant for 200 ug/L initial concentration of chemical.

K_{500} = Biotransformation rate constant for 500 ug/L initial concentration of chemical.

K_{1000} = Biotransformation rate constant for 1000 ug/L initial concentration of chemical.

K_d = Biodegradation rate constant derived from ^{14}C-tagged chemical after acidification.

K_u = Biodegradation rate constant derived from $^{14}CO_2$ evolved.

[a] ± one standard deviation.

*This initial concentration level was not used.

Biotransformation first-order rate constants indicate that phenol was biotransformed more quickly in Roselawn Cemetery Pond, followed by naphthalene and then lindane. For the other two waters, however, phenol biotransformation was much slower. In the Indiana Quarry and Cross Lake waters, naphthalene had a consistently faster biotransformation rate than either lindane or phenol. While lindane has been shown to be a persistent chemical in the environment (1, 4, 11, 13), phenol biotransformation is thought to be relatively rapid (14). Only in Roselawn Cemetery Pond water did this appear to be so.

Initial chemical concentration over the range tested (100 to 1000ug/L) did not appear to cause much alteration of the observed biotransformation rate as seen in Figures 3-1, 3-2, and 3-3. While no lag phase was observed for naphthalene and lindane biotransformation, phenol showed about a three-day lag. The raw data from the biotransformation and biodegradation procedures are given in the Appendix in Tables 5-1 and 5-2.

Prior to comparing biotransformation and biodegradation rate constants, the biotransformation treatments were tested for homogeneity of their slopes, or rate constants. Treatment C (K_{100}) designates those samples with 100ug/L initial concentration. Treatment D (K_{200}) represents 200ug/L initial concentration and V (K_{500}) and M

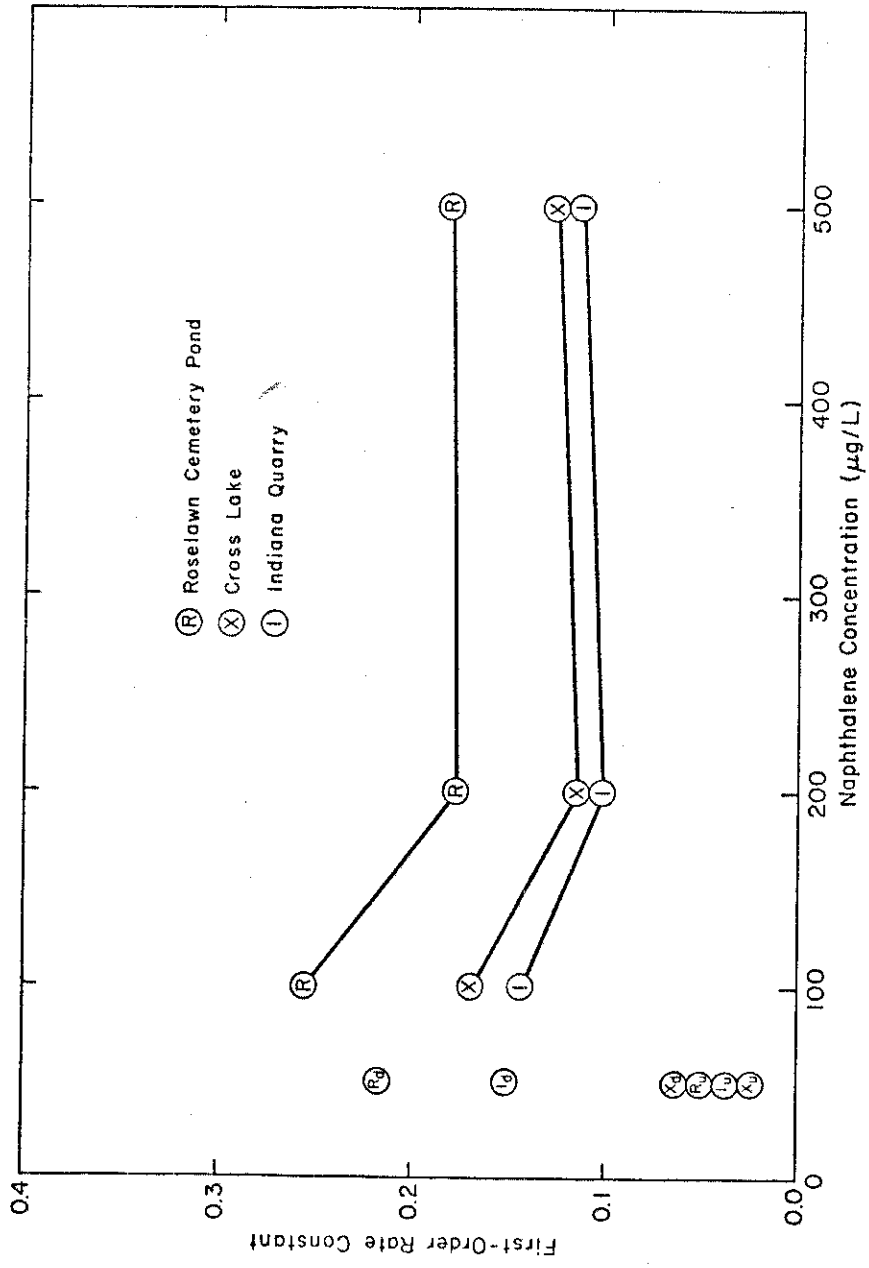

Figure 3-1. Relationship of biotransformation and biodegradation rate constants to naphthalene concentration. Rate constants denoted with d represent primary biodegradation and u represents ultimate biodegradation.

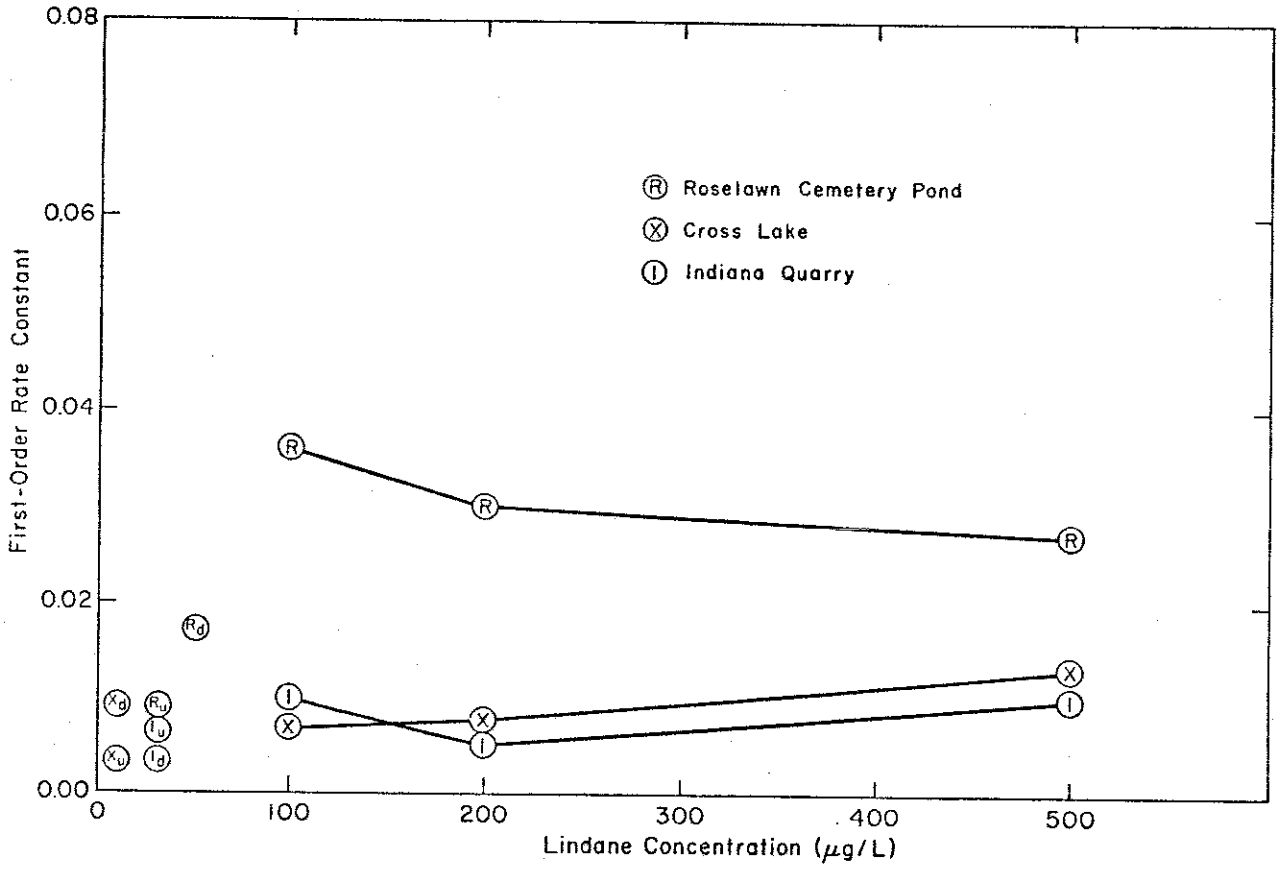

Figure 3-2. Relationship of biotransformation and biodegradation rate constants to lindane concentration. Rate constants denoted with d represent primary biodegradation and u represents ultimate biodegradation.

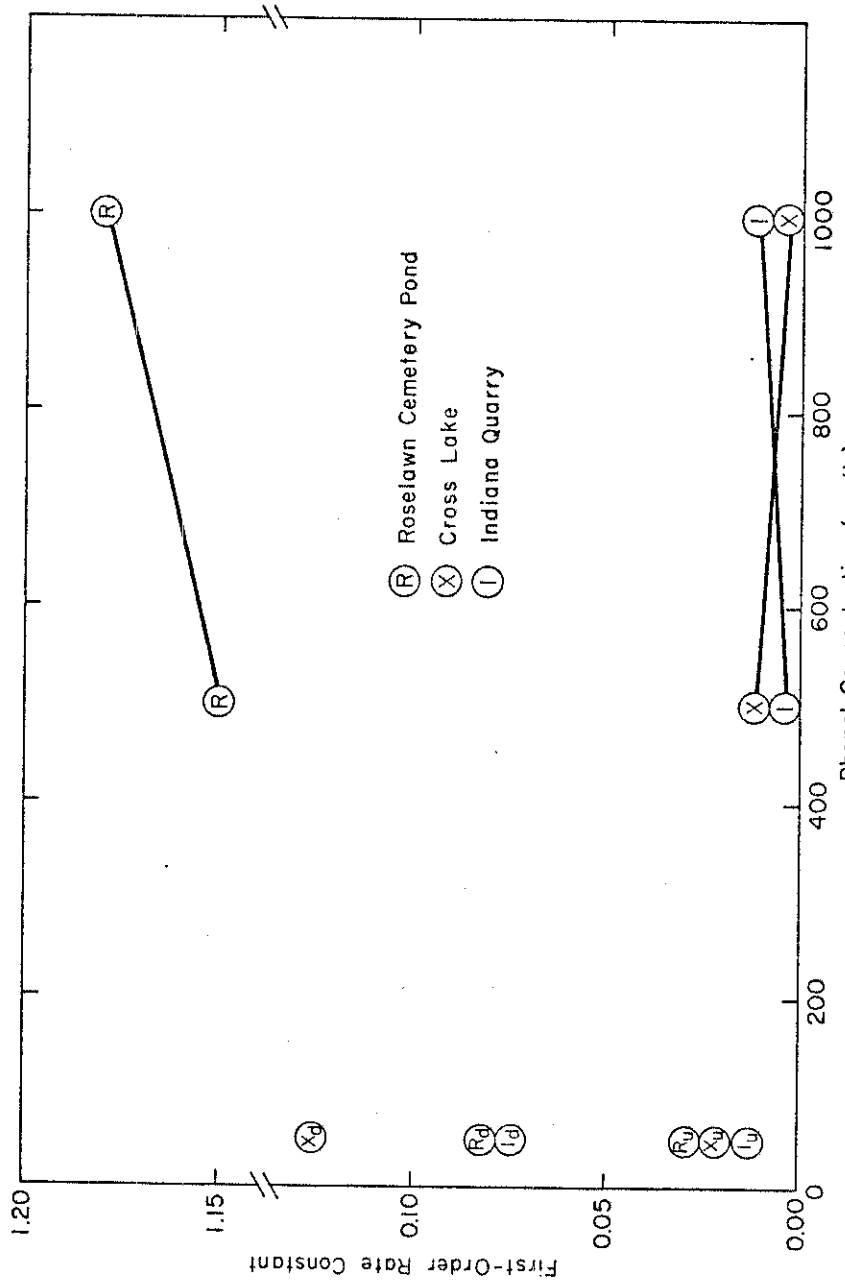

Figure 3-3. Relationship of biotransformation and biodegradation rate constants to phenol concentration. Rate constants denoted with d represent primary biodegradation and u represents ultimate biodegradation.

(K_{1000}) correspond, respectively, to 500ug/L and 1000ug/L initial concentration.

The same testing was done among the biodegradation treatments with treatment A (K_d) designating those rate constants, or slopes, derived from ^{14}carbon-labeled naphthalene, lindane, and phenol after acidification. Treatment B (K_u) represents those rate constants derived from $^{14}CO_2$ evolved. The results of these analyses are in Table 3-4.

In many combinations, but not all, biotransformation treatment rate constants indicated homogeneity. All three chemicals showed some similarity between the initial concentrations used. With the exception of naphthalene in Cross Lake, all biodegradation treatment slopes were found to be heterogeneous, indicating a greater variation in the two biodegradation methods used than in the biotransformation procedures. The naphthalene K_d biodegradation treatment for Cross Lake has a much higher standard deviation than the others, which may indicate a greater variability associated with it and may be responsible for the lack of significant difference between those slopes.

An analysis of covariance test, Table 3-5, was done to compare slopes of similar treatments for each of the chemicals. For example, all biodegradation treatment A

TABLE 3-4

COMPARISON OF RATE CONSTANTS BETWEEN TREATMENTS

Treatment designations indicate initial chemical concentration, for biotransformation data, and method, for biodegradation data.

Lake	K_{100} vs K_{200}	K_{100} vs K_{500}	K_{200} vs K_{500}	K_u vs K_d
		NAPHTHALENE		
Roselawn Pond	P=0.0031[a]	P=0.0291	P=0.9010[b]	P=0.0001
Indiana Quarry	P=0.0028	P=0.0845[b]	P=0.3051[b]	P=0.0001
Cross Lake	P=0.0001	P=0.0064	P=0.4433[b]	P=0.8525[b]
		LINDANE		
Roselawn Pond	P=0.2930[b]	P=0.0827[b]	P=0.4855[b]	P=0.0010
Indiana Quarry	P=0.0001	P=0.8323[b]	P=0.0001	P=0.0006
Cross Lake	P=0.7759[b]	P=0.0009	P=0.0008	P=0.0001
		PHENOL		
			K_{500} vs K_{1000}	K_u vs K_d
Roselawn Pond	*	*	P=0.8974[b]	P=0.0001
Indiana Quarry	*	*	P=0.2349[b]	P=0.0001
Cross Lake	*	*	P=0.0001	P=0.0001

[a]p is the probability of no difference in an analysis of covariance test.

[b]Indicates rate constants showing no significant difference.

*Phenol was not used at this level.

TABLE 3-5

COMPARISON OF RATE CONSTANTS OF CORRESPONDING TREATMENTS FOR THE CHEMICALS IN EACH LAKE

Analysis of Covariance was Used for Rate Constant Comparisons.

	A (K_d)	B (K_u)	C (K_{100})	D (K_{200})	V (K_{500})	M (K_{1000})
Roselawn Cemetery Pond	P=0.0001[a]	P=0.0001	P=0.0001	P=0.0001	P=0.0001	*
Indiana Quarry	P=0.0001	P=0.0001	P=0.0001	P=0.0001	P=0.0001	*
Cross Lake	P=0.0083	P=0.0001	P=0.0001	P=0.0001	P=0.0001	*

[a]p-value less than 0.0500 indicates that there is a significant difference among the rate constants, or slopes of each treatment.

*Treatment M is used only with the chemical phenol so comparisons cannot be made with naphthalene and lindane.

Treatment designations are as follows: A=Primary Biodegradation, B=Ultimate Biodegradation, and for Biotransformation, C=initial chemical concentration of 100ug/L, D=200ug/L, V=500ug/L, and M=1mg/L.

rate constants for the three chemicals were compared. In all cases there is a significant difference shown among the rate constants or slopes of each treatment, indicating that there is a difference between the biotransformation rates and among the biodegradation rates for each chemical.

When biotransformation and biodegradation rate constants were compared, one biotransformation rate constant, treatment V (K_{500}), was used to test for homogeneity with one biodegradation rate constant, either treatment A (K_d) or treatment B (K_u). Treatment V was chosen from the biotransformation data because, unlike some of the other biotransformation treatments, it is a concentration level that was used with all the chemicals. The result of this analysis is in Table 3-6. It shows that the only homogeneity occurs between treatment V and treatment A for naphthalene in two of the waters.

Therefore, this analysis indicates that the rate constants are different for each chemical within a lake. Biodegradation rate constants are different from the biotransformation rate constants.

In order to determine if there is a difference in biotransformation and biodegradation rate constants due to water quality of the water sources, the rate constant data have been sorted by chemical for the three lakes in Tables 3-7, 3-8, and 3-9.

TABLE 3-6

COMPARISON OF BIODEGRADATION AND BIOTRANSFORMATION RATE CONSTANTS

	Roselawn Cemetery Pond	Indiana Quarry	Cross Lake
Naphthalene	P=0.0001	P=0.0001	P=0.0001
Lindane	P=0.0001	P=0.0001	P=0.0001
Phenol	P=0.0001	P=0.0001	P=0.0001
	Biodegradation (Treatment A) Versus Biotransformation (Treatment V)		
Naphthalene	P=0.1380*	P=0.0192	P=0.5614*
Lindane	P=0.0019	P=0.0208	P=0.0035
Phenol	P=0.0001	P=0.0001	P=0.0001

Analysis of covariance with the Statistical Analysis Computer System was used to determine comparisons. Biotransformation treatment V (K_{500}) was analyzed against the biodegradation treatments because all three chemicals were tested with 500 ug/L initial concentration.

Biodegradation treatment B rate constants are derived from ^{14}C-tagged chemical after acidification and treatment A rate constants are from $^{14}CO_2$ evolved.

*Probability is greater than 0.05.

TABLE 3-7

BIOTRANSFORMATION AND BIODEGRADATION FIRST-ORDER

RATE CONSTANTS FOR NAPHTHALENE

Units are Day^{-1}

Sample	Roselawn Cemetery Pond	Indiana Quarry	Cross Lake
K_{100}	0.2525 ± 0.0176[a]	0.1443 ± 0.0091	0.1705 ± 0.0085
K_{200}	0.1790 ± 0.0140	0.1024 ± 0.0088	0.1159 ± 0.0078
K_{500}	0.1826 ± 0.0246	0.1177 ± 0.0117	0.1270 ± 0.0120
K_d	0.2179 ± 0.0086	0.1514 ± 0.0080	0.0522 ± 0.1092
K_u	0.0461 ± 0.0045	0.0317 ± 0.0023	0.0317 ± 0.0012

K_{100} = Biotransformation rate constant for 100 ug/L initial concentration.

K_{200} = Biotransformation rate constant for 200 ug/L initial concentration.

K_{500} = Biotransformation rate constant for 500 ug/L initial concentration.

K_d = Biodegradation rate constant for ^{14}C-tagged naphthalene after acidification.

K_u = Biodegradation rate constant for $^{14}CO_2$ evolved.

[a] ± one standard deviation.

TABLE 3-8

BIOTRANSFORMATION AND BIODEGRADATION FIRST-ORDER

RATE CONSTANTS FOR LINDANE

Units are Day^{-1}

Sample	Roselawn Cemetery Pond	Indiana Quarry	Cross Lake
K_{100}	0.0360 ± 0.0046[a]	0.0097 ± 0.0008	0.0073 ± 0.0005
K_{200}	0.0299 ± 0.0034	0.0056 ± 0.0003	0.0071 ± 0.0006
K_{500}	0.0271 ± 0.0018	0.0100 ± 0.0007	0.0129 ± 0.0014
K_d	0.0168 ± 0.0024	0.0030 ± 0.0001	0.0079 ± 0.0007
K_u	0.0075 ± 0.0006	0.0069 ± 0.0010	0.0033 ± 0.0001

K_{100} = Biotransformation rate constant for 100 ug/L initial concentration.

K_{200} = Biotransformation rate constant for 200 ug/L initial concentration.

K_{500} = Biotransformation rate constant for 500 ug/L initial concentration.

K_d = Biodegradation rate constant for ^{14}C-tagged lindane after acidification.

K_u = Biodegradation rate constant derived from $^{14}CO_2$ evolved.

[a] ± one standard deviation.

TABLE 3-9

BIOTRANSFORMATION AND BIODEGRADATION FIRST-ORDER

RATE CONSTANTS FOR PHENOL

Units are Day^{-1}

Sample	Roselawn Cemetery Pond	Indiana Quarry	Cross Lake
K_{500}	1.1513 ± 0.1698[a]	0.0044 ± 0.0004	0.0087 ± 0.0006
K_{1000}	1.1800 ± 0.1422	0.0050 ± 0.0002	0.0027 ± 0.0002
K_d	0.0824 ± 0.0094	0.0749 ± 0.0119	0.1264 ± 0.0115
K_u	0.0264 ± 0.0013	0.0131 ± 0.0010	0.0245 ± 0.0014

K_{500} = Biotransformation rate constant for 500 ug/L initial concentration.

K_{1000} = Biotransformation rate constant for 1000 ug/L initial concentration.

K_d = Biodegradation rate constant for ^{14}C-tagged phenol after acidification.

K_u = Biodegradation rate constant for $^{14}CO_2$ evolved.

[a] $\pm$ one standard deviation.

Naphthalene was biotransformed and biodegraded more rapidly in Roselawn Cemetery pond than in Cross Lake and Indiana Quarry, both of which had somewhat similar rate constants.

The two biodegradation experimental treatments (K_d and K_u) showed little correlation with one another. The $^{14}CO_2$ evolved method (K_u) was uniformly low throughout the lakes. The acidified ^{14}carbon-tagged naphthalene sample (K_d) showed the fastest biodegradation rate in Roselawn Pond but was followed by Indiana Quarry and Cross Lake, which is in contrast to the biotransformation rate data.

Half-lives for naphthalene in this study ranged from 2.8 days in Roselawn Pond to 6.8 days in Indiana Quarry. In a controlled ecosystem enclosure experiment, naphthalene was significantly degraded by microbes, with up to 5% of the chemical removed per day from previously non-polluted water (8). Herbes and Schwall reported naphthalene first-order rate constants as high as 0.14 per hour in an oil-contaminated stream (6).

Lindane biotransformation rate constants in Table 3-8, indicate that Roselawn Pond had the highest biotransforming rates, with the other two waters lower and similar to each other.

The biodegradation rate constants are consistently lower

for the K_u treatment. The K_d treatment shows the fastest rate in Roselawn Pond, followed by Cross Lake, which is somewhat comparable to the biotransformation rate constants.

Lindane rate constants ranged from 0.0360 day^{-1} in Roselawn Pond to 0.0056 day^{-1} in Indiana Quarry water. The disappearance of lindane has been studied in the Indiana Quarry previously, with about 50% chemical decrease occuring after 123 days (5). Alexander reported that lindane could persist in soil longer than 15 years (1). Another study found no measurable degradation or transformation of lindane in raw river water over a period of eight weeks (4). Sharom et al. found that about 75% of the pesticide was biotransformed in 16 weeks during incubation in water from a drainage canal (13).

The two phenol biotransformation rate constants, Table 3-9, indicated the same trend as the other chemicals, with the highest rate constants achieved in Roselawn Pond. Visser et al. reported degradation rates of 30 ug/L per hour in St. Lawrence River water at an initial concentration of 125 ug/L (14).

As before, the biodegradation K_u treatment is the lowest of the two and is similar for all the waters. In general, the biodegradation rate constants, although somewhat different from each other, are relatively constant

for all three lakes. This trend is in contrast with the biotransformation data.

Table 3-10 gives the chemical characterization, along with some biological parameters, of the experimental waters. The higher levels of phosphorus, nitrates, and ATP in Roselawn Cemetery Pond give an indication of its potentially higher productivity. Nesbitt and Watson (10), in a river water study, found that microbial activity was primarily affected by temperature and secondly by low concentrations of available nutrients of which phosphorus was the most important followed by nitrogen.

Other parameters, such as dissolved organic carbon, plate count, and chlorophyll-a, seem unexpectedly low. However, rate constants are consistently higher in the water samples from the pond, which is apparently more capable of allowing faster chemical biotransformation and biodegradation. This is probably due to its support of a larger microbial population, even though biomass estimates such as plate counts and chlorophyll-a may not reveal that.

Table 3-11 illustrates the results of an analysis of covariance among like treatments for each chemical. The only case of no significant difference is found in the biodegradation treatment K_d which was shown in the previous three tables to be not only relatively constant for each chemical but also different from the trends of the

TABLE 3-10

CHEMICAL CHARACTERIZATION OF EXPERIMENTAL WATERS

Parameters	Roselawn Cemetery Pond			Indiana Quarry			Cross Lake		
	Summer Unfiltered	Fall Unfiltered	Fall Filtered*	Summer Unfiltered	Fall Unfiltered	Fall Filtered	Summer Unfiltered	Fall Unfiltered	Fall Filtered
pH	9.3	9.3	9.3	8.7	8.6	8.6	8.0	8.0	8.0
Alkalinity (mg/L $CaCO_3$)	370.0	290.0	270.0	37.0	30.0	25.0	90.0	5.0	5.0
Acidity (mg/L $CaCO_3$)	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0
Chlorides (mg/L)		14.0	11.0		28.0	28.0		5.0	6.0
Sulphates SO_4-S (mg/L)	44.0	33.0	18.0	47.0	37.0	27.0	42.0	105.0	98.0
Orthophosphate PO_4-P (mg/L)	0.30	0.70	0.63	0.01	0.20	0.003	0.0	0.006	0.003
Total Phosphate PO_4-P (mg/L)	0.30	0.86	0.78	0.01	0.005	0.012	0.0	0.007	0.005
Nitrate NO_3-N (mg/L)	1.33	0.26	0.08	0.37	0.05	0.06	0.21	0.02	0.02
Total Dissolved Solids (mg/L)		830.0	550.0		284.0	198.0		726.0	380.0
Total Suspended Solids (mg/L)		24.0	2.0		56.0	44.0		24.0	8.0

Table 3-10 continued on next page.

TABLE 3-10 (continued)

CHARACTERIZATION OF EXPERIMENTAL WATERS

Parameters	Roselawn Cemetery Pond			Indiana Quarry			Cross Lake		
	Summer Unfiltered	Fall Unfiltered	Fall Filtered	Summer Unfiltered	Fall Unfiltered	Fall Filtered	Summer Unfiltered	Fall Unfiltered	Fall Filtered
Turbidity (NTU)	5.0	39.0	18.0	67.0	20.0	3.0	4.0	5.0	5.0
Apparent Color	60.0	100.0	45.0	210.0	80.0	5.0	35.0	25.0	0.0
True Color	20.0	35.0	35.0	5.0	5.0	5.0	3.0	5.0	0.0
Dissolved Organic Carbon (mg/L)		7.1			8.2			1.9	
ATP (ug/L)		2.370	1.380		0.058	0.022		0.042	0.034
Chlorophyll-a (mg/L)		0.0185	0.0036		0.0133	0.0028		0.0036	0.0012
Plate Count (colonies/ml)	$3.5x10^3$	$1.7x10^3$		$3.1x10^5$	$3.4x10^4$		$1.4x10^4$	$3.6x10^4$	

*Water samples were filtered through #30 glass filters which had previously been burned at 500 C for 60 minutes.

TABLE 3-11

COMPARISON OF RATE CONSTANTS OF CORRESPONDING TREATMENTS AMONG THE WATERS.

Analysis of Covariance was Used for Rate Constant Comparisons.

Chemical	Treatments of Each Chemical					
	A (K_d)	B (K_u)	C (K_{100})	D (K_{200})	V (K_{500})	M (K_{1000})
Naphthalene	P=0.1809[a]	P=0.0012	P=0.0001	P=0.0001	P=0.0228	*
Lindane	P=0.0001	P=0.0001	P=0.0001	P=0.0001	P=0.0001	*
Phenol	P=0.0021	P=0.0001	*	*	P=0.0001	P=0.0001

[a]p-value less than 0.0500 indicates that there is a significant difference among the slopes.

*The chemical concentration corresponding to these treatments was not used.

Treatment designations are as follows: A=Primary Biodegradation, B=Ultimate Biodegradation, and for Biotransformation, C=initial chemical concentration of 100ug/1, D=200ug/L, V=500ug/L, and M=1ug/L.

other treatments. The remainder of the table suggests that the rate constants of similar treatments vary from water to water.

Because of the variation exhibited between the water sources for the chemical biodegradation and biotransformation, it is likely that there is a difference in the rate constants due to the water quality.

The third hypothesis is concerned with learning if there is a difference in biotransformation and biodegradation rate constants of the three chemicals due to the microbial biomass present. It has been suggested that degradation rates are proportional to the concentration of the chemical substrate as well as the microbial biomass (2, 12). The biomass of the three experimental waters was determined by two methods so that second-order rate constants could be established and compared to the first-order rate constants.

Plate count and ATP results are listed in Tables 3-12 and 3-13, respectively. Chlorophyll-a data were disregarded because the majority of the calculated values were negative, indicating a problem in handling, procedure, or analysis.

Plate counts did not reflect the trophic status of the waters, nor did they correspond to the ATP results. Roselawn Pond, being the eutrophic example, was expected to yield the most colony-forming units.

TABLE 3-12

NUMBERS OF COLONY-FORMING UNITS PER MILLILITER FOR THE WATERS USED IN BIOTRANSFORMATION AND BIODEGRADATION STUDIES.

Chemical	Concentration (ug/L)	Source of Water					
		Roselawn Cemetery Pond		Indiana Quarry		Cross Lake	
		Initial	Final	Initial	Final	Initial	Final
Naphthalene	100	3.3×10^3	3.5×10^3	3.45×10^4	1.4×10^4	6.2×10^4	3.1×10^5
	200	*	1.7×10^3	*	5.0×10^3	*	8.3×10^3
	500	*	8.4×10^2	*	6.3×10^3	*	1.0×10^4
Lindane	100	1.7×10^3	1.2×10^4	3.6×10^4	4.6×10^2	3.4×10^4	1.7×10^3
	200	*	8.6×10^3	*	3.6×10^2	*	9.7×10^2
	500	*	7.5×10^3	*	2.4×10^2	*	5.2×10^2
Phenol	500	7.5×10^3	3.0×10^3	1.0×10^4	TFTC[a]	7.5×10^4	TFTC[a]
	1000	*	2.0×10^3	*	TFTC[a]	*	TFTC[a]

[a]TFTC represents Too-Few-To-Count because there were less than 10 colony-forming units per ml.

*There is only one initial sample plated for each water.

Plate counts were done at the start of the experiment (initial) and at the termination of an experiment (final). The numbers represent the means of three replicate plates.

TABLE 3-13

ATP MEASUREMENT FOR THE WATERS USED IN BIOTRANSFORMATION AND BIODEGRADATION STUDIES.

Measurement Units are ug/L and Represent the Means of

Three Replicate Determinations.

Chemical	Concentration (ug/L)	Source of Water					
		Roselawn Cemetery Pond		Indiana Quarry		Cross Lake	
		Initial	Final	Initial	Final	Initial	Final
Naphthalene	100	2.11	<0.25	1.06	1.16	0.12	1.65
	200	*	<0.25	*	1.58	*	1.11
	500	*	<0.25	*	0.12	*	0.96
Lindane	100	9.72	1.93	0.45	<0.25	0.27	0.41
	200	*	0.98	*	<0.25	*	0.52
	500	*	1.58	*	0.92	*	0.61
Phenol	500	1.86	1.35	0.85	0.32	1.17	0.18
1000	*	1.35	*	0.30	*	0.30	

*There is only one replicate-set initially determined for each water and it is without chemical added.

Initial values are from water without chemical at the beginning of each experiment.

Final values are from water samples with chemical at the end of each experiment.

The values of these colony counts may be due to any of several factors, such as (1.) clumping of the bacteria, thus reducing the number of colonies formed; (2.) adhesion of bacteria to the vessel walls or the particulates within the water sample; or (3.) anaerobic microorganisms, algae, and organisms not growing on this media and not accounted for in this biomass procedure.

ATP data, however, was more indicative of the nutrient levels of the waters (Table 3-13), with Roselawn Pond having the highest values. This biomass estimate would include algae and other organisms which may not be detected on standard plate count agar. Indiana Quarry and Cross Lake ATP values were somewhat inconsistent. This may be due to the long storage period that these two waters underwent.

Both of the estimators of biomass (plate counts and ATP values) were used to determine second-order rate constants, which appear in Table 3-14. The second-order rate constants obtained from ATP values are consistently higher and have little or no correlation with the corresponding rate constants derived from plate count data.

Second-order biotransformation rate constants from the ATP data for naphthalene ranged from a questionable 0.981 in Indiana Quarry (the next largest was 0.132 in Cross Lake) to 0.065 also in Indiana Quarry. The corresponding biodegradation rate constants varied from 0.435 in Cross

TABLE 3-14

BIOTRANSFORMATION AND BIODEGRADATION SECOND-ORDER RATE CONSTANTS

From Plate Count Data, Units in L org^{-1} day^{-1}

and From ATP Data, Units in L ug^{-1} day^{-1}

Chemical	Concentration*	Roselawn Cemetery Pond		Indiana Quarry		Cross Lake	
		Plate Count	ATP	Plate Count	ATP	Plate Count	ATP
Naphthalene	K_{100}	7.21×10^{-8}	0.120	1.03×10^{-8}	0.124	5.50×10^{-10}	0.103
	K_{200}	1.02×10^{-7}	0.085	2.05×10^{-8}	0.065	1.40×10^{-8}	0.104
	K_{500}	2.18×10^{-7}	0.087	1.87×10^{-8}	0.981	1.27×10^{-8}	0.132
	K_d	6.60×10^{-8}	0.103	4.39×10^{-9}	0.143	8.42×10^{-10}	0.435
	K_u	1.40×10^{-8}	0.022	9.19×10^{-10}	0.030	5.11×10^{-10}	0.264
Lindane	K_{100}	3.00×10^{-9}	0.019	2.11×10^{-8}	0.022	4.29×10^{-9}	0.018
	K_{200}	3.48×10^{-9}	0.031	1.56×10^{-8}	0.012	7.32×10^{-9}	0.014
	K_{500}	3.61×10^{-9}	0.017	4.17×10^{-8}	0.011	2.48×10^{-8}	0.021
	K_d	9.88×10^{-9}	0.002	8.33×10^{-11}	0.007	2.32×10^{-10}	0.029
	K_u	4.41×10^{-9}	0.001	1.92×10^{-10}	0.015	9.71×10^{-11}	0.012
Phenol	K_{500}	3.84×10^{-7}	0.853	4.40×10^{-10}	0.014	1.16×10^{-10}	0.048
	K_{1000}	5.90×10^{-7}	0.874	5.00×10^{-10}	0.017	3.60×10^{-11}	0.009
	K_d	1.10×10^{-8}	0.044	7.49×10^{-9}	0.088	1.69×10^{-9}	0.018
	K_u	3.52×10^{-9}	0.014	1.31×10^{-9}	0.015	3.27×10^{-10}	0.021

*K_{100}, K_{200}, K_{500} express the biotransformation rate constants, while K_d and K_u, respectively, are biodegradation rate constants from acidified residual ^{14}C-tagged chemical and $^{14}CO_2$ evolved.

Lake to 0.022 in Roselawn Pond. Plate count biotransformation rate constants varied from 2.18×10^{-7} in Roselawn Pond to 5.5×10^{-10} in Cross Lake, with biodegradation rate constants ranging from 6.6×10^{-8} in Roselawn Pond to 5.11×10^{-10} in Cross Lake.

Lindane ATP second-order biotransformation rate constants extended from 0.031 in Roselawn Pond to 0.011 in Indiana Quarry. The biodegradation range was 0.029 for Cross Lake to 0.002 for Roselawn Pond. The plate count biotransformation data varied from 1.56×10^{-8} in Indiana Quarry to 3.0×10^{-9} in Roselawn Pond. The corresponding biodegradation rate constants from plate count information varied from 9.88×10^{-9} for Roselawn Pond to 8.33×10^{-11} for Indiana Quarry.

Phenol ATP biotransformation rate constants showed a range of 0.874 in Roselawn Pond to 0.009 in Cross Lake, while the biodegradation rate constants extended from 0.088 in Indiana Quarry to 0.014 in Roselawn Pond. The phenol plate count biotransformation rate constant interval was 5.9×10^{-7} in Roselawn Pond to 3.6×10^{-11} in Cross Lake. The biodegradation data varied from 1.1×10^{-8} for Roselawn Pond to 3.27×10^{-10} for Cross Lake. There are few if any consistencies between the biotransformation and biodegradation second-order rate constants or between ATP and plate count data, let alone between the chemicals

themselves.

Tables 3-15, 3-16, and 3-17 show the corresponding first- and second-order rate constants for each chemical in each water source. As before, there are few or inconsistent correlations. Among the chemicals in each water, naphthalene nearly always has the fastest rate constants of either order. Lindane is generally the lowest, while phenol varies widely.

The more consistent nature of the naphthalene values in this investigation may be due to the relatively low experimental time (eight days for naphthalene compared to sixty days for lindane). Naphthalene was studied before lindane and phenol. Perhaps limiting levels of nutrients, decreased algae biomass, or chemical changes within the water containers may have occurred, causing microbial population of the stored Indiana Quarry and Cross Lake waters to decline or change. Roselawn Pond water was collected directly from the pond for each experiment.

Biotransformation and biodegradation coefficients of variation for the first- and second-order rate constants are found in Table 3-18. The coefficient of variation is a statistic that expresses sample variability relative to the mean of the sample. It is used to compare the amount of variation in populations having different means. The coefficient of variation values are generally lower with

TABLE 3-15

FIRST AND SECOND-ORDER BIOTRANSFORMATION AND BIODEGRADATION RATE CONSTANTS FOR ROSELAWN CEMETERY POND.

	Treatments					
Chemical	K_{100}	K_{200}	K_{500}	K_{1000}	K_d	K_u
Naphthalene						
1st-order[a]	0.253	0.179	0.183	*	0.218	0.046
2nd-order(PC)[b]	7.21×10^{-8}	1.02×10^{-7}	2.18×10^{-7}	*	6.60×10^{-8}	1.40×10^{-8}
2nd-order(ATP)[c]	0.120	0.085	0.087	*	0.103	0.022
Lindane						
1st-order	0.036	0.030	0.027	*	0.017	0.007
2nd-order(PC)	3.00×10^{-9}	3.48×10^{-9}	3.61×10^{-9}	*	9.88×10^{-9}	4.41×10^{-9}
2nd-order(ATP)	0.019	0.031	0.017	*	0.002	0.001
Phenol						
1st-order	*	*	1.151	1.180	0.082	0.026
2nd-order(PC)	*	*	3.84×10^{-7}	5.90×10^{-7}	1.10×10^{-8}	3.52×10^{-9}
2nd-order(ATP)	*	*	0.853	0.874	0.044	0.014

[a]First-order rate constant units are day^{-1}.

[b]PC signifies that the rate constant is derived from plate count data and is in L org^{-1} day^{-1}.

[c]ATP signifies that the rate constant is derived from ATP data and is in L ug^{-1} day^{-1}.

Treatment designations indicate initial chemical concentration, for biotransformation data, and method, for biodegradation data.

TABLE 3-16

FIRST AND SECOND-ORDER BIOTRANSFORMATION AND BIODEGRADATION RATE CONSTANTS FOR INDIANA QUARRY.

Chemical	Treatments					
	K_{100}	K_{200}	K_{500}	K_{1000}	K_d	K_u
Naphthalene						
1st-order[a]	0.144	0.102	0.118	*	0.151	0.032
2nd-order(PC)[b]	1.03×10^{-8}	2.05×10^{-8}	1.87×10^{-8}	*	4.39×10^{-9}	9.19×10^{-10}
2nd-order(ATP)[c]	0.124	0.065	0.981	*	0.143	0.030
Lindane						
1st-order	0.010	0.006	0.010	*	0.003	0.007
2nd-order(PC)	2.11×10^{-8}	1.56×10^{-8}	4.17×10^{-8}	*	8.33×10^{-11}	1.92×10^{-10}
2nd-order(ATP)	0.022	0.012	0.011	*	0.007	0.015
Phenol						
1st-order	*	*	0.004	0.005	0.075	0.013
2nd-order(PC)	*	*	4.40×10^{-10}	5.00×10^{-10}	7.49×10^{-9}	1.31×10^{-9}
2nd-order(ATP)	*	*	0.014	0.017	0.088	0.015

[a]First-order rate constant units are day^{-1}.

[b]PC signifies that the rate constant is derived from plate count data and is in L org^{-1} day^{-1}.

[c]ATP signifies that the rate constant is derived from plate count data and is in L ug^{-1} day^{-1}.

Treatment designations indicate initial chemical concentration, for biotransformation data, and method, for biodegradation data.

TABLE 3-17

FIRST AND SECOND-ORDER BIOTRANSFORMATION AND BIODEGRADATION RATE CONSTANTS FOR CROSS LAKE.

Chemical	Treatments					
	K_{100}	K_{200}	K_{500}	K_{1000}	K_d	K_u
Naphthalene						
1st-order[a]	0.171	0.116	0.127	*	0.052	0.032
2nd-order(PC)[b]	5.50x10-10	1.40x10-8	1.27x10-8	*	8.42x10-10	5.11x10-10
2nd-order(ATP)[c]	0.103	0.104	0.132	*	0.435	0.264
Lindane						
1st-order	0.007	0.007	0.013	*	0.008	0.003
2nd-order(PC)	4.29x10-9	7.32x10-9	2.48x10-8	*	2.32x10-10	9.71x10-11
2nd-order(ATP)	0.018	0.014	0.021	*	0.029	0.012
Phenol						
1st-order	*	*	0.009	0.003	0.126	0.024
2nd-order(PC)	*	*	1.16x10-10	3.60x10-11	1.69x10-9	3.27x10-10
2nd-order(ATP)	*	*	0.048	0.009	0.018	0.021

[a]First-order rate constant units are day^{-1}.

[b]PC signifies the rate constant is derived from plate count data and is in L org^{-1} day^{-1}.

[c]ATP signifies the rate constant is derived from ATP data and is in L ug^{-1} day^{-1}.

Treatment designations indicate initial chemical concentration, for biotransformation data, and method, for biodegradation data.

TABLE 3-18

BIOTRANSFORMATION AND BIODEGRADATION COEFFICIENTS OF VARIATION

FOR FIRST AND SECOND-ORDER RATE CONSTANTS

Units are percent

	FIRST ORDER				SECOND ORDER					
	Biotransformation		Biodegradation		Biotransformation			Biodegradation		
						CFU/L[a]	ugATP/L[b]		CFU/L	ugATP/L
Naphthalene	K_{100}	29.83	K_d	59.35	K_{100}	140.33	9.64	K_d	154.30	78.84
	K_{200}	30.88	K_u	22.80	K_{200}	107.78	23.03	K_u	149.18	130.51
	K_{500}	24.62			K_{500}	140.54	125.92			
Lindane	K_{100}	90.15	K_d	51.61	K_{100}	106.71	10.59	K_d	165.19	111.65
	K_{200}	95.80	K_u	54.80	K_{200}	70.39	54.95	K_u	157.25	78.98
	K_{500}	55.01			K_{500}	81.67	30.82			
Phenol	K_{500}	170.28	K_d	29.44	K_{500}	172.83	155.70	K_d	69.90	40.93
	K_{1000}	171.51	K_u	33.66	K_{1000}	172.97	165.71	K_u	143.48	22.72

[a]Second order rate constant derived from plate count data.

[b]Second order rate constant derived from ATP data.

K_{100}, K_{200}, K_{500}, and K_{1000} indicate initial chemical concentrations used in the biotransformation experiment.

K_d and K_u indicate primary and ultimate biodegradation respectively.

the first order rate constants for both biotransformation and biodegradation. An exception is some of the biotransformation data between first-order rate constants and their corresponding second-order rate constants which were derived from ATP data.

Table 3-18 illustrates that there is, in general, less variability connected to first-order rate constants, and that, therefore, these rates may be more reliable than those determined with microbial biomass estimates. Nesbitt and Watson (9) found no continuous relationship between 2,4-D biodegradation in water and the standard plate counts. They theorized that there may have been an inadequate availability of nutrients for microbial activity.

Larson and Payne (7) estimated microbial numbers in a biodegradation study by most-probable-number (MPN) techniques because they felt plate count methods would be imprecise and would not give accurate estimates of microbial biomass. Another study, comparing MPN techniques with plate counts for the enumeration of aromatic degraders, demonstrated an agreement between the two methods but added that the MPN process would be advantageous when used with potentially toxic chemicals which may inhibit visible colony growth, and for counting slow-growing organisms, as well as utilizing the spectrophotometric assay which can easily assess substrate

disappearance (3).

Boethling and Alexander (2) suggest that other circumstances, such as the chemical structure and concentration of the compound are important factors in the removal of chemicals from the environment, and that the biodegradation rate may be directly proportional to the substrate concentration where little or no biodegradation may occur at low concentrations.

Between the two bacterial biomass estimators, plate counts seem the least significant. While it appears likely that microbes do play an important role in the breakdown of chemicals in our environment, more investigation is warranted in the determination of accurate biomass estimation. The correlation of that information to their actual biodegradation and biotransformation activities are also needed in order to obtain reasonably accurate predictions of chemical behaviors in the environment.

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CHAPTER IV

CONCLUSIONS

In this investigation three hypothesis were examined, with the following conclusions:

1. H_o: There is no difference between the biotransformation rate constants and the biodegradation rate constants of naphthalene, lindane, and phenol, derived from three water sources.

The results indicate that the null hypothesis must be rejected. The alternate hypothesis, which states that there is a difference between the rate constants of biodegradation and biotransformation for these chemicals, is assumed. It is not too surprising that the chemicals themselves have different rate constants because chemical structure is known to be influential in the breakdown rate of a substance. Assuming an adequate substrate concentration, microbes probably transform molecules or sections of molecules that can be processed more easily before attacking the more difficult structures.

Since biotransformation does not necessarily go to the mineralization of the compound and evolution of CO_2, it

may not be expected to have the same rate constants as the biodegradation process. This study showed that biotransformation rates were often, but not always, more rapid than the corresponding biodegradation rate.

The distinction between biotransformation and biodegradation needs to be clear, especially among those interested in the fate of chemicals in the environment. Investigation of the two processes needs to continue, and the resulting information should be exchanged and considered, so that the most effective methods of analyzing the biotransformation and biodegradation of chemicals can be utilized and eventually standardized.

2. H_o: There is no difference in the biodegradation rate constants and the biotransformation rate constants of the three chemicals due to the water source and its intrinsic water quality.

As before, the results indicate that the null hypothesis must be rejected and the alternate hypothesis assumed. There appears to be a difference in the biotransformation and biodegradation of a chemical due to the type of water into which it is introduced.

During this experiment the basic physical parameters of temperature, container size and shape, mixing, and light were identical for the three water sources and chemicals. The specific chemical makeup of each water would be most

influential on its resident biota.

The eutrophic example, Roselawn Cemetery Pond, consistently yielded the highest rate constants, suggesting that it contained materials making it more conducive to supporting populations which are capable of transforming and degrading added chemical substrates. A greater availability of nutrients, concentration of the chemical, toxicity of the chemical, and presence of populations already acclimated to a chemical should be very important to the degradation rate.

That the mesotrophic water (Cross Lake) and the oligotrophic water (Indiana Quarry) often had similar results could be due to their long containment in metal barrels, which eventually produced similar water conditions. It is possible that they may have had somewhat similar water chemistry profiles to begin with.

A large database of information from many differing water systems, both lotic and lentic, from fresh-water, marine, and estuarine waters, and from temperate and tropical climates would be very useful. Chemical rate constants derived from such a variety should give a reasonable picture of the influence of each type of water source.

3. H_o: There is no difference in the biotransformation rate constants and the biodegradation

rate constants of the chemicals due to the microbial biomass.

With these data, it is not possible to accept or reject this null hypothesis with much confidence. While the plate count estimates showed very little real difference in the numbers over the whole experiment, the ATP measurements seemed to produce values more consistent with the trophic levels of the waters. However, when the second-order rate constant ranges were compared by plate count and ATP and by biodegration and biotransformation there was little or no pattern or consistent behavior detectable.

Biomass estimation methods that apply to some situations may not apply to others. In using rate constants that must be determined from biomass values, it makes little sense to compare those rate constants unless there is a reasonable correlation between the population estimates. This will entail the development of more precise and accurate methods of enumeration, especially for the non-cultured, naturally occurring populations present in waters being investigated.

APPENDIX

TABLE 5-1

DATA FROM BIODEGRADATION EXPERIMENTS

Chemicals N, L, and P are naphthalene, lindane, and phenol, respectively. Lakes R, I, and X are Roselawn Cemetery Pond, Indiana Quarry, and Cross Lake, respectively. Time is in days. Water data is from unacidified water samples. Acid data is from acidified water samples. Base data is from $^{14}CO_2$-evolved and trapped in the NaOH trap. Type E refers to experimental samples, CA refers to autoclaved controls, and CB refers to filtered and autoclaved controls. Type C in phenol data refers to autoclaved controls.

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
N	R	0	16329	16300	37	E
N	R	0	17062	16897	32	E
N	R	0	17097	16886	29	E
N	R	0	16874	16523	36	E
N	R	0	17251	16999	33	CA
N	R	0	17006	16823	33	CA
N	R	0	16955	16826	37	CB
N	R	0	16993	16797	29	E
N	R	1	13677	13069	234975	E
N	R	1	14032	13997	194060	E
N	R	1	12968	12645	184100	E
N	R	1	12879	12318	297460	CA
N	R	1	15624	14999	35	CA
N	R	1	16002	15606	37	CB
N	R	1	16321	14811	35	CB
N	R	1	15876	15114	278280	E
N	R	2	9718	8501	273116	E
N	R	2	8634	8209	185160	E
N	R	2	10133	9381	284540	E
N	R	2	10067	9597	36	CA
N	R	2	14837	13510	28	CA
N	P	2	14976	13582	30	CB
N	R	2	14635	13746	32	CB
N	R	5	6237	5876	153817	E
N	R	5	8105	6985	152874	E
N	R	5	6643	5851	169671	E
N	R	5	6024	5095	166537	E
N	R	5	12891	11961	37	CA
N	R	5	12002	11832	38	CA
N	R	5	12735	11994	32	CB
N	R	5	11969	11800	36	CB
N	R	8	3188	3015	152155	E
N	R	8	2969	2600	168172	E
N	R	8	2856	2713	166386	E
N	R	8	3223	2810	189294	E
N	R	8	10642	9897	37	CA
N	R	8	9986	9638	32	CA
N	R	8	10037	9209	33	CB

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
N	I	0	17241	17200	37	E
N	I	0	16376	16360	29	E
N	I	0	16892	16849	32	E
N	I	0	18122	18107	34	E
N	I	0	17226	17203	30	CA
N	I	0	17241	17005	29	CA
N	I	0	17682	17523	33	CB
N	I	0	16937	16869	33	CB
N	I	1	15230	15064	185017	E
N	I	1	15061	15006	190129	E
N	I	1	14923	14371	160332	E
N	I	1	16189	16055	181174	E
N	I	1	17024	16932	30	CA
N	I	1	16021	15829	36	CA
N	I	1	16835	16323	33	CB
N	I	1	15007	14892	53	CB
N	I	2	12329	11934	160201	E
N	I	2	13061	12097	171077	E
N	I	2	12646	11651	161928	E
N	I	2	13008	12293	163818	E
N	I	2	14172	13177	37	CA
N	I	2	15106	14660	27	CA
N	I	2	14687	13167	31	CB
N	I	2	13898	12596	39	CB
N	I	5	9274	8196	133961	E
N	I	5	8298	8075	127538	E
N	I	5	8937	8904	141195	E
N	I	5	8863	8653	125325	E
N	I	5	12632	11972	29	CA
N	I	5	13041	12066	183	CA
N	I	5	12517	12317	30	CB
N	I	5	11039	11000	28	CB
N	I	8	6318	6210	141372	E
N	I	8	5371	5097	131309	E
N	I	8	3899	3662	136372	E
N	I	8	6511	5371	114179	E
N	I	8	11672	10966	36	CA
N	I	8	10544	10329	38	CA
N	I	8	10379	10229	38	CB
N	I	8	11567	10875	34	CB

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
N	X	0	16020	15016	32	E
N	X	0	17123	16027	30	E
N	X	0	16119	16019	31	E
N	X	0	18106	17246	29	E
N	X	0	18100	18004	32	CA
N	X	0	16036	16041	36	CA
N	X	0	16732	16333	32	CB
N	X	0	17241	16967	31	CB
N	X	1	10748	10499	90617	E
N	X	1	11700	11002	110323	E
N	X	1	12686	11295	121298	E
N	X	1	11606	11951	106326	E
N	X	1	12174	11436	35	CA
N	X	1	13118	11555	35	CA
N	X	1	13146	11548	37	CB
N	X	1	12124	11162	32	CB
N	X	2	10573	317	103354	E
N	X	2	9415	855	113210	E
N	X	2	9470	509	103436	E
N	X	2	9289	617	103520	E
N	X	2	11733	11681	70	CA
N	X	2	12562	12526	66	CA
N	X	2	11183	10104	27	CB
N	X	2	13043	12156	29	CB
N	X	5	8407	8296	231640	E
N	X	5	8602	81170	203317	E
N	X	5	8594	8400	212135	E
N	X	5	11468	9632	158862	E
N	X	5	9520	9471	32	CA
N	X	5	8805	8379	82	CA
N	X	5	9904	9996	113	CB
N	X	5	9378	9300	39	CB
N	X	8	4304	4304	191128	E
N	X	8	3696	3674	163002	E
N	X	8	3474	3342	149014	E
N	X	8	3727	3648	130786	E
N	X	8	6594	6566	112	CA
N	X	8	5444	5423	230	CA
N	X	8	6292	6265	230	CB
N	X	8	5552	5476	42	CB

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
L	R	0	13756	13636	32	E
L	R	0	13601	13438	34	E
L	R	0	13832	13671	30	E
L	R	0	13719	13654	33	E
L	R	0	13004	12966	30	CA
L	R	0	13725	13521	31	CA
L	R	0	13823	13618	36	CB
L	R	0	13897	13604	30	CB
L	R	20	6836	6445	297441	E
L	R	20	6559	6057	187642	E
L	R	20	7959	8265	194663	E
L	R	20	8128	7821	192433	E
L	R	20	3633	3481	32	CA
L	R	20	6196	5691	359	CA
L	R	20	4546	4305	60	CB
L	R	20	4671	4585	54	CB
L	R	40	4938	5216	293167	E
L	R	40	4902	4768	194315	E
L	R	40	6338	6381	286061	E
L	R	40	6448	6862	184192	E
L	R	40	2177	2128	43	CA
L	R	40	3794	3750	53	CA
L	R	40	2899	2794	40	CB
L	R	40	3291	3184	117	CB
L	R	60	3108	3124	281716	E
L	R	60	3349	5287	283606	E
L	R	60	4859	6158	178080	E
L	R	60	4619	5098	281644	E
L	R	60	1751	1847	57	CA
L	R	60	2926	2941	71	CA
L	R	60	2223	2248	45	CB
L	R	60	2679	2708	167	CB

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
L	I	0	13732	13700	32	E
L	I	0	13868	13336	36	E
L	I	0	13631	13479	34	E
L	I	0	12967	12896	29	E
L	I	0	13076	12972	33	CA
L	I	0	13597	13264	32	CA
L	I	0	13962	13872	31	CB
L	I	0	13606	13563	34	CB
L	I	20	12128	11885	114087	E
L	I	20	12229	11450	101055	E
L	I	20	13661	12955	121786	E
L	I	20	13128	12603	105521	E
L	I	20	13009	12403	67	CA
L	I	20	2250	2072	35	CA
L	I	20	14496	14016	80	CB
L	I	20	15536	14222	124	CB
L	I	40	12098	12484	103184	E
L	I	40	9368	9572	114469	E
L	I	40	12187	12112	121322	E
L	I	40	11505	11236	106313	E
L	I	40	11175	11255	31	CA
L	I	40	2092	2054	33	CA
L	I	40	11269	13811	43	CB
L	I	40	12195	12746	46	CB
L	I	60	8585	8654	102639	E
L	I	60	8059	7863	109911	E
L	I	60	9024	9612	111670	E
L	I	60	8617	8611	113347	E
L	I	60	8348	8296	70	CA
L	I	60	2112	2724	55	CA
L	I	60	9151	9255	67	CB
L	I	60	10743	11398	58	CB

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
L	X	0	13652	13510	34	E
L	X	0	13881	13686	35	E
L	X	0	13097	13004	33	E
L	X	0	13605	13435	30	E
L	X	0	13761	13528	31	CA
L	X	0	13244	13106	34	CA
L	X	0	13377	13185	30	CB
L	X	0	13810	13633	33	CB
L	X	20	10360	10245	151773	E
L	X	20	10609	10645	109096	E
L	X	20	11615	11090	113847	E
L	X	20	11674	11365	110620	E
L	X	20	20311	19783	229	CA
L	X	20	11791	11801	225	CA
L	X	20	12668	12133	122	CB
L	X	20	12808	10777	31	CB
L	X	40	8668	8760	109404	E
L	X	40	8974	8721	172413	E
L	X	40	9888	9772	113576	E
L	X	40	9986	9697	106193	E
L	X	40	17562	16688	371	CA
L	X	40	10898	10714	86	CA
L	X	40	11088	11037	72	CB
L	X	40	9729	9708	59	CB
L	X	60	8354	9050	113182	E
L	X	60	7008	7521	124579	E
L	X	60	8375	8383	112266	E
L	X	60	8505	8487	133972	E
L	X	60	13650	13648	324	CA
L	X	60	9006	9069	101	CA
L	X	60	8798	8720	70	CB
L	X	60	7847	7874	47	CB

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
P	R	0	30017	27917	40	E
P	R	0	33780	33040	33	E
P	R	0	32040	31067	41	E
P	R	0	32960	31233	580	E
P	R	0	32520	34460	34	C
P	R	0	33920	33180	43	C
P	R	1	28383	28117	213875	E
P	R	1	24800	20162	214712	E
P	R	1	26667	27066	208965	E
P	R	1	26983	26728	230317	E
P	R	1	34660	32780	46	C
P	R	1	35500	33620	1596	C
P	R	2	25887	22739	315752	E
P	R	2	30967	22470	210497	E
P	R	2	23447	21043	209756	E
P	R	2	37557	25312	221714	E
P	R	2	35769	34428	694	C
P	R	2	34986	34212	1491	C
P	R	3	23672	20013	27301	E
P	R	3	27686	25926	1240	E
P	R	3	20762	16976	1013	E
P	R	3	23067	19968	24639	E
P	R	3	31692	27726	48	C
P	R	3	30821	25311	115	C
P	R	6	13904	13068	209706	E
P	R	6	24873	20626	300621	E
P	R	6	24606	21034	216722	E
P	R	6	19890	18647	272981	E
P	R	6	34595	33822	36	C
P	R	6	34442	32626	66	C
P	R	7	13673	13077	229318	E
P	R	7	19563	16698	191881	E
P	R	7	19872	17077	161233	E
P	R	7	21064	19891	160147	E
P	R	7	32617	30814	46	C
P	R	7	30821	29676	68	C
P	R	8	13342	13021	150619	E
P	R	8	16058	15068	117592	E
P	R	8	16193	15894	109675	E
P	R	8	13109	12952	116074	E
P	R	8	30174	29894	68	C
P	R	8	31622	29997	74	C

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
P	I	0	30950	31066	552	E
P	I	0	31150	30483	37	E
P	I	0	22900	29100	66	E
P	I	0	31250	32200	27	E
P	I	0	33380	33640	478	C
P	I	0	35140	33760	322	C
P	I	1	29729	27233	50475	E
P	I	1	27583	21563	72655	E
P	I	1	27721	26057	84331	E
P	I	1	25689	28183	75347	E
P	I	1	34856	34440	821	C
P	I	1	35068	34400	90	C
P	I	2	28835	28173	104553	E
P	I	2	27902	27761	113170	E
P	I	2	26457	26863	92441	E
P	I	2	24431	25778	40940	E
P	I	2	34264	34585	513	C
P	I	2	34677	34781	287	C
P	I	3	23736	21552	162390	E
P	I	3	21634	20072	47756	E
P	I	3	20898	19286	120912	E
P	I	3	21617	14836	92800	E
P	I	3	33886	33472	190	C
P	I	3	34391	34139	202	C
P	I	6	21716	20616	40689	E
P	I	6	1990.	18997	36231	E
P	I	6	15679	14820	34361	E
P	I	6	13954	13013	33586	E
P	I	6	33695	31672	82	C
P	I	6	34379	32809	104	C
P	I	7	23100	20103	16338	E
P	I	7	20291	19118	90643	E
P	I	7	15541	18302	120047	E
P	I	7	11962	15135	119037	E
P	I	7	34311	33358	2046	C
P	I	7	35388	33817	1463	C
P	I	8	20770	22466	179532	E
P	I	8	17511	17416	116529	E
P	I	8	13501	17175	103219	E
P	I	8	9716	10530	50628	E
P	I	8	33997	33726	3537	C
P	I	8	34344	35006	3902	C
P	I	9	18540	18765	59504	E
P	I	9	15374	15978	28067	E
P	I	9	13600	12466	21262	E
P	I	9	8189	7291	36027	E
P	I	9	33870	34243	2844	C
P	I	9	35410	35083	80	C
P	I	10	17840	20086	74872	E
P	I	10	15492	19037	13657	E
P	I	10	16848	16328	6463	E
P	I	10	5451	7730	45921	E
P	I	10	34572	34056	3217	C
P	I	10	35144	35072	291	C

Table 5-1 continued on next page.

TABLE 5-1 (continued)

CHEM	LAKE	TIME	WATER	ACID	BASE	TYPE
P	X	0	32040	33700	175	E
P	X	0	32940	33840	30	E
P	X	0	32660	33000	43	E
P	X	0	33920	34980	96	E
P	X	0	38100	45800	33	C
P	X	0	34620	31367	37	C
P	X	1	24818	16200	140800	E
P	X	1	25696	15609	265600	E
P	X	1	23421	21963	101649	E
P	X	1	33204	36080	28533	E
P	X	1	34777	33900	2113	C
P	X	1	34062	33140	43	C
P	X	2	25558	21780	140348	E
P	X	2	22102	21725	120148	E
P	X	2	35109	19899	106041	E
P	X	2	22785	19782	116737	E
P	X	2	33776	35026	612	C
P	X	2	34885	34186	58	C
P	X	3	26782	25631	262293	E
P	X	3	25321	24837	174256	E
P	X	3	20684	19079	144253	E
P	X	3	25891	24933	143751	E
P	X	3	30762	29042	498	C
P	X	3	30004	28976	124	C
P	X	6	10907	12809	149706	E
P	X	6	12955	14079	103621	E
P	X	6	13663	16076	197257	E
P	X	6	14687	18236	108925	E
P	X	6	34374	34233	86	C
P	X	6	33528	33791	29	C
P	X	7	6179	9730	101033	E
P	X	7	12778	12242	94109	E
P	X	7	12563	18181	91357	E
P	X	7	13712	13191	110210	E
P	X	7	33098	33373	1380	C
P	X	7	33230	33039	677	C
P	X	8	8780	9372	118876	E
P	X	8	11230	11051	98575	E
P	X	8	10388	20308	130994	E
P	X	8	13989	14099	188005	E
P	X	8	33416	33786	179	C
P	X	8	33583	33171	36	C
P	X	9	10917	8320	151987	E
P	X	9	9929	9519	204822	E
P	X	9	11889	9387	172584	E
P	X	9	15706	9411	261487	E
P	X	9	33830	33308	5369	C
P	X	9	33325	32979	2092	C
P	X	10	6736	5605	151720	E
P	X	10	8873	6681	241930	E
P	X	10	15830	8968	156640	E
P	X	10	8050	7526	153683	E
P	X	10	32825	32968	792	C
P	X	10	33527	32805	139	C

TABLE 5-2

DATA FROM BIOTRANSFORMATION EXPERIMENTS

Chemicals N, L, and P are naphthalene, lindane, and phenol, respectively. Lakes R, I, and X are Roselawn Cemetery Pond, Indiana Quarry, and Cross Lake, respectively. Time is is in days. Controls are associated with their initial chemical concentration and are autoclaved (CA) and filtered and autoclaved (CB). Experimental data are C, CC, V, and M which designate initial chemical concentrations of 100ug/L, 200ug/L, 500ug/L, and 1000ug/L, respectively.

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
N	R	0	100	200	500	100	95	195	500	95	200	495
N	R	0	100	200	500	100	95	195	500	95	195	495
N	R	0	100	200	500	100	95	195	500	97	200	500
N	R	1	98	195	450	98	90	195	490	87	195	495
N	R	1	98	195	450	98	90	195	490	83	195	470
N	R	1	98	195	450	98	90	195	490	89	195	450
N	R	2	96	188	440	96	90	185	480	89	180	430
N	R	2	96	188	440	96	90	185	480	88	160	430
N	R	2	96	188	440	96	90	185	480	83	140	430
N	R	5	56	115	106	56	45	127	152	28	114	242
N	R	5	56	115	106	56	45	127	152	34	87	155
N	R	5	56	115	106	56	45	127	152	23	60	99
N	R	8	38	110	103	38	38	110	104	15	60	121
N	R	8	38	110	103	38	38	110	104	12	49	193
N	R	8	38	110	103	38	38	110	104	19	48	123

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
N	I	0	100	200	500	100	100	200	500	100	200	500
N	I	0	100	200	500	100	100	200	500	100	198	500
N	I	0	100	200	500	100	100	200	500	97	200	500
N	I	1	87	170	423	87	86	168	436	80	160	413
N	I	1	87	170	423	87	86	168	436	82	141	393
N	I	1	87	170	423	87	86	168	436	86	154	405
N	I	2	70	151	208	70	78	154	256	61	143	286
N	I	2	70	151	208	70	78	154	256	65	147	334
N	I	2	70	151	208	70	78	154	256	61	139	341
N	I	5	39	114	216	39	39	114	216	39	102	215
N	I	5	39	114	216	39	39	114	216	41	113	209
N	I	5	39	114	216	39	39	114	216	39	102	208
N	I	8	35	98	217	35	36	98	220	35	92	194
N	I	8	35	98	217	35	36	98	220	30	68	208
N	I	8	35	98	217	35	36	98	220	30	92	194

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
N	X	0	100	200	500	100	100	200	500	100	200	495
N	X	0	100	200	500	100	100	200	500	98	200	495
N	X	0	100	200	500	100	100	200	500	98	200	495
N	X	1	82	178	420	82	84	178	440	77	140	406
N	X	1	82	178	420	82	84	178	440	77	180	326
N	X	1	82	178	420	82	84	178	440	77	172	372
N	X	2	74	160	376	74	76	162	377	70	160	375
N	X	2	74	160	376	74	76	162	377	64	148	270
N	X	2	74	160	376	74	76	162	377	59	154	377
N	X	5	53	127	295	53	54	127	295	43	126	281
N	X	5	53	127	295	53	54	127	295	53	117	275
N	X	5	53	127	295	53	54	127	295	43	120	274
N	X	8	36	86	194	36	36	86	194	23	71	179
N	X	8	36	86	194	36	36	86	194	23	78	147
N	X	8	36	86	194	36	36	86	194	23	71	147

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
L	R	0	100	200	500	100	100	200	500	100	200	500
L	R	0	100	200	500	100	100	200	500	100	200	500
L	R	0	100	200	500	100	100	200	500	100	200	500
L	R	0	100	200	500	100	100	200	500	100	200	500
L	R	20	95	190	450	95	95	190	450	50	100	260
L	R	20	95	190	450	95	95	190	450	45	110	280
L	R	20	95	190	450	95	95	190	450	47	105	240
L	R	20	95	190	450	95	95	190	450	55	110	200
L	R	40	90	180	425	90	90	180	425	50	100	200
L	R	40	90	180	425	90	90	180	425	53	100	200
L	R	40	90	180	425	90	90	180	425	50	100	190
L	R	40	90	180	425	90	90	180	425	50	100	210
L	R	60	80	160	410	80	80	160	410	10	20	90
L	R	60	80	160	410	80	80	160	410	8	40	80
L	R	60	80	160	410	80	80	160	410	8	25	90
L	R	60	80	160	410	80	80	160	410	10	30	90

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
L	I	0	100	200	500	100	100	200	500	100	200	500
L	I	0	100	200	500	100	100	200	500	100	200	500
L	I	0	100	200	500	100	100	200	500	100	200	500
L	I	0	100	200	500	100	100	200	500	100	200	500
L	I	20	94	195	450	94	92	195	450	80	190	400
L	I	20	94	195	450	94	92	195	450	85	180	390
L	I	20	94	195	450	94	92	195	450	88	180	360
L	I	20	94	195	450	94	92	195	450	80	180	390
L	I	40	90	190	410	90	90	190	410	70	150	.
L	I	40	90	190	410	90	90	190	410	80	150	360
L	I	40	90	190	410	90	90	190	410	72	155	360
L	I	40	90	190	410	90	90	190	410	68	158	350
L	I	60	80	175	400	80	80	180	400	62	150	260
L	I	60	80	175	400	80	80	180	400	60	140	240
L	I	60	80	175	400	80	80	180	400	58	140	280
L	I	60	80	175	400	80	80	180	400	50	140	280

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
L	X	0	100	200	500	100	100	200	500	100	200	500
L	X	0	100	200	500	100	100	200	500	100	200	500
L	X	0	100	200	500	100	100	200	500	100	200	500
L	X	0	100	200	500	100	100	200	500	100	200	500
L	X	20	95	190	450	95	95	190	450	90	188	320
L	X	20	95	190	450	95	95	190	450	92	190	330
L	X	20	95	190	450	95	95	190	450	86	194	350
L	X	20	95	190	450	95	95	190	450	90	186	310
L	X	40	90	180	425	90	90	180	440	80	150	250
L	X	40	90	180	425	90	90	180	440	70	170	240
L	X	40	90	180	425	90	90	180	440	80	150	200
L	X	40	90	180	425	90	90	180	440	75	150	280
L	X	60	80	160	410	80	80	160	400	70	140	240
L	X	60	80	160	410	80	80	160	400	60	140	230
L	X	60	80	160	410	80	80	160	400	65	130	230
L	X	60	80	160	410	80	80	160	400	65	120	235

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
P	R	0	.	.	500	.	.	.	.	.	.	500
P	R	0	.	.	500	.	.	.	.	.	.	500
P	R	0	.	.	500	.	.	.	.	.	.	500
P	R	0	.	.	500	.	.	.	.	.	.	500
P	R	1	.	.	500	.	.	.	.	.	.	500
P	R	1	.	.	500	.	.	.	.	.	.	500
P	R	1	.	.	500	.	.	.	.	.	.	500
P	R	1	.	.	500	.	.	.	.	.	.	500
P	R	2	.	.	490	.	.	.	.	.	.	440
P	R	2	.	.	490	.	.	.	.	.	.	460
P	R	2	.	.	490	.	.	.	.	.	.	450
P	R	2	.	.	490	.	.	.	.	.	.	460
P	R	3	.	.	480	.	.	.	.	.	.	1
P	R	3	.	.	480	.	.	.	.	.	.	300
P	R	3	.	.	480	.	.	.	.	.	.	205
P	R	3	.	.	480	.	.	.	.	.	.	1
P	R	6	.	.	470	.	.	.	.	.	.	1
P	R	6	.	.	470	.	.	.	.	.	.	1
P	R	6	.	.	470	.	.	.	.	.	.	1
P	R	6	.	.	470	.	.	.	.	.	.	1

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
P	I	0	.	.	500	.	.	.	.	.	.	500
P	I	0	.	.	500	.	.	.	.	.	.	500
P	I	0	.	.	500	.	.	.	.	.	.	500
P	I	1	.	.	500	.	.	.	.	.	.	500
P	I	1	.	.	500	.	.	.	.	.	.	500
P	I	1	.	.	500	.	.	.	.	.	.	500
P	I	1	.	.	500	.	.	.	.	.	.	500
P	I	2	.	.	500	.	.	.	.	.	.	490
P	I	2	.	.	500	.	.	.	.	.	.	490
P	I	2	.	.	500	.	.	.	.	.	.	490
P	I	2	.	.	500	.	.	.	.	.	.	490
P	I	3	.	.	500	.	.	.	.	.	.	480
P	I	3	.	.	500	.	.	.	.	.	.	490
P	I	3	.	.	500	.	.	.	.	.	.	490
P	I	3	.	.	500	.	.	.	.	.	.	495
P	I	6	.	.	495	.	.	.	.	.	.	490
P	I	6	.	.	495	.	.	.	.	.	.	480
P	I	6	.	.	495	.	.	.	.	.	.	490
P	I	6	.	.	495	.	.	.	.	.	.	485
P	I	7	.	.	495	.	.	.	.	.	.	485
P	I	7	.	.	495	.	.	.	.	.	.	485
P	I	7	.	.	495	.	.	.	.	.	.	485
P	I	7	.	.	495	.	.	.	.	.	.	485
P	I	8	.	.	495	.	.	.	.	.	.	495
P	I	8	.	.	495	.	.	.	.	.	.	485
P	I	8	.	.	495	.	.	.	.	.	.	490
P	I	8	.	.	495	.	.	.	.	.	.	485
P	I	9	.	.	495	.	.	.	.	.	.	475
P	I	9	.	.	495	.	.	.	.	.	.	480
P	I	9	.	.	495	.	.	.	.	.	.	480
P	I	9	.	.	495	.	.	.	.	.	.	480
P	I	10	.	.	495	.	.	.	.	.	.	480
P	I	10	.	.	495	.	.	.	.	.	.	480
P	I	10	.	.	495	.	.	.	.	.	.	470
P	I	10	.	.	495	.	.	.	.	.	.	470

Table 5-2 continued on next page.

TABLE 5-2 (continued)

CHEM	LAKE	TIME	CA100	CA200	CA500	CA100	CB100	CB200	CB500	C	CC	V
P	X	0	.	.	500	.	.	.	.	.	.	500
P	X	0	.	.	500	.	.	.	.	.	.	500
P	X	0	.	.	500	.	.	.	.	.	.	500
P	X	0	.	.	500	.	.	.	.	.	.	500
P	X	0	.	.	500	.	.	.	.	.	.	500
P	X	1	.	.	500	.	.	.	.	.	.	495
P	X	1	.	.	500	.	.	.	.	.	.	495
P	X	1	.	.	500	.	.	.	.	.	.	500
P	X	1	.	.	500	.	.	.	.	.	.	500
P	X	2	.	.	499	.	.	.	.	.	.	495
P	X	2	.	.	499	.	.	.	.	.	.	495
P	X	2	.	.	499	.	.	.	.	.	.	495
P	X	2	.	.	499	.	.	.	.	.	.	495
P	X	3	.	.	495	.	.	.	.	.	.	490
P	X	3	.	.	495	.	.	.	.	.	.	480
P	X	3	.	.	495	.	.	.	.	.	.	480
P	X	3	.	.	495	.	.	.	.	.	.	475
P	X	6	.	.	490	.	.	.	.	.	.	460
P	X	6	.	.	490	.	.	.	.	.	.	470
P	X	6	.	.	490	.	.	.	.	.	.	470
P	X	6	.	.	490	.	.	.	.	.	.	465
P	X	7	.	.	490	.	.	.	.	.	.	470
P	X	7	.	.	490	.	.	.	.	.	.	465
P	X	7	.	.	490	.	.	.	.	.	.	470
P	X	7	.	.	490	.	.	.	.	.	.	470
P	X	8	.	.	490	.	.	.	.	.	.	470
P	X	8	.	.	490	.	.	.	.	.	.	470
P	X	8	.	.	490	.	.	.	.	.	.	470
P	X	9	.	.	485	.	.	.	.	.	.	470
P	X	9	.	.	485	.	.	.	.	.	.	470
P	X	9	.	.	485	.	.	.	.	.	.	470
P	X	9	.	.	485	.	.	.	.	.	.	465
P	X	10	.	.	485	.	.	.	.	.	.	460
P	X	10	.	.	485	.	.	.	.	.	.	450
P	X	10	.	.	485	.	.	.	.	.	.	460
P	X	10	.	.	485	.	.	.	.	.	.	450

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