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ENVIRONMENTAL PATHWAYS OF SELECTED CHEMICALS IN FRESHWATER SYSTEMS
Part I: Background and Experimental Procedures

by

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FOREWORD

Environmental protection efforts are increasingly directed towards prevention of adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Processes Branch studies the microbiological, chemical, and physico-chemical processes that control the transport, transformation, and impact of pollutants in soil and water.

Delineation of the environmental pathways followed by aquatic pollutants is a key element in assessing the effects of low concentrations of pollutants. Based on concepts developed over a number of years at this laboratory, the extramural work reported here provides an approach that can be used to predict potential exposure of aquatic organisms.

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ABSTRACT

This research program was initiated to develop environmental exposure assessment procedures that can be used to predict the pathways of potentially harmful chemicals in freshwater environments.

The fundamental premises on which the environmental exposure assessment approach is based are that (1) the overall rate of disappearance of a chemical from the aquatic environment is controlled only by the dominant transformation and transport processes, (2) these processes can be studied independently in the laboratory, and (3) the laboratory data can be extrapolated to environmental conditions.

Laboratory procedures have been developed for measuring the rates of volatilization, photolysis, oxidation, hydrolysis, and biotransformations as well as the sorption partition coefficients on natural sediments and on a mixture of four bacteria. Two models have been used to extrapolate the laboratory results to the environment. The one-compartment model assumes that the aquatic system is a single, well-mixed reactor in which chemicals are transformed, degraded, and/or transported. It can be used to analyze acute discharges such as spills and to establish priorities for in-depth laboratory studies. The nine-compartment computer model is used to study the effect of the transport and transformation processes studied in the laboratory program on the distribution of a chemical in ponds, streams, and eutrophic and oligotrophic lakes.

This report is Part I of a two-part report and describes the environmental exposure assessment models and the laboratory procedures. Part II will report the results of using these procedures to study eleven chemicals: p-cresol, benz[a]anthracene, benzo[a]pyrene, quinoline, benzo[f]quinoline, 9H-carbazole, 7H-dibenzo[c,g]carbazole, benzo[b]thiophene, dibenzothiophene, methyl parathion, and mirex.

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ABBREVIATIONS AND SYMBOLS

AA 4,4'-Azobis(4-cyanovaleric acid)

H Henry's law constant

I_o Light flux (photons $time^{-1}$ $liter^{-1}$)

K_D Partition coefficient for sorption on biota

K_p Partition coefficient for sorption on sediments

K_s Concentration of substrate at which $\mu = \frac{1}{2} \mu_m$ (mass ml^{-1})

M Moles $liter^{-1}$

M_{Bi} Mass of chemical in biota in compartment i

$M_{\ell i}$ Total mass of substrate in aqueous phase (ℓ) of compartment i before sorption

$M'_{\ell i}$ Total mass of substrate in aqueous phase (ℓ) of compartment i after sorption

M_s Mass of suspended sediment

M_{Si} Total mass of substrate in the suspended sediment of compartment i before sorption

M'_{Si} Total mass of substrate in the suspended sediment of compartment i after sorption

M_w Mass of water

P Vapor pressure of pure substrate (torr)

R Gas constant

S Substrate concentration (mass per unit volume)

[S] Substrate concentration (moles per liter)

$S_{\ell i}$ Substrate concentration in the aqueous phase of compartment i

T Temperature (°K)

V_i Volume of compartment i

X Bacterial mass or cell count (cells ml^{-1})*

X_i Microbial population in compartment i (cells ml^{-1})

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*Cell count was used in biokinetic studies and biomass was used in biosorptions.

Y Biomass or cell yield per mass of substrate utilized (cells μg substrate)

Z_λ Solar radiance intensity (photons cm^{-3} sec^{-1} nm^{-1})

e Efficiency of production of $RO_2\cdot$ from AA

f_{ei} Total transformation rate in the aqueous phase in compartment i

k_A Rate constant for acid-catalyzed hydrolysis (M^{-1} sec^{-1})

k_B Rate constant for base-catalyzed hydrolysis (M^{-1} sec^{-1})

k_N Rate constant for neutral hydrolysis (sec^{-1})

k_a First-order rate constant for light absorption by chemical (sec^{-1})

k_b Rate constant for biodegradation (μg $cell^{-1}$ hr^{-1})

k_b' Pseudo-first-order rate constant for biodegradation (hr^{-1})

k_{b2} Second-order rate constants for biodegradation (ml $cell^{-1}$ hr^{-1})

k_h Rate constant for hydrolysis (sec^{-1})

k_1 Rate constant for decomposition of AA

k_{ox} Rate constant for oxidation (M^{-1} $time^{-1}$)

k_p Rate constant for photolysis ($time^{-1}$)

k_v^O Oxygen reaeration rate ($time^{-1}$)

k_v^S Rate constant for volatilization (μg ml^{-1} $time^{-1}$)

m_{bi} Mass of biota in compartment i

M_{wi} Mass of water in compartment i

r_b Biodegradation rate (μg ml^{-1} $time^{-1}$)

r_h Hydrolysis rate (μg ml^{-1} $time^{-1}$)

r_o Oxidation rate (μg ml^{-1} $time^{-1}$)

r_p Photolysis rate (μg ml^{-1} $time^{-1}$)

r_v Volatilization rate (μg ml^{-1} $time^{-1}$)

$t_{1/2}$ Half-life (time)

ε Absorption coefficient (M cm^{-1})

λ Wavelength (nm)

λ_{max} Wavelength of an absorption maximum (nm)

μ Specific growth rate (hr^{-1})

μ_m μ_{max} = Maximum specific growth rate (hr^{-1})

ϕ Reaction quantum yield

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1. INTRODUCTION

This study was designed to develop objective, well-documented procedures that can be used to predict the pathways of potentially harmful chemicals in freshwater systems before extensive damage occurs or major investments are made in production facilities. Although either field or laboratory studies might provide the data necessary for environmental assessment, field experiments are limited to those chemicals already present in the aquatic environment and are costly because of the large number of samples that must be collected and analyzed. Laboratory studies, on the other hand, are relatively inexpensive and more easily controlled, and the results are potentially more amenable to generalization to different environmental conditions.

Two important laboratory approaches that are now under development are: the microcosm or ecosystem study (Isensee et al., 1973; Metcalf et al., 1971; Taub, 1973) and the integration of independent transformation and transport processes (Wolfe et al, 1976; Paris et al., 1975; Hill et al., 1976). The use of microcosms can provide an overall assessment of complex interactions in a specific environment, but provides little or no basis for extrapolating the results to other kinds of environments because the relative rates of many of the component processes cannot be determined.

We have used the second approach, which we call environmental exposure analysis. Many of the concepts of this approach were first suggested to us by the staff of the Athens Environmental Research Laboratory. The approach uses the results of laboratory measurements of specific physical, chemical, and biological processes in a computer model that integrates the data with hydrologic parameters of selected aquatic systems. This approach can provide information on environmental exposure in many kinds of aquatic environments.

This study was designed to achieve three objectives:

- Develop laboratory procedures for a general environmental exposure analysis of a chemical, based on measurements of the rates of physical, chemical, and microbiological transformations believed to be important for that chemical in natural freshwater ecosystems.

- Develop an integration procedure for extrapolating the laboratory data to a variety of natural waters.

- Demonstrate the procedures using a series of selected organic chemicals.

Part I of this report describes the theory and methods of the laboratory measurements and computer modeling used for environmental exposure assessment.

Part II describes the results of specific laboratory and modeling studies with eleven organic chemicals selected for this program.

The scope of the environmental assessment was limited to transport and transformation processes that might occur under steady-state environmental exposure, such as would result from the continued release from manufacturing plants, agricultural field runoff, or desorption from contaminated sediments. Laboratory experiments were performed on homogeneous water solutions of selected chemicals below their solubility limits (usually at less than 1 μg ml^{-1}) to measure their rates of volatilization, oxidation, hydrolysis, photolysis, and microbiological transformations using adapted mixed cultures, as well as their partition coefficients for sorption to sediments and biomass, under conditions representative of, or extrapolatable to, freshwater aquatic systems. The results of the laboratory studies were integrated with simple one- and nine-compartment computer models to predict the pathways of the chemicals in ponds, streams, and lakes (see Part II).

A potential shortcoming of this approach to environmental assessment is that it may not measure important transformation or transport processes that occur in a natural aquatic system. To minimize this possibility, we have compared idealized laboratory experiments in pure water with experiments using natural sediments and waters. Nonetheless, the possibility for more complex interactions in natural systems does exist, and, if they occur, could lead to incorrect estimates of persistence, distribution, and pathways.

Three potentially important pathways were deliberately omitted: chemical and biochemical transformations that might take place in or on sediments, biodegradation by bacteria not obtained in mixed culture systems by enrichment procedures or by microorganisms other than bacteria, and biomagnification. The effect of these omissions will be discussed in specific sections of Part II, but we believe that these omissions will probably not significantly affect the general conclusions.

Figure 1.1 shows the sequence of the various phases of this study, beginning with selection of the chemicals, followed by literature review, laboratory programs, and environmental assessment. Eleven organic chemicals were selected for study. Nine of these were aromatic compounds typical of those likely to be found in effluent streams from fossil fuel processing plants. These compounds are p-cresol, benz[a]anthracene, benzo[a]pyrene, quinoline, benzo[f]quinoline, 9H-carbazole, 7H-dibenzo[c,g,]carbazole, benzo[b]thiophene, and dibenzothiophene. The two other compounds, methyl parathion and mirex, are pesticides that have been used extensively in field applications where runoff to streams and ponds is likely. In most cases, the literature data on these compounds were insufficient to allow us to decide which environmental processes might be important for detailed study. Therefore, screening studies were conducted to obtain an estimate of the relative importance of each process. Pathways that appeared to be important were studied in detail to obtain rate data and to identify products. To maximize the amount of relevant data produced and minimize the cost, processes that did not appear to be significant were not carried past the screening stage.

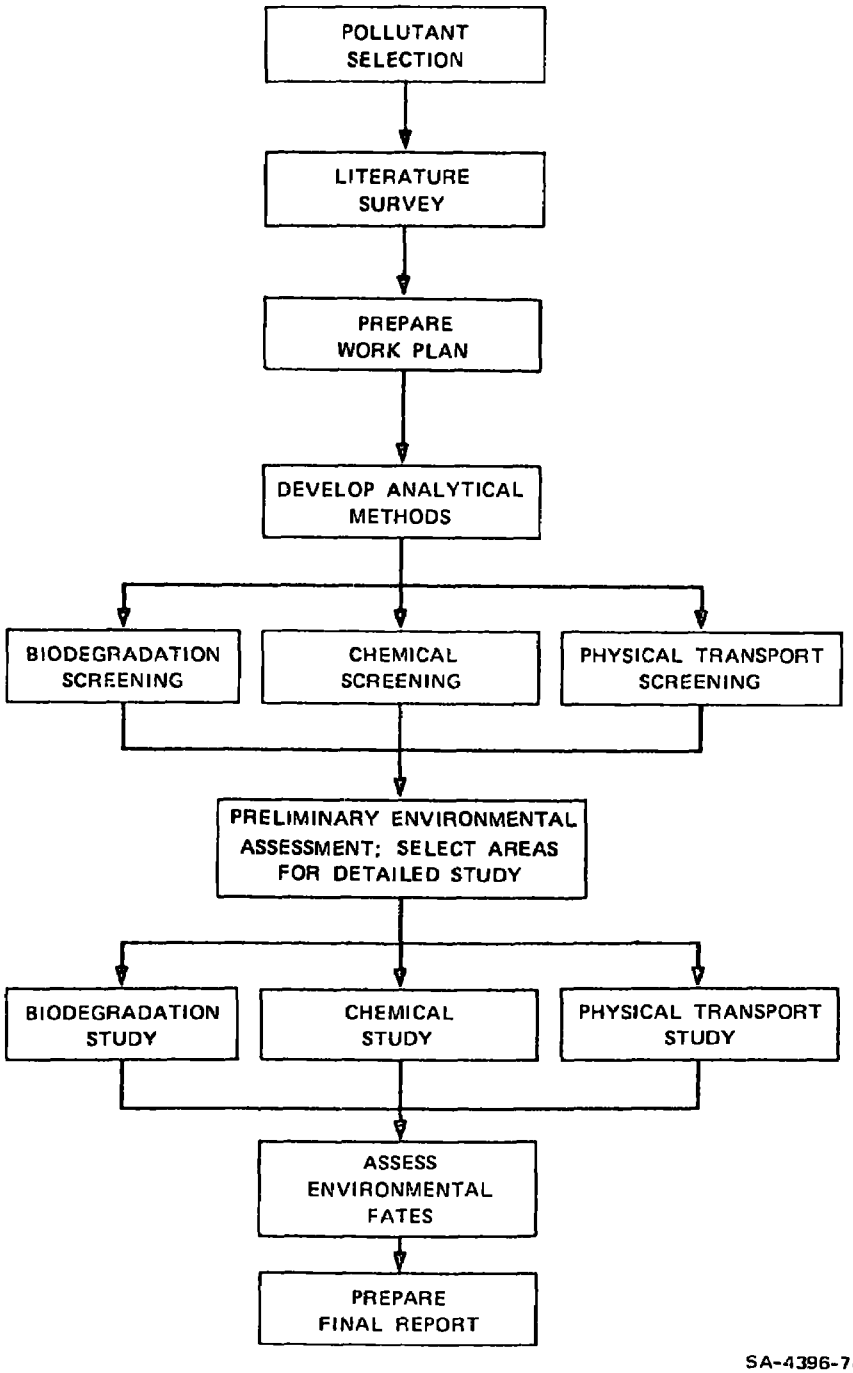

FIGURE 1.1 FLOWCHART FOR TECHNICAL APPROACH

Brief descriptions of the program elements shown in Figure 1.1 are listed below. Details are given in the sections noted in parentheses.

- Screening studies were designed to measure:

  -- Solubility in water at 20 to 25°C (Section 5.1).

  -- Absorption spectra at wavelengths greater than 290 nm (Section 5.2).

  -- Volatilization rates under high turbulence conditions (Section 5.3).

  -- Sorption partition coefficients (Section 5.4) for a Ca-montmorillonite clay, one high organic content natural sediment, and a mixture of four species of bacteria.

  -- Photolysis rates in sunlight and monochromatic light above 300 nm (Section 6.2).

  -- Oxidation rates in air-saturated water using a free-radical initiator at 50°C (Section 6.3).

  -- Hydrolysis rate at constant pH and temperature (Section 6.4).

  --Biodegradation susceptibility, by attempting to develop within 6 weeks enrichment cultures that would degrade substrate when it was the sole carbon source (Section 7.2).

- Preliminary assessments, using a one-compartment model with rate constants based on screening studies, were used to decide which processes should be studied in detail (Section 4.1).

- Detailed studies were designed to:

  -- Measure volatilization rates under several low turbulence conditions (Section 5.3).

  -- Measure sorption partition coefficients on additional natural sediments (Section 5.4).

  -- Measure photolysis rates and quantum yield in pure water and in natural waters and identify major products (Section 6.2).

  -- Measure oxidation rates and identify major oxidation products (Section 6.3).

  -- Measure hydrolysis rates at several temperatures and over a pH range 3 to 10 and identify major hydrolysis products (Section 6.4).

  -- Measure biodegradation rates and identify major metabolites (Section 7.3).

- Final environmental assessments were made with the one-compartment model and the nine-compartment computer model.

  -- The one-compartment model was used to refine the preliminary assessments, using rate constants obtained in detailed studies. This assessment provided a comparison of the relative transformation rates of the substrate under different aquatic conditions (Section 4.1).

  -- The nine-compartment computer model* was used to predict in detail the transport, distribution, and steady-state concentrations in four representative aquatic environments (Section 4.2).

The following chapters present and evaluate the laboratory procedures used in this study and the procedures for integrating laboratory measurements. These evaluations are important in the application of the methods and conclusions of the assessment and must be considered in any critical evaluation of the environmental exposure analysis.

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* The number of compartments can be increased to 99 if necessary. Individual compartments represent various parts of the water column and sediment layer of a representative pond, lake, or river.

2. CONCLUSIONS

1. The approach described in this report is a simple, a priori method for evaluating many of the possible environmental pathways of chemical pollutants in natural aquatic environments. This technique can provide useful predictions of the potential environmental exposure of chemicals in freshwater systems before they are introduced into the environment.

2. Calculations based on first-order kinetics and a homogeneous water body are useful for rapid assessment of laboratory screening studies and can be used to estimate both the volatilization and transformation rates of the substrate in solution and the importance of sorption by sediments following spills or long-term exposure.

3. The nine-compartment computer model developed during this study for use in extrapolating laboratory data to typical water bodies can predict the persistence, distribution, and pathways of chemicals in ponds, lakes, and streams. This model sacrifices the simplicity of the single compartment model for realism, but it is still much simpler than many of the computer models now in use and it allows the users to adjust parameters selectively to conform to their best judgment or knowledge.

4. Laboratory procedures have been developed to measure the rate constants for volatilization, photolysis, oxidation, hydrolysis, and biodegradation and the sorption partition coefficients on natural sediments and bacteria at substrate concentrations from 0.1 to 1000 ng ml^{-1}. The procedures have been designed so that the rate constants can be extrapolated to the environmental conditions simulated by the one-compartment model and nine-compartment computer models.

3. RECOMMENDATIONS

The work done under this program has laid the basis for further work in this field. We have developed the following recommendations as a result of our experience in developing an environmental exposure assessment model.

1. Verify this environmental exposure assessment model by comparing the predicted pollutant concentrations with those measured in an ecosystem and, if possible, in the field.

2. Develop procedures for measuring and expressing the rates of biodegradation and chemical transformations of substrates sorbed on sediments. These processes should be included in the environmental exposure assessment model.

3. Investigate further the observation that natural waters and humic acid had varying effects on the photolysis rates of different substrates. These investigations could focus on the role of natural substances as photosensitizers, quenchers, and free radical photoinitiators as well as on how other natural waters affect the reaction rates and products of specific substrates.

4. Intensify efforts to identify the major products of chemical transformation and the metabolites from the biodegradations. The latter may require changes in fermentation schedules, use of cell-free enzyme systems, or use of mutated organisms. The toxic properties of these products and metabolites should be measured, perhaps by the B. Ames mutagenic assay, by unscheduled DNA synthesis, or by animal culture systems.

5. Continue work on refinement of the nine-compartment model and the laboratory procedures by incorporating the following recommendations:*

   a. Expand the usefulness of the nine-compartment model by making the following modifications:

      - Develop sets of input data that define compartment size, flow rates, microbial populations, and the like for a larger variety of water bodies to make it easier for nonspecialists to use the model.

      - Introduce equations describing interactions among phenomena such as pH, temperature, light intensity, and turbidity to allow more realistic and sophisticated simulations.

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* These refinements have been listed in roughly the order of discussion in the report.

b. Obtain additional data concerning the relationship of substrate solubility and n-octanol partition coefficient and sediment organic content to the sorption partition coefficients on natural sediments. These correlations may provide simpler techniques for estimating partition coefficients for some types of compounds under different environmental conditions.

c. Develop improved experimental procedures for measuring volatilization rates of very volatile materials under conditions of low turbulence.

d. Use radiolabelled substrates for measurement of sorption partition coefficients, biodegradation rates, and metabolites to improve the precision of the analytical techniques at very low, environmentally realistic concentrations.

e. Increase the variety of organisms in conducting biosorptions, including phytoplankton and protozoa. Selected protozoa could be added after a predetermined sorption period, and the viability of the protozoa could serve as an indicator of potential biomagnification dangers of a pollutant and its metabolites.

f. Develop refinements to the procedures described in this report to increase the likelihood of obtaining cultures capable of degrading recalcitrant substrates:

- Increase the number of locations for sampling and take samples during different seasons.

- Maintain incubation temperatures at the temperature of the sample at the time of sampling.

- With recalcitrant compounds, conduct some screening with pure cultures or with young enrichment cultures isolated from aquatic sources. Analog enrichment procedures could be used in the isolation of these cultures, and fermentation conditions could be somewhat different from those existing in the environment.

- Replace the buffering salts used in the enrichment culture procedure by automatic addition of alkali or CO_2 to more realistically simulate environmental conditions.

4. ENVIRONMENTAL ASSESSMENT

The objectives of the environmental assessment were to:

- Estimate the probable concentrations and distributions of selected chemicals in aquatic systems resulting from continuous discharge of low concentrations of these chemicals in industrail waste and surface runoff.

- Assess the relative importance of photolysis, hydrolysis, oxidation, volatilization, sorption, and biotransformation in the removal of selected chemicals from solution in natural freshwater systems, exclusive of transfer through food chains or transformation on sediments.

To accomplish these objectives, it was necessary to make two fundamental assumptions concerning the various possible environmental transport and transformation processes in aquatic systems. These are:

- The overall rate of disappearance of a pollutant from solution is controlled only by the transformation and transport processes that were studied separately in the laboratory and by hydraulic and hydrological processes of the aquatic systems.

- Each transformation and transport process can be studied independently by laboratory experiments, and the results of these experiments can be extrapolated to natural waters.

On the basis of these assumptions, laboratory procedures were used to acquire data for discrete physical, chemical, and biological processes that are believed to be important in aquatic systems by using solutions of the selected chemicals in pure water below their solubility limits. The data from laboratory studies were integrated by a computer model that can simulate streams, ponds, and stratified lakes by suitable combinations of compartments and hydrologic parameters.

In its simplest application, the model is fixed as one compartment, and all data on transport and transformation processes are put in the form of simple first-order relations in which only the concentration of the chemical is a variable. Rate constants and reactive environmental intermediates are lumped together as constants typical of a specific water body. This simple and preliminary assessment provides a good method of evaluating the relative importance of different transformation and transport processes and thereby eliminating additional laboratory studies on subordinate processes.

More elaborate analyses, based on our multicompartment computer model, which allows for the heterogeneity of actual water bodies, give somewhat greater accuracy and greatly facilitate computation of the pollutant concentrations in different parts of the simulated water bodies.

The following subsections present the assumptions that are specific to the mathematical formulations of the models used and discuss the computational approaches in detail. Subsection 4.1 discusses the computation of overall transformation rates, and subsection 4.2 discusses the multicompartment model developed for this study. The assumptions that are independent of the mathematical formulations are presented in subsection 4.3.

4.1 ONE-COMPARTMENT MODEL

Analysis of the data under the assumption of first-order kinetics, referred to hereafter as the one-compartment model, assumes that the system is a single, completely mixed reactor from which the chemicals disappear through transformation and transport. This model allows analysis of acute discharges such as spills or deliberate use of pesticides and was used to establish priorities for detailed laboratory studies.

4.1.1 Assumptions of the One-Compartment Model

The equations of the one-compartment model make the following assumptions:

- The water body is homogeneous with respect to all physical, chemical, and biological properties.
- Chemical, physical, and biological properties (other than changes in the concentrations of the pollutant and solid masses within the compartments) remain constant.
- The effects of physical, chemical, and biological variables such as temperature, pH, and species composition are included implicitly in the rate factors used in the simulations, but are otherwise excluded.
- Exogenous environmental parameters such as sunlight intensity are constant for a given water body.
- The pollutant is introduced as a pulse at time zero.
- Sorption occurs only between solids and solution and between solution and biota; no sorption occurs directly between the biota and solids.
- The sorption equilibrium is rapidly established compared with all other transformation and transport processes. The relative proportions of sorbed and dissolved chemical are those calculated from the equilibrium constant or partition (sorption partition coefficient) for the chemical between water and a natural sediment.

- A portion of the microbial population is acclimated to the specific substrate at all times. The rate of microbial transformation is a function of the number of acclimated microbes and is first order with respect to substrate concentration.
- The microbial yield factor is constant.

The assumption that an acclimated microbial population is present implies that the pollution is chronic or that it consists of repeated, discrete releases of the chemical. Since several hours or days may be required for acclimation, the model tends to overestimate the loss rate of chemicals from solution in cases of acute pollution caused by spills. Also, the assumption that the water body is homogeneous precludes appraisal of the distribution of the chemical within large, incompletely mixed water bodies, such as large stratified lakes. However, this model is a useful approximation of transport and transformation in small water bodies such as ponds and has been especially useful as a preliminary assessment tool to establish priorities for the detailed laboratory studies.

4.1.2 Mathematical Formulations

Given the assumptions listed above, each process may be described by a first-order or pseudo-first-order rate law:

$$R_j = k_j[S] \tag{4.1}$$

where R_j is the transformation or transport rate for process j, k_j is the first-order or pseudo-first-order rate constant for process j, and [S] is the concentration of substrate. If k_j is a pseudo-first-order rate constant, then

$$k_j = k_{2_j}[E] \tag{4.2}$$

where k_{2_j} is the second-order rate constant for process j, and [E] is the concentration of the environmental component. We have assumed that the net rate of loss of substrate from the water body is

$$R = \sum_j R_j = \sum_j k_j \quad [S] \tag{4.3}$$

The half-life of a chemical in any first-order process ($t_{1/2}$) is

$$(t_{1/2})_j = \frac{\ln 2}{k_j} \tag{4.4}$$

Also, since all the transformation and transport processes have been expressed as first- or pseudo-first-order rate expressions, it is possible to calculate an overall or net half-life for the pollutant, since

$$t_{1/2} = \frac{\ln 2}{\sum_j k_j} \tag{4.5}$$

To be realistic, allowance should be made for flow of a pollutant out of the system by adding a rate constant for the movement of water through the system. Thus, equation (4.5) becomes

$$t_{1/2} = \frac{\ln 2}{k_d + \sum_j k_j} \tag{4.6}$$

where k_d is the dilution rate constant for the system as a whole. k_d is defined as the mass of chemical per unit time in the outflow divided by the total mass of chemical in the system. Equation (4.6) shows that the effective half-life is determined by two terms: the dilution rate constant and the sum of transformation rate constants. If the system is assumed to be completely mixed, the system dilution rate than becomes the outflow rate divided by the total volume. Two different cases of water movement are discussed: In the case of zero or low dilution rate, equation (4.6) becomes

$$t_{1/2} = \frac{\ln 2}{\sum_j k_j} \tag{4.7}$$

In the case of rapid dilution rate, equation (4.6) becomes

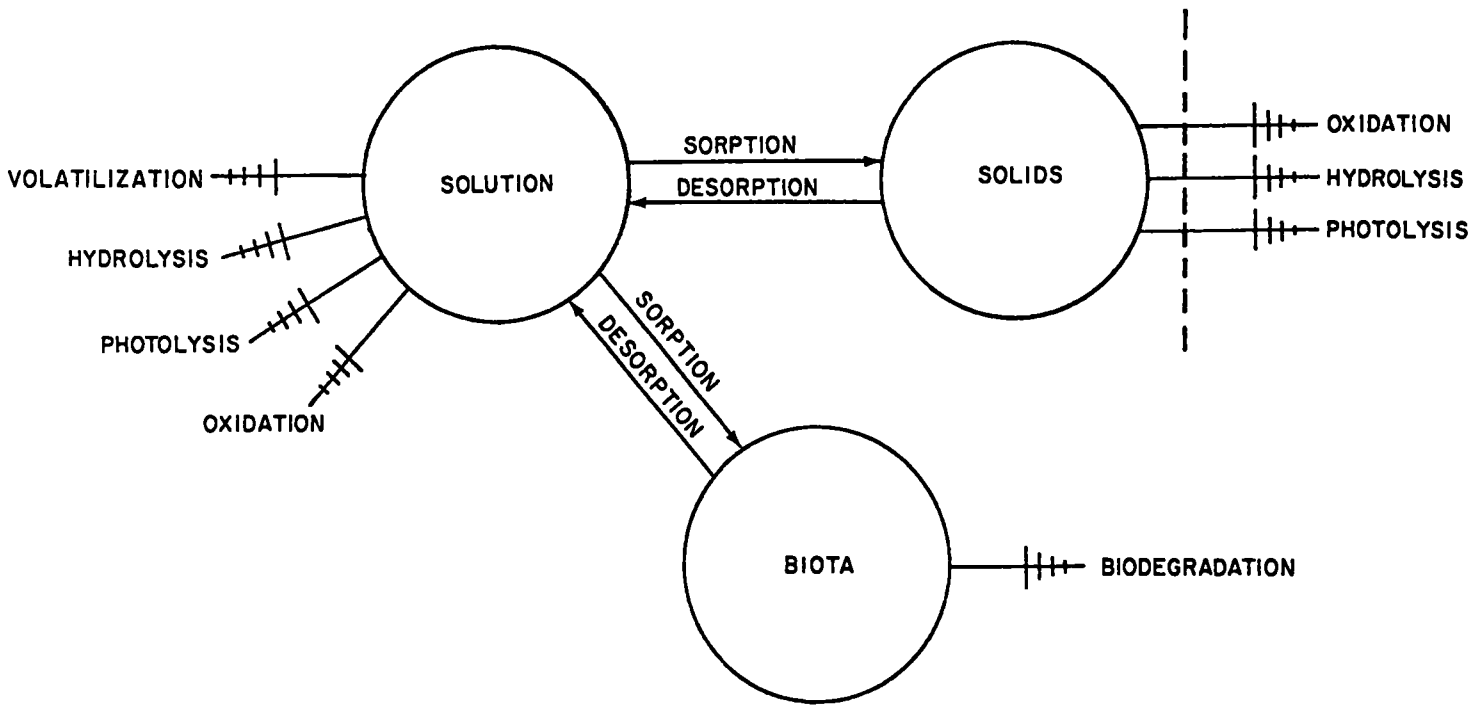
$$t_{1/2} = \frac{\ln 2}{k_d} \tag{4.8}$$

We have further assumed that the value for exogenous parameters [E] for any particular process will differ in different water bodies and will affect the values of k_j. The details of the differences we have assumed are given in subsection 4.3.

4.2 NINE-COMPARTMENT MODEL

The nine-compartment computer model* was designed to explore the impact of water body heterogeneity on the transformation and transport mechanisms covered in the laboratory phases of this project (Figure 4.1). Transformation of sorbed chemicals and accumulation of chemicals within food chains were not considered, but the model does permit assessment of the variations in concentrations to which specific segments of food chains will be exposed.

*The computer program for this model has been modified to accommodate up to 99 compartments, to allow for three-dimensional simulations, and to allow continuous flows between compartments. However, for the sake of consistency of methodology, the nine-compartment, two-dimensional, batch-flow version described here has been used throughout this study. The assumptions underlying the two versions of the model are otherwise unchanged.



Note : Items to the right of the dotted line are not used in current versions of the model

FIGURE 4.1 TRANSPORT AND TRANSFORMATION ROUTES SIMULATED

4.2.1 Assumptions

The model contains nine compartments of arbitrary size and composition, which are used to represent segments of the water column or sediments. By selectively removing compartments and appropriately adjusting the constants for water quality and water movement parameters, we can use the model to simulate streams, ponds, or stratified lakes.

Inputs can be made to any compartment, allowing simulation of atmospheric inputs, offshore outfalls, or accidental spills. For this study, we have assumed that inputs are restricted to a single surface compartment.

Transfers between compartments are assumed to be dominated by rates of water or solid particle movement and hence are assumed to be specific to the ecosystem rather than to the chemical. Solid and solution flows are allowed between compartments, and they can flow at different rates, but the biota remain in place. Inflows of new solution and solids are allowed as are equivalent outflows of solution and solids. Estimates of these rates of transfers between compartments are based on published data.

Sorption and desorption are allowed within compartments in the model and can occur between solution and solid particles and between solution and biota. Although exchange of sorbed substrate between solid particles and biota can be important, it has been excluded because it is not being measured in our current project. No distinction is made between organic and inorganic particles.

Calculations are made on a time-sequenced basis. We assume that flows and mixing occur at the end of each time interval and that volatilization, sorption, and transformations occur within each time interval. A short time interval, generally less than 0.2 hour, is used in the simulations.

The model assumes that a number of base conditions are constant within each compartment but may vary between the nine compartments. These conditions include temperature, pH, light, mass and species of biota, and so on, the effects of which are included implicitly in the input, transformation, and sorption rate factors. Changing base conditions, such as day to night, can be approximated by several sequential computer runs in which the output of one run regarding substrate concentration in the daytime is input to the next run where rate factors are modified to represent the nighttime conditions.

An initial concentration of the substrate can be arbitrarily assumed in either the solutions, solids, or biota in any of the compartments. Loading rates of the chemicals and suspended solids can be input as the specifications of the system of study.

Rate constants for chemical and biological transformation and volatilization measured in the laboratory are used directly when feasible or, when necessary, they are used to estimate the rate constants for combinations of temperature, light, and acidity that were not appraised in the laboratory.

Laboratory data for hydrolysis, oxidation, and volatilization are used directly or adjusted by empirical equations or coefficients. Data for sorption are used directly even though there is some uncertainty in any extrapolation of the complex and poorly understood phenomena collectively known as sorption. Environmental parameters related to sorption, volatilization, photolysis, and biodegradation are adjusted subjectively to approximate the net effect of the numerous differences between laboratory and field environments. A simple coefficient is used to adjust the photolysis rate constant. Biotransformation rates are adjusted by varying the number of bacteria assumed to be acclimated to the chemical of interest.

The complete structure of the model is presented schematically in Figure 4.2. The arrows indicate direction of flows and the rectangles represent the three-dimensional compartments. The model can direct flows between any pair of compartments (for example, from 4 to 8); however, those illustrated are the ones typically used. Compartments 1, 2, and 3 are generally used to represent surface waters; 4, 5, and 6 deep waters; and 7, 8, and 9 sediments. However, these compartments can be used in other ways if appropriate. The configurations of compartments actually used in this study and the dimensions assumed are shown in Figure 4.3.

Each compartment of the computer model can be considered as a completely mixed batch reactor. Transformation of a pollutant follows its transformation kinetics during the simulation time interval, and masses of the pollutant interchange among the compartments and between the aqueous and solid phases within compartments between each simulation time step.

The nine-compartment model lacks the assumptions of overall homogeneity, irreversible sorption, episodic discharge of pollutants, and first-order kinetics for biodegradation used in the one-compartment model, but shares the remaining assumptions listed in subsection 4.1.1. In addition, the nine-compartment model assumes that:

- Inputs, outputs, and transfers between compartments are limited to nonliving solids and solutions.
- Movements between compartments occur in discrete time steps.
- The contents of each compartment are thoroughly mixed after each intercompartment transfer.
- Volatilization, sorption, desorption, and transformation occur simultaneously within each compartment within each time interval.
- Chemical reactions are assumed to be pseudo-first order in pollutant concentration; biodegradation is assumed to follow Monod kinetics.
- Degradation is assumed to occur only in the liquid phase or in (or on) microorganisms.

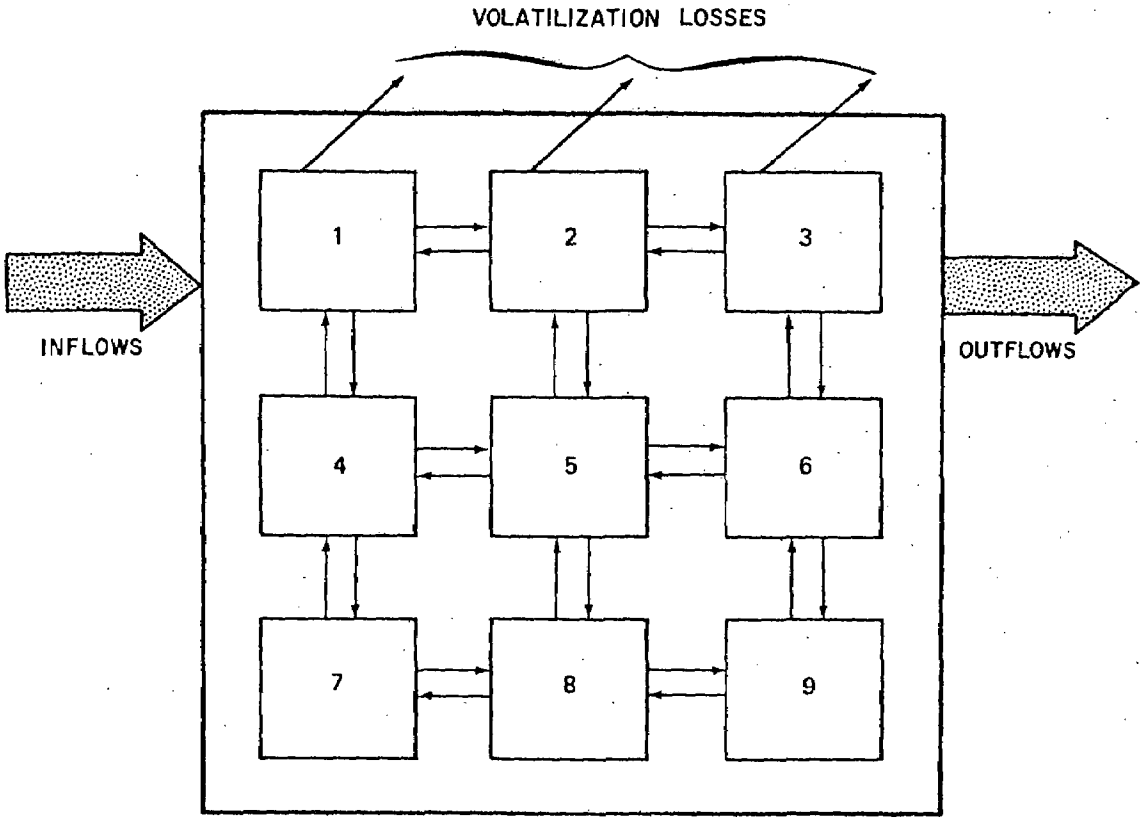


FIGURE 4.2 SCHEMATIC OF ASSUMED FLOWS BETWEEN COMPARTMENTS IN AN AQUATIC SYSTEM

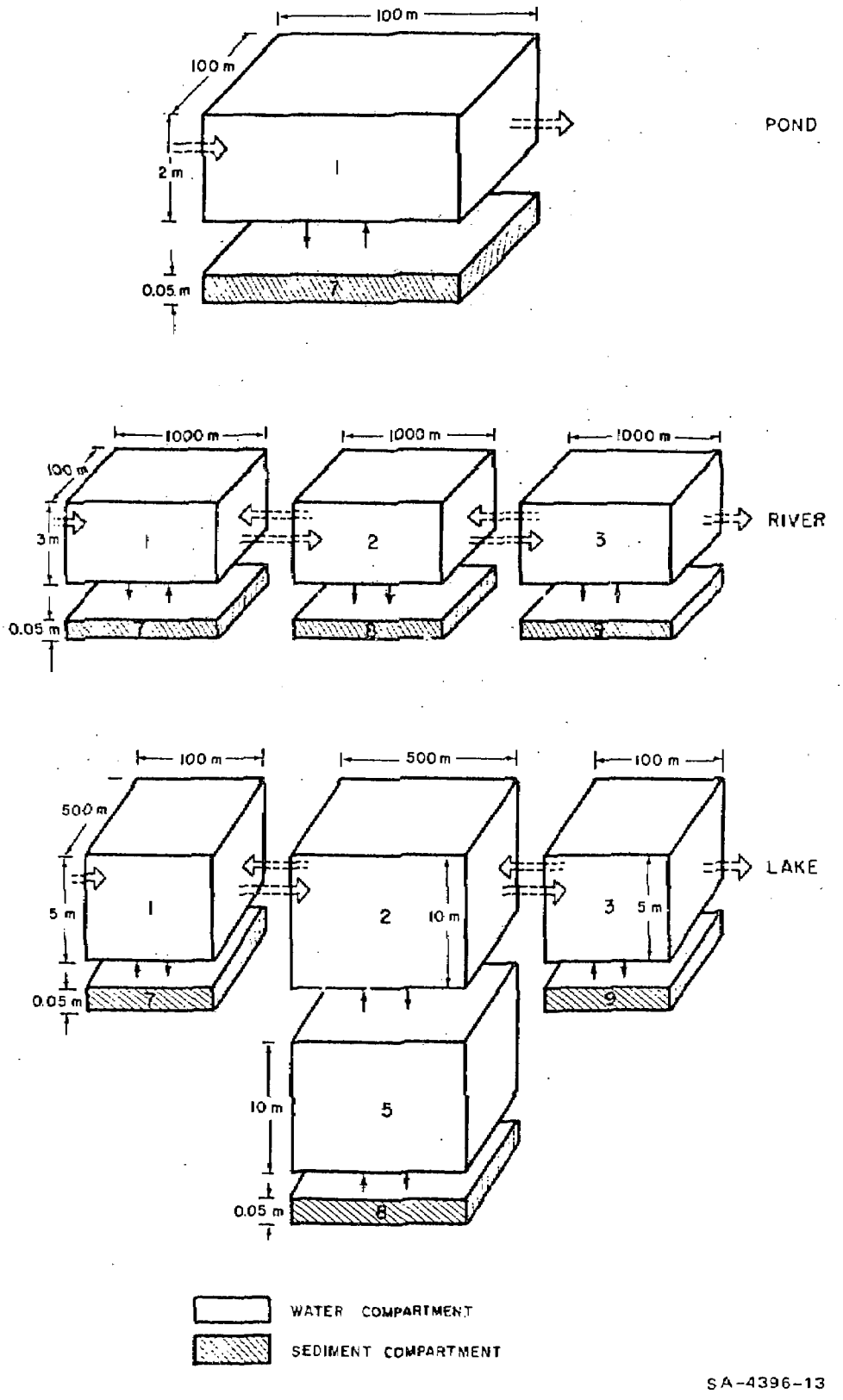


FIGURE 4.3 PHYSICAL CONFIGURATIONS OF THE POND, RIVER, AND LAKE SIMULATIONS

- In the current version of the computer program, bottom sediments are half water and half solids by volume.
- Outflows of solids are based on the ratio of solution in the compartment from which the outflow occurs.
- Inflows of solids are equal to outflows plus losses to the sediments.

The model also requires explicit assumptions for the environmental components, including physical dimensions and water quality, which are presented in Section 4.3.

4.2.2 Mathematical Formulations

The mass of pollutants in the aqueous phase and suspended solid phase in each compartment during the simulation time interval (Δt) is determined by the concentration of the external inflow and outflow, and interflows among the compartments. The mass of pollutants in the aqueous phase of compartment i after a time step can be written as follows:

$$M''_{\ell i} = I(\Delta t)_i + \sum_{in} M_{\ell ji} + M_{\ell i} - \sum_{out} M_{\ell ij} \qquad (4.9)$$

where $I(\Delta t)$ is the external input of pollutant to compartment i during the simulation time interval, $M_{\ell i}$ is the original mass in the compartment, $\sum_{in} M_{\ell ji}$ is the mass of pollutant in the aqueous phase added from the jth adjacent compartments, and $\sum_{out} M_{\ell ij}$ is the mass of pollutant in the aqueous phase that flowed out of compartment i to compartment j.

For the pollutant adsorbed on the suspended solids, a mass balance similar to equation (4.9) is written as follows:

$$M''_{si} = IS(\Delta t)_i + \sum_{in} M_{sji} + M_{si} - \sum_{out} M_{sij} \qquad (4.10)$$

where $IS(\Delta t)$ is the external input of solids to compartment i during the simulation time interval, $\sum_{in} M_{sji}$ is the mass of pollutant in the suspended solids added from the jth adjacent compartments, and $\sum_{out} M_{sij}$ is the mass of pollutant in the suspended solids that flowed from compartment i to compartment j.

Because many transformation processes may occur simultaneously in the aquatic system, a function $f_{\ell i}$ is defined as the total transformation rate of the pollutant, on a mass basis, in the aqueous phase in compartment i.

$$f_{\ell i} = \frac{dM'_{\ell i}}{dt} = (r_p + r_h + r_o + r_v + r_b)_i V_i \qquad (4.11)$$

where

$M'_{\ell i}$ is total mass of the pollutant in aqueous phase (ℓ) of compartment i

r_p is photolysis rate

r_h is hydrolysis rate

r_o is oxidation rate

r_v is volatilization rate

r_b is biodegradation rate

V_i is the volume of compartment i.

The photolysis rate is expressed as follows:

$$r_p = k_p[S]_{\ell i} \tag{4.12}$$

where k_p is the photolysis rate constant (Section 6.2) and $[S]_{\ell i}$ is the chemical concentration in the aqueous phase in compartment i. Note that k_p is a function of quantum yield, absorption spectrum, and solar irradiance; k_p will vary with time of day, season, and location.

The hydrolysis rate is written as follows:

$$r_h = k_h[S]_{\ell i} \tag{4.13}$$

where k_h is the hydrolysis rate constant, which depends on the temperature, $[H^+]$, and $[OH^-]$ in the aquatic system.

The oxidation rate is expressed by the equation:

$$r_o = k_{ox}[S]_{\ell i} \tag{4.14}$$

where k_{ox} is a pseudo-first-order rate constant equal to $k'_{ox}[RO_2\cdot]$ in which the concentration of $RO_2\cdot$ is fixed at 10^{-9} M (Section 6). Oxidation by other oxidants such as HO· or O_3 would follow similar kinetic relationships but were not measured here.

The volatilization rate is proportional to the difference between the chemical concentrations in the aqueous and the air phases:

$$r_v = k_v([S]_{\ell i} - [S]_g) \tag{4.15}$$

where k_v^S is the mass transfer rate constant and $[S]_g$ is the chemical concentration in the air phase. In a normal atmospheric environment $[S]_g$ is usually so small that it can be assumed equal to zero. Therefore, equation (4.15) reduces to

$$r_v = k_v^S[S]_{\ell i} \tag{4.16}$$

Clearly, k_V^S should depend on the surface area, wind speed, air and water temperature, and so on. Laboratory measurements of k_V^S for chemicals have been described elsewhere (Hill et al., 1976). The ratio of the gas transfer constant of chemical to that for oxygen is constant for a wide range of turbulence conditions. The gas transfer constant of chemicals in various water bodies can be estimated if the oxygen reaeration constants in the corresponding water bodies are also known (Section 5.3).

The biodegradation rate is described by the following equation:

$$r_b = \frac{1}{Y} \frac{\mu_{max}[S]_{\ell i}}{(K_s + [S]_{\ell i})} X_i \tag{4.17}$$

where μ_{max}, K_s, and Y are the kinetic constants of the Monod expression (Stumm-Zollinger and Harris, 1971; Monod, 1949). μ_{max} is defined as the maximum growth rate, K_s is the half-saturation rate, which is defined as the pollutant concentration at one-half of the maximum growth rate, and Y is the yield factor, which describes the efficiency of converting chemical mass into microbial mass. In the model, $[X_i]$, the microbial mass or concentration, is considered to be an environmental parameter and is assumed to be constant.

Sorption of the chemical on the suspended solid particles and the suspended biota (Section 5.4) is assumed to be an equilibrium process.

The partition coefficient for distribution between the suspended sediment phase and the aqueous phase is defined by:

$$K_P = \frac{m_{wi}}{m_{si}} \frac{M_{si}}{M_{\ell i}} \tag{4.18}$$

where m_{wi} is the mass of water and m_{si} is the mass of suspended sediment in compartment i. M_{si} and $M_{\ell i}$ are the masses of chemical in suspended sediment phase and the aqueous phase, respectively.

Similarly, the partition coefficient for distribution between biota and aqueous phase is:

$$K_b = \frac{m_{wi}}{m_{Bi}} \frac{M_{Bi}}{M_{\ell i}} \tag{4.19}$$

where m_{Bi} and M_{Bi} are the masses of biota and chemical in biota, respectively. Since it is assumed that no biodegradation or chemical transformation processes take place on the surface or inside the sorbent phase, the total mass of pollutant in a compartment before and after sorption will be the same. Therefore, the relationship of the total mass of chemical in the aqueous phase ($M_{\ell i}$), the suspended sediment (M_{si}), and biota (M_{Bi}) before (primed) and after (not primed) sorption can be written as:

$$M'_{\ell i} + M'_{si} + M'_{Bi} = M_{\ell i} + M_{si} + M_{Bi} \tag{4.20}$$

The mass distribution after sorption, therefore, can be calculated by solving equations (4.18), (4.19), and (4.20).

The relative amounts of bacteria and sediments found in the water column and sediment layer of eutrophic and oligotrophic water bodies are summarized in Table 4.1.

TABLE 4.1. RATIO OF BACTERIA TO SEDIMENTS IN NATURAL WATER BODIES

	Weight bacteria/weight sediment[a]	
	Water column	Sediments
Entrophic	1×10^{-5}	1.6×10^{-2}
Oligotrophic	1×10^{-7} to 1×10^{-6}	1.6×10^{-4} to 1.6×10^{-3}

[a]Dry weights.

Sources: Tables 28 and 31 of Kuznetsov (1970), pp. 592-595 of Wetzel (1975), and an empirical bacteria density to dry weight conversion factor of 4 g (dry weight) per 10^{13} cells.

The estimates show that even if the biosorption is ten times as much as the sorption on sediments, the bacteria contribute little to the total amount of substrate sorbed. The model developed in the study, therefore, excludes the biosorption from the calculations. The mass distribution after sorption can be directly calculated from equations (4.18) and (4.20) with omission of the M'_{Bi} and M_{Bi} terms in the equations.

To integrate equation (4.11), the predictor-corrector method (ESØDEQ) (Rollins, 1968) is used. ESØDEQ uses a four-point Adams-Bashforth-Moulton predictor-corrector method to carry out its integration. The predictor-corrector can be executed only if four points are available. To estimate the first four points (including the initial point), the fourth-order Runge-Kutta method is chosen because it is quite accurate over small intervals. ESØDEQ also provides a feature whereby the integration can be carried out only with the Runge-Kutta method by selecting an appropriate control index.

The functional relationships of the computer program subroutines are illustrated in Figure 4.4. The sequence for the time interval Δt is:

(1) Calculate the mass of chemical transformed.

(2) Calculate the distribution of chemical between the sediment and aqueous phases, according to the equilibrium partition coefficient.

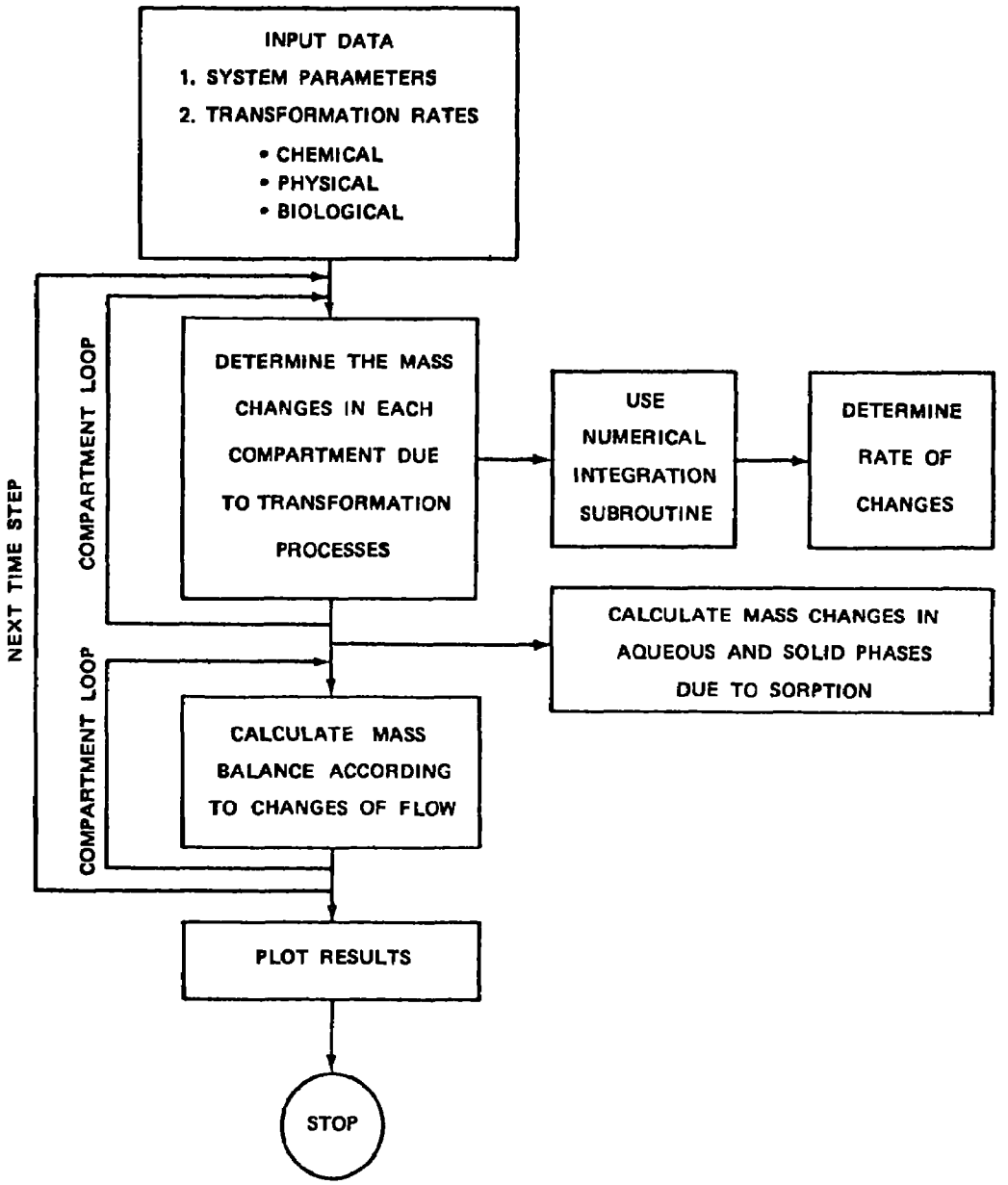


FIGURE 4-4. FLOWCHART FOR THE ENVIRONMENTAL ASSESSMENT MODEL

(3) Calculate the new masses in each compartment based on the assumed inflow-outflow conditions.

4.3 ENVIRONMENTAL PARAMETERS

The constants used to define the volumes of the compartments, the fluxes of materials, and the inflow of pollutant at a concentration of 1 $\mu g\ ml^{-1}$ are given in Table 4.2 (input pollutant concentrations are set at 1 $\mu g\ ml^{-1}$ or below the solubility, whichever is lower). The values used to define the water quality characteristics that directly affect the transformation and transport processes studied in the laboratory are also given in Table 4.2. The values of the constants presented in this table are based on values given in Wetzel (1975) and Leopold et al. (1964), except for the reaeration rates, which are discussed in Section 5.3.1.

TABLE 4.2. PHYSICAL DIMENSIONS AND WATER QUALITY CHARACTERISTICS ASSUMED IN THE ENVIRONMENTAL ANALYSIS

Parameter	River	Pond	Eutrophic lake	Oligotrophic lake
Physical dimension				
Total water volume (m^3)	9×10^5	2×10^4	5.5×10^6	5.5×10^6
Inflow ($m^3\ hr^{-1}$)	1.0×10^6	20	9.7×10^2	9.7×10^2
Mean residence time (hr)	8.3×10^{-1}	1.0×10^3	5.7×10^3	5.7×10^3
Pollutant inflow ($kg\ hr^{-1}$)[a]	1.0×10^3	0.02	0.97	0.97
Water quality				
Total bacteria ($cells\ ml^{-1}$)	10^6	10^6	10^6	10^2
Active bacteria ($cells\ ml^{-1}$)	10^5	10^5	10^5	10
pH	7	8	8	6
Sediment loading ($\mu g\ ml^{-1}$)	100	300	50	50
Photolysis activity index[b]	0.5	0.2	0.2	1.0
Oxygen reaeration rate (hr^{-1})	0.04	0.008	0.01	0.01
$[RO_2\cdot]$ (M)	10^{-9}	10^{-9}	10^{-9}	10^{-9}

[a] The flow rates between compartments are given in Appendix A.

[b] Factor to account for differences in light transmission through different types of water. Distilled water has an index value of 1.

5. PHYSICAL PROPERTIES

Four physical properties of each substrate were measured as part of this study: solubility in water, ultraviolet (uv) and visible absorption spectra, volatilization rate constant, and sorption partition coefficients. The solubility in pure water must be known because the substrate must be in solution for meaningful rate constants and sorption partition coefficients to be measured. The solubility is also used to estimate volatilization rates. The uv/visible absorption spectrum is used to estimate the photolysis rate. Volatilization, photolysis, and sorption are possible important environmental pathways for the substrate.

5.1 SOLUBILITY

The general procedure described by Campbell (1930) is simple to use to measure the solubilities of solids in the $\mu g\ ml^{-1}$ range. A small amount of the solid substrate is placed in an all-glass apparatus, which is immersed in a water bath and shaken gently. This apparatus has two compartments separated by a glass frit. When the flask is inverted, the aqueous solution is filtered through the frit to remove solid substrate. The filtration step can be carried out without removing the apparatus from the water bath. After equilibration in the water for several days, the sample is filtered in the apparatus and the filtrate is analyzed for the substrate.

Generally, at least three measurements are made. Also, measurements are made on samples that have been heated to 35° to 40°C, allowed to equilibrate, and then cooled in the water bath. Since the concentrations are fairly high, potential problems that are encountered with low-solubility materials, such as adsorption onto the frit during filtration and the possibility that finely divided particulate substrate is not removed during filtration, are not likely to be significant.

Solutions of compounds having a solubility in the $ng\ ml^{-1}$ range were prepared by the procedure described by Haque and Schmedding (1975). The substrate is dissolved in an organic solvent and put on the walls of a 5-gallon (18.8-liter) carboy. The carboy is rotated slowly on its side while the solvent evaporates, so that a thin film of substrate coats the wall of the carboy. A large Teflon-coated magnetic stirring bar is added, and the carboy is filled with the purest water available. Care must be taken to prevent the substrate from coating the bottom of the carboy so that it is not dislodged by the stirring bar. The solution is allowed to stir gently for at least a week to assure that equilibration has taken place. Samples of water are withdrawn with a glass siphon and analyzed for the substrate.

In several cases, we found that even with these precautions particulate matter, presumably substrate, could be observed in the carboy, and the substrate concentration was reduced by centrifugation at 10,000 rpm. The solubility measurements of low-solubility (less than 0.1 μg ml^{-1}) compounds should be made on centrifuged samples.

5.2 ABSORPTION SPECTRA

The absorption spectrum of the substrate is measured to determine if photochemical transformation or degradation is possible. If the substrate does not absorb light in some region of the solar spectrum, then direct photochemical transformation in the environment is not possible. Sensitized photolyses may also be possible, but their importance is quantitatively assessed by experiments with humic acid (Section 6.2).

Zepp and Cline (1977) and Wolfe et al. (1976) have developed a computer program that will calculate the direct photochemical transformation rate of a substrate, provided the substrate absorption spectrum and transformation quantum yield and the solar spectrum are known (see Section 6.2 for details). This program requires the average molar extinction coefficient and the solar irradiance for specific wavelength intervals, which are listed in Table 5.1.

TABLE 5.1. NOMINAL WAVELENGTHS AND WAVELENGTH INTERVALS FOR UV AND VISIBLE ABSORPTION SPECTRA

Nominal wavelength (nm)	Wavelength interval (nm)
297.5	± 1.25
300.0 to 320.0	± 1.25
323.1	+ 1.9, - 1.85
330.0	± 5.0
340.0 and higher	± 5.0

*These numbers represent the wavelength intervals used by the computer (Wolfe et al., 1976). The actual precision of a measured wavelength is about ± 0.5 nm.

Most measurements of absorption spectra reported in the literature have focused on the location and intensity of the absorption maxima. Since the solar irradiance rises rapidly between 295 and 350 nm, the low absorption "tail" that is often present in molecules at the longer wavelengths can have a significant contribution to the photolysis rate. Therefore, it is necessary to measure the magnitude of the absorption tail as well as the more intense portions of the absorption spectrum.

It is well known that the absorption spectra of many compounds are slightly different in polar and nonpolar solvents.* Since the substrate will be in water in the aquatic environments, we have used pure water as the solvent for measurement of the absorption spectra whenever possible. It is important to ensure that the absorption tail of low-solubility substrates can be measured accurately; the concentration of the substrate should be in the 10^{-2} to 10^{-6} M range. Therefore, it is often necessary to increase the solubility by adding a water-soluble cosolvent, such as acetonitrile. Since acetonitrile is also very polar, at concentrations less than about 20% by volume, acetonitrile does not significantly affect the absorption spectrum. Also, the absorption spectrum should be measured in 1-cm and 10-cm cells; the 10-cm cell is necessary to maximize the precision of the measurement of the absorption tail at low substrate concentrations. The general procedure has been to prepare a substrate solution of a minimum of about 10^{-5} M in water, using acetonitrile to dissolve any solid, undissolved substrate. Obviously, some trial and error is required to minimize the amount of acetonitrile. To obtain satisfactory spectra with 10-cm cells, we have always run solvent versus solvent to obtain a baseline.† Then, the cell containing solvent in the sample beam is refilled with the substrate solution, and the absorption spectrum is measured. The molar extinction coefficients ε_λ are obtained from Beer's law.

$$\text{Absorbance} = -\log \frac{I}{I_o} = \varepsilon_\lambda 1 S \qquad (5.1)$$

where I_o is the incident light flux and I is the transmitted light flux in the spectrophotometer. If 1, the cell path length, is in centimeters and if S, the substrate concentrations, is in M, then the molar extinction coefficient ε is in units of $cm^{-1}\ M^{-1}$. The average molar extinction coefficient for each nominal wavelength is calculated from the average of the molar extinction coefficients at the lower and upper limits of the wavelength interval (Table 5.1).

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*This may not be true for substrates that are sorbed onto sediments or biota, especially since absorption spectra are known to shift in some cases when molecules are adsorbed.

†We have used Cary model 14 and 15 spectrophotometers in these studies. However, any high quality, uv/visible spectrophotometer that will accept 10-cm cells can be used. Suitable standards should be prepared to assure that the same absorbance is obtained in the 10-cm cell with a solution that is one-tenth the concentration used in the 1-cm cell.

5.3 VOLATILIZATION RATE

5.3.1 Background

To assess the importance of volatilization as a pathway for pollutant substrates in natural water bodies, it is desirable to have an expression of the form

$$\frac{d[S]}{dt} = k_v^S [S] \tag{5.2}$$

where k_v^S is the volatilization rate of the substrate S. Compounds of low molecular weight and high vapor pressure, such as vinyl chloride (Hill et al., 1976), have been shown to volatilize rapidly as one might expect. However, some high molecular weight, low solubility compounds, such as DDT (Acree et al., 1963), also volatilize at an appreciable rate, since the Henry's Law constant for these compounds is very high because the activity coefficients are also very high. The details are discussed in Appendix B.

Several authors have suggested ways to estimate volatilization rates of compounds from water, using theoretical considerations and laboratory measurements. Mackay and Wolkoff (1973) have suggested simple equations that can be used to estimate the volatilization rate of an organic solute from a water body under certain conditions. When their assumptions for the evaporation rate of water, etc., are used and a 1-m depth for homogenous mixing is used, their equation (10) for the volatilization half-life of substrate in a representative lake reduces to

$$\tau_{\frac{1}{2}}(\text{days}) = \frac{0.108\ S_{sat}}{P_s M_s} \tag{5.3}$$

where P_s is the vapor pressure (torr), M_s is the molecular weight of the substrate, and S_{sat} is the solubility of the substrate ($\mu g\ ml^{-1}$). While estimates using this equation are simple to make, the assumption of a 1-m depth for homogeneous mixing is a serious deficiency. In most water bodies, mass transfer across the boundary layers, which is not accounted for in equation (5.3), is the rate-determining step for volatilization.

Mackay and Leinonen (1975) recognized the problem with assuming homogeneous mixing and developed equations that included the mass transfer across the boundary layers (equations B.3, B.4, and B.5 in Appendix B of this report). To use this method it is necessary to measure the mass transfer coefficients and the Henry's Law constants. Mackay and Cohen (1976) have described several methods for measuring these values in the laboratory. However, these measurements require a special apparatus and some experimental care. The principal difficulty with this approach is that there is no way to relate the mass transfer coefficients determined in the laboratory to those in the real body of water with varying wind and water flow conditions. There is no convenient way to measure the mass transfer coefficients directly in the real water bodies.

Tsivoglou has made an important observation. He showed that the ratio of the volatilization rates of several low-molecular weight gases from water is constant over a wide range of turbulence conditions (Tsivoglou et al., 1965; Tsivoglou, 1967). Thus, for compounds A and B, the ratio

$$\frac{k_v^A}{k_V^B} = \text{constant} \tag{5.4}$$

It is convenient to choose the substrate for A and oxygen for B. The law of microscopic reversibility requires that the rate of volatilization equal the rate of dissolution into the liquid for identical conditions. The term, oxygen reaeration rate, is commonly used to express the rate at which oxygen from the atmosphere dissolves in oxygen-deficient water. The oxygen reaeration rate is defined by

$$\frac{d[O_2]}{dt} = k_v^O([O_2]_{sat} - [O_2]) \tag{5.5}$$

where $[O_2]$ is the oxygen concentration, $[O_2]_{sat}$ is the oxygen concentration when the water is saturated, and k_v^O is the oxygen reaeration rate constant. The oxygen reaeration rate has the additional advantage that it has been measured for many different water bodies. Values for representative water bodies are given in Table 5.2.

TABLE 5.2. OXYGEN REAERATION RATES IN REPRESENTATIVE WATER BODIES

	Literature values (day^{-1})	Values used in this study (day^{-1})	(hr^{-1})
Pond	0.11 - 0.23[a]	0.19	0.008
River	0.2[b], 0.1 - 9.3[c]	0.96	0.04
Lake	0.10 - 0.30[a]	0.24	0.01

[a] Metcalf and Eddy (1972).

[b] Grenney et al. (1976).

[c] Langbein and Durum (1967); taken from Table 2 for rivers such as the Allegheny, Kansas, Rio Grande, Tennessee, and Wabash.

Therefore, if the ratio of the substrate volatilization rate to the oxygen reaeration rate constant can be measured in the laboratory, the

volatilization rate of the substrate in a real water body for which the oxygen reaeration rate is known can be estimated:

$$(k_v^S)_{\text{water body}} = (k_v^O)_{\text{water body}} \, (k_v^S/k_v^O)_{\text{laboratory}} \tag{5.6}$$

Hill et al. (1976) used this procedure successfully to estimate the volatilization rate constants for vinyl chloride.

The purpose of Appendix B is to tie all the theoretical work together and to show that the simple relationship

$$\frac{k_v^S}{k_v^O} = \frac{d^O}{d^S} \tag{5.7}$$

where d^O and d^S are the molecular diameters of O_2 and S (assuming that they are spherical), can be used to estimate values of k_v^S/k_v^O at the low turbulence values likely to be found in natural water bodies. In Part II of this report, this theory will be compared with the laboratory measurements.

5.3.2 Experimental Procedures

Volatilization rates were measured using the method described by Hill et al. (1976). A solution of the substrate in pure water is prepared at a concentration that is below its saturation value. About 1 liter of this solution is placed in a 2-liter beaker equipped with a stirring bar. The solution is purged with nitrogen to remove most of the dissolved O_2. At the start of the experiment (t = 0), the concentration of substrate is measured* and the O_2 concentration is measured with an O_2-analyzer. Successive substrate and O_2 measurements are made at regular time intervals.

The substrate concentration versus time data are fit to an exponential decay curve of the form

$$[S_t] = [S_o]e^{-k_v^S t} \tag{5.8}$$

which is the integrated form of the first-order rate expression

$$-\frac{dS}{dt} = k_v^S[S] \tag{5.9}$$

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*Usually, an aliquot was removed and saved for subsequent extraction or direct analysis.

The oxygen concentration data are fit to the integrated form of equation (5.10)

$$\frac{d[O_2]}{dt} = k_v^0 \left([O_2]_{sat} - [O_2]t\right) \tag{5.10}$$

which is

$$[O_2]_t = [O_2]_{sat} - \left([O_2]_{sat} - [O_2]_o\right) e^{-k_v^0 t} \tag{5.11}$$

where $[O_2]_{sat}$ is the saturation concentration of O_2 in water at the temperature of the measurement and is a constant because the concentration of O_2 in the air is constant.

To calculate values of k_v^0 and k_v^S, we used the linear least squares routine supplied with the Hewlett-Packard Model 65 calculator. This program gives a linear least squares fit to lnS versus t, plus the variance of the parameter estimates.* The value of k_v^S/k_v^0 was calculated from these values. Other curve fitting procedures could also be used.

Experimental problems arise if k_v^S is either very low or very high. If k_v^S is low, evaporation of water becomes significant. When that happens, the mixing rate in the beaker changes because the stirring bar, which rotates at a nearly constant rate, imparts more turbulence to the solution as the amount of water decreases. Therefore, the value of k_v^0 must be measured several times and the variance in k_v^0 is significantly larger.

If k_v^S is very high, as was the case for benzo[b]thiophene , the substrate volatilization rate can be comparable to the O_2 reaeration rate. In that case, the stirring rate must be reduced to bring k_v^S within a range that can be measured. If the stirring is too low, the solution becomes inhomogeneous and the data do not fit the theoretical scheme above. These problems were overcome by using a reasonable stirring rate but reducing the liquid surface area exposed to the atmosphere. A 1-liter Erlenmeyer flask, filled nearly to the rim, was used in the volatilization experiments with benzo[b]thiophene.

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*Note that the natural logarithms of [S] or $([O_2]_{sat} - [O_2]_t)$ must be entered. Obviously, the exponential curve-fitting routine could also be used, but it does not provide the variance of the parameter estimates.

SORPTION OF ORGANIC SUBSTRATES

Background

Sorption* of organic substrates onto sediments and biota can be a very important phenomenon in the aquatic environment. The sediments can act as a sink for sorbed materials, removing them from the water column. However, the substrate can also be released (desorbed) from the sediments at a later time. In this way sorbed material can also be a source of pollution. Sorption of pollutants by sediments often results in high concentrations of low-solubility pollutants in a part of the water column where uptake by biomagnification may become significant.

Three types of sorbents have been evaluated in this study: a montmorillonite clay, several natural sediments, and a mixture of bacteria. The clay was used as a reference sorbent because it could be readily obtained and prepared by other workers. The sediments were collected from a variety of sources chosen to represent different types of freshwater bodies in different parts of the United States. The bacteria cultures were obtained from the American Type Culture Collection (ATCC). They were chosen because they are representative of the types of microorganisms found in freshwater bodies and had not been exposed to the types of substrate being studied.

The low proportion of bacteria to other materials (such as clays, detritus, humic substances) in both suspended and bottom sediments (see Table 4.1) suggests that the bacterial population does not contribute significantly to the total amount of sorption. The effects of bacteria in sediments on the sorption partition coefficients are already included because the procedures we have used to collect and preserve the sediments are such that any bacteria originally present will be in the sediment samples used to measure the sorption properties.

The separate biosorption measurements are useful, even if bacterial adsorption is not an important fate, because biosorption is often the first step of biomagnification and is therefore important environmentally. Biosorption studies indicate whether biomagnification could be an important pathway for a particular compound. Sorption takes place, in general, when solutions containing a dissolved substrate contact a solid phase surface. If the total amount of substrate is increased, the amount of substrate that is sorbed is also increased.

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*The term "sorption" includes any type of process whereby the substrate is physically or chemically bound to a solid surface. The term "adsorption" implies to us that the process that holds the substrate on the sediment is strictly physical, such as the Van der Waals type of attraction. We have used the term "sorption" (or "biosorption" when the solid sorbent is a microorganism) throughout this report to avoid questions about the details of the sorption mechanism.

Experimental data for sorption have generally been found to fit one of two mathematical forms: the Langmuir and Freundlich isotherms. First, S_w and S_s are defined as:

$$S_w = \frac{\text{weight substrate in solution}}{\text{ml solution}} \tag{5.12}$$

$$S_s = \frac{\text{weight substrate sorbed}}{\text{g sorbent}} \tag{5.13}$$

at equilibrium. The substrate weights must be in the same units (e.g., ng, g). For a dilute aqueous solution, 1 ml of solution equals 1 g of solution, and

$$S_w = \frac{\text{weight substrate in solution}}{\text{g solution}} \tag{5.14}$$

The Langmuir isotherm equation is defined as

$$S_s = \frac{abS_w}{1 + bS_w} \tag{5.15}$$

where a and b are constants and

$$a = X_c \tag{5.16}$$

$$b = \frac{K_p}{X_c} \tag{5.17}$$

where X_c is the sorption capacity of the sorbent and K_p is a partition coefficient. Data for gas-solid sorption generally and data for organic substrates sorbed on clay minerals usually fit the Langmuir isotherm. However, natural sediments are not homogeneous--sorbed complex organic material such as humic substances is already present on the clay particles--and sorption by natural sediments usually fails to fit the Langmuir isotherm.

Data for sorption of multiple substrates from solution on nonuniform surfaces generally fit the Freundlich isotherm, which is an empirical equation,*

$$S_s = KS_w^{1/n} \tag{5.18}$$

At low substrate concentrations, n is often very nearly equal to 1. If n = 1 and S_s and S_w are in the same units, the units of each side of equation (5.18) cancel and K becomes a partition coefficient, as defined by

$$S_s = K_p S_w \tag{5.19}$$

This equation has been used to describe the laboratory sorption data obtained in this study and is equivalent to equation (4.18), which is used in the environmental assessment models.

5.4.2 Sorption on Clays and Sediments

5.4.2.1 Clay and Natural Sediments Selected for This Study--The montmorillonite clay used in these studies was a Wyoming montmorillonite obtained from Dr. William John, Department of Geology, University of Missouri, Columbia, Missouri. Clay suspensions (about 1% by weight) were prepared by soaking a measured weight of clay in distilled-deionized water for at least one week. The clay suspension was then passed through an ion exchange column that had been presaturated with calcium ions to convert the clay from the sodium form to the calcium form. This was necessary because the Na-montmorillonite suspension could not be centrifuged but the Ca-montmorillonite could. The particle size was less than 1 μm.

The procedures that were used to prepare and store the natural sediments were designed to preserve the sediments in their natural state as well as possible. Natural sediments were screened to remove large rocks, twigs, and other debris. The mesh sizes of the screens used were 4, 16, and 28 per 2.54 cm. Following the screening, the sediment was mixed, using a Humbolt splitter to be sure the sediment was uniform. A small volume of each sediment was mixed with two volumes of 0.1 M calcium chloride and the pH was recorded. The remainder of the screened and split sediment was stored in 100-ml Nalgene bottles at 4°C until use. The sediments were never allowed to dry out, because the drying would change the characteristics of the clay-organic complexes in the sediments. Similarly, no attempts were made to kill or remove the bacteria in the sediments. Therefore, the contribution of the sediment bacteria

*Hamaker and Thompson (1972) point out that the exponent in equation (5.18), 1/n, is ". . . an archaic remnant of an attempt to give the Freundlich equation physical meaning and is retained only because its use is embedded in the literature." We have continued this practice.

to the total sorption properties of the sediment has been included in our data.

It is very difficult to make reproducible transfers of whole sediments if the sediments are in suspension. The sand fractions settle rapidly, and they constitute a significant portion of the total mass of many sediments.* Therefore, we used only the sediment particles smaller than 100 μm. Our experience indicates that less than 100-μm sediment can be reproducibly transferred as a slurry. The less than 100-μm sediment was prepared from as-received sediment by screening and a final settling of 30 seconds to remove coarse sand.

The characteristics of the Ca-montmorillonite clay and natural sediments used in these studies are given in Table 5.3. The organic carbon (OC) values, expressed as percent carbon by weight, were determined using the Walkley and Black procedure, which involves oxidation of the organic material by chromate followed by back-titration with ferrous ammonium sulfate (Hesse, 1971). Other methods of determining OC values, such as combustion and determination of evolved CO_2, could have been used and would most likely give different OC values. However, the trend in the organic carbon levels in the sediments should not change.

5.4.2.2 General Procedures--Screening studies for sorption were made to estimate the magnitude of the partition coefficient K_p. This value was used in the one-compartment model to provide an estimate of the importance of sorption as an environmental pathway. Biosorption studies were not carried out on substrates that were not strongly sorbed on the natural sediments and/or were rapidly biodegraded (see Section 7). The screening isotherm measurement was usually made on the Coyote Creek sediment, which was arbitrarily selected because we had collected a large sample and because it has an intermediate organic content.

To set up these screening isotherms, it is necessary to make an estimate of the partition coefficient, based on the solubility of the substrate in water. In general, as the substrate solubility decreases, the value of the partition coefficient increases. For instance, Bailey et al. (1968) studied the sorption of several series of organic herbicides including amines and acids on montmorillonite clays. They found that the log K_p was related to solubility in water "within an analog series basic in chemical character." In their case, cation exchange and surface acidity of the clay and the pK_a of the herbicide determined the sorption within a chemical family. The chemicals studied in this program do not fit within the families studied by Bailey et al. because they do not have acid or basic character (except p-cresol). The sorption of the compounds studied here is probably due entirely to Van der Waals type of sorption.

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*Reproducible transfers of dried, whole sediments could be made, but the nature of the sediment would probably be significantly altered by the drying process.

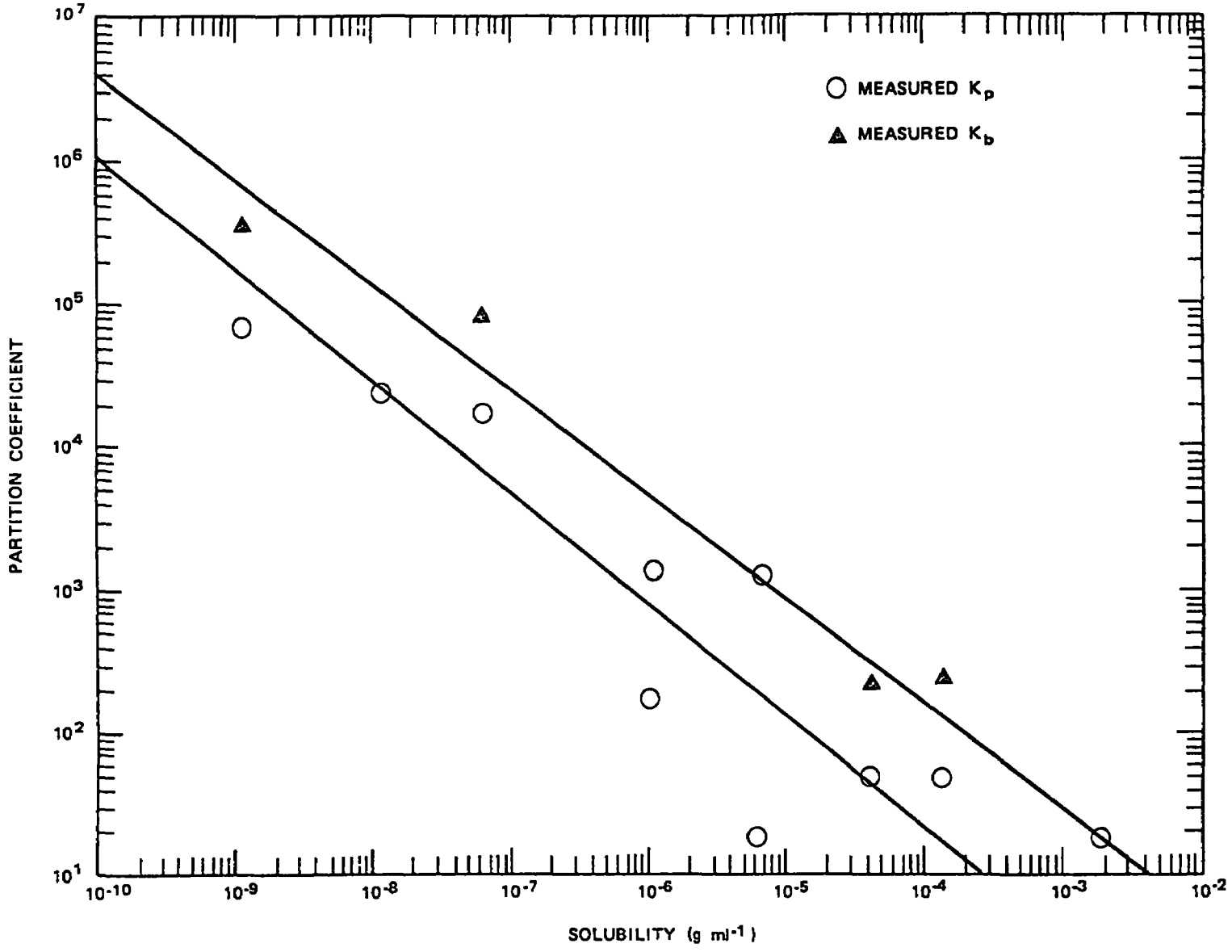

FIGURE 5-1. SOLUBILITY VERSUS PARTITION COEFFICIENT ON COYOTE CREEK SEDIMENTS (K_p) AND ON A MIXED POPULATION OF BACTERIA (K_b)

TABLE 5.3. SOURCES AND CHARACTERISTICS OF Ca-MONTMORILLONITE CLAY AND NATURAL SEDIMENTS

Source	Location and description	Sediment pH	OC[a] (%)	Cation exchange capacity (meq/100 g)
	Ca-montmorillonite clay	--	0.05	69.0
Navarro River	Mendocino County, California An unpolluted river that drains redwood forests, orchards, and pasture	6.7	0.5	4.5
Des Moines River	Iowa	7.1	0.8	10.5
Oconee River	Georgia	6.2	0.8	8.5
Coyote Creek	San Jose, California A eutrophic, polluted stream	6.5	1.9	13.5
Searsville Pond	Woodside, California A small eutrophic but unpolluted pond	6.7	5.0	34.5

[a]Organic carbon, Walkley and Black value, corrected for recovery by multiplying experimental value by 1.33.

Figure 5.1 is a plot of the logarithm of the partition coefficient data obtained on this project versus the logarithm of the substrate solubility in water at about 20°C. The values of K_p were obtained from the Coyote Creek sediment (Table 5.3); the values of K_b were obtained from the mixed population of bacteria described in Section 5.4.3. While there is some scatter of the data, the correlation is surprisingly good. This correlation can be used to estimate the order of magnitude of the sorption partition coefficient for other compounds. Compounds that interact with sediments via an ion exchange mechanism probably would not fit this plot.

Many experimental designs for sorption studies are possible. In most cases, the clay and sediment isotherm measurements were made at two sediment loadings and two substrate concentrations. Biosorption studies were made at one level of the mixed bacteria culture and two levels of substrate. Replicate flasks were used at each level, and at least three analyses of each flask were made. Suitable blanks of both sorbent and substrate were carried through the experimental steps and analyzed. Contact times of 1 to 16

hours were used. The equilibrium partitioning is probably reached in about 1 to 2 hours. There were no experimental problems in the sediment sorption studies with the 16-hour time, except for p-cresol, which biodegraded rapidly during the experiment. In that case, a 1-hour exposure was used. For the biosorption studies, the partitioning time was about 1 hour. At longer times, sorption by the glassware was a problem with low-solubility substrates.

As the experiments progressed, the experimental plan for the isotherm measurements evolved into the experimental plan described in Table 5.4. We consider this to be the minimum number of data points that will permit a sound statistical analysis of the data.

TABLE 5.4. RECOMMENDED EXPERIMENTAL PLAN FOR ISOTHERM MEASUREMENTS

Substrate concentration	Number of flasks[a]		
	No sediment	Low sediment	High sediment
None	1	2	2
Low	2	2	2
High	2	2	2

[a]Four replicate measurements of the substrate concentration at equilibrium in each flask should be made.

The mechanics of the isotherm measurements were generally the same whether the sorbent was Ca-montmorillonite clay, a natural sediment, or our bacterial mixture. A solution* of the substrate in water was prepared. An aliquot of the substrate solution and an aliquot of a suspension of the sorbent were mixed and allowed to shake for a specified period of time. The mass of dry sorbent used was determined, normally by a gravimetric procedure, in a separate experiment. A portion of the mixture was centrifuged to separate the substrate remaining in solution and the sorbent. The supernatant and usually the sorbent were analyzed separately to measure the substrate concentration.

5.4.2.3 Statistical Analysis of Isotherm Data--The statistical analysis of the isotherm data on clays and sediments was considered carefully.

*The importance of using true solutions below the substrate solubility limit cannot be overemphasized, especially with low-solubility substrates.

The simplest, and often adequate, procedure is to fit the data to the Freundlich isotherm equation with n = 1,

$$S_s = K_p S_w \tag{5.19}$$

The value of S_w was always measured. If S_s was not measured independently, then it was calculated from

$$S_s = (S_o - S_w)V_w/m_s \tag{5.20}$$

where S_o is the initial concentration of substrate used (determined from the concentration in flasks without sorbent), V_w is the volume of solution (in ml), and m_s is the mass of sediment (in grams) added to the flask. Preliminary estimates of K_p were obtained by two methods: when only S_s was measured, every concentration measurement was used, and when both S_s and S_w were measured, average S_s and S_w were calculated for each flask. In both cases the data were then fit to an equation of the form

$$y = bx \tag{5.21}$$

(notice the similarity to equation 5.19) using a linear least squares regression method. The regression equations are:

$$b = \frac{\Sigma x_i y_i}{\Sigma x_i^2} \tag{5.22}$$

$$S_{yx} = \left\{ \left[\Sigma y_i^2 - \frac{\left(\Sigma x_i y_i\right)^2}{\Sigma x_i^2} \right] (n-1)^{-1} \right\}^{\frac{1}{2}}. \tag{5.23}$$

$$r^2 = \left[-\frac{1}{n}(\Sigma y_i)^2 + \frac{\left(\Sigma x_i y_i\right)^2}{\Sigma x_i^2} \right] \left[\Sigma y_i^2 - \frac{1}{n}(\Sigma y_i)^2 \right]^{-1} \tag{5.24}$$

$$95\% \text{ confidence interval} = \left(t_{n-1,\alpha}\right) S_{yx} \left(\Sigma x_i^2\right)^{\frac{1}{2}} \tag{5.25}$$

where $b = K_p$ is the slope, x_i and y_i are the individual or average measurements from each flask of S_w and S_s, respectively, S_{yx} is the standard error, r^2 is the correlation coefficient, and $t_{n-1,\alpha}$ is the t-value from Student's

t-test for n measurements at α - 0.05 confidence. These expressions are in the form suitable for use in hand calculators, such as the HP-65.

It is also possible to test that the linear Freundlich isotherm (n = 1, equation 5.19) does pass through the origin. To do this, the data are fit to linear equation of the form

$$S_s = K_p S_w + a_o \tag{5.26}$$

where a_o is the intercept. The HP-65 Stat-Pac routine is used to estimate the 95% confidence intervals about a_o. If these confidence intervals include the origin, then equation (5.19) can be used. In the data we have tested so far, the linear Freundlich equation, passing through the origin gives the best fit of the data, based on the values of the correlation coefficient.

The isotherm data have also been fit to other equations, using linear regressions available in the HP-65 Stat-Pac routines. The Freundlich isotherm $n \neq 1$

$$S_s = KS_s^{1/n} \tag{5.18}$$

becomes

$$y = ax^b \tag{5.27}$$

The Langmuir equation can be written as

$$\frac{1}{S_s} = \frac{1}{K_p S_w} + \frac{1}{X_c} \tag{5.28}$$

and then fit to the form y = ax + b. However, this is not a good statistical analysis because the variables $1/S_w$ and $1/S_s$ are not normally distributed even if S_s and S_w are.

Strictly speaking, these linear least squares methods are not statistically correct. When only S_w is measured, S_s is calculated using equation (5.20) and a linear least squares method then has the response variable S_w on both sides of the regression equation. This makes the confidence limits for the parameter values invalid. The linear least squares procedure is also inefficient because it does not use all the data to estimate S_o.

The limitations of the linear method can be removed by stating the problem as:

- When sorbent is present

$$S_w = \frac{\hat{S}_i - \hat{a}_o}{1 + M_s \hat{K}_p / V_w} \quad ; \; i = h \text{ or } \ell \tag{5.29}$$

- When sorbent is not present

$$S_w = S_i \quad ; \; i = h \text{ or } \ell \tag{5.30}$$

The hat on a variable indicates that its value is estimated by the regression procedure. $\hat{S}_i$ is equivalent to S_o and represents the original amount of substrate present in each flask, $\hat{S}_h$ being the concentration in flasks with the high amount of substrate and $\hat{S}_\ell$ being the concentration in flasks with the low amount of substrate. M_s represents the amount (grams) of sorbent present. In this procedure, $\hat{S}_h$ or $\hat{S}_\ell$ is estimated using all the flasks as in the linear method.

Since each flask is handled separately and at a different time, the above procedure was modified to include "flask effects" as an estimated parameter. Flask effects include such things as biases due to instrument drift and systematic errors on the part of the analyst. The resulting problem formulation is suitable for input to a nonlinear regression program that estimates values for S_h, S_ℓ, a_o, K_p, and the flask effects. The actual results of the nonlinear regressions are comparable to the estimates obtained from the linear least squares regressions, except that the nonlinear approach gives smaller confidence limits for the parameter estimates.

When both S_s and S_w are measured, the linear least squares procedures using average values for S_s and S_w are not statistically correct because again response variables appear on both sides of the regression equation. In addition, the averaging procedure throws away valuable information about experimental variance. To deal more correctly with this situation, we use eight simultaneous nonlinear regression equations. Four regressions use only the substrate concentrations measured on the sediment from the various flasks.

$$S_s = \hat{K}_p \hat{S}_i \qquad i = 1, 2, 3, 4 \tag{5.31}$$

and four regressions use only the concentrations measured in the supernatant, S_w,

$$S_w = \hat{S}_i \qquad i = 1, 2, 3, 4 \tag{5.32}$$

where $\hat{S}_i$ is the estimated value of substrate concentration in a particular flask. With this formulation, the response variables appear only on the left-hand side of the regression equation. The subscript "i" on the right-hand side is the independent variable, since it indicates the conditions used to set up the flask. For example, i = 1 indicates the two duplicate flasks that contained high sediment and high substrate concentrations. The common parameters $\hat{K}_p$ and $\hat{S}_i$ tie the simultaneous regressions together and assure that the resulting estimate for K_p is conditioned on both the sediment and supernatant concentrations measured.

The method is superficially similar to the simple linear least squares procedure used to estimate K_p. The regression equations (5.32) use the supernatant concentrations to estimate a single concentration that best represents the concentration in the flasks for each different substrate and sediment level. This representative concentration is then used with the concentration of supernatant on the sediment in equations (5.31) to estimate K_p. An important difference between the two approaches, however, is that, with the nonlinear approach, the supernatant concentration, $\hat{S}_i$, that represents a particular substrate and sediment level is not necessarily the average supernatant concentration. The values of $\hat{S}_i$ determined by the method are almost always very close to the average except when concentration measurements are highly scattered.

An important feature of nonlinear approach is that it does not require that the same number of observations of both S_s and S_w be made in each flask. The formulation also does not require that individual measured values of S_s and S_w from a particular flask be paired (see equation 5.19). This is an important feature because any pairing of data points is artificial and could bias the estimate of K_p.

5.4.3 Biosorption and Desorption

Biosorption and desorption on biomass are important because they may affect biomagnification up the food chain and the "available" concentration of a substrate for biodegradation. They may also affect the viability or growth of micro- and macroorganisms that may participate in biodegradative reactions. All these factors have been demonstrated in various studies with polyaromatic hydrocarbons, which are of much concern as potential carcinogens.

Our biosorption and desorption studies were conducted with mixtures of four species of gram-positive and gram-negative aquatic-origin bacteria that had frequently been used in various microbiological assays and had no record of functioning as degraders of the types of compounds studied. The mixtures contained equal optical densities of Azotobacter beijerinckii ATCC 19366, Bacillus cereus ATC 11778, Escherichia coli ATCC 9637, and Serratia marcescens ATCC 13880. In the early stages of this program, Flavobacterium capsulatum ATCC 14666 was used, but because this organism was difficult to centrifuge to a compact pellet and clear supernatant, it was replaced with the above indicated Serratia marcescens.

The test organisms were transferred several times in Trypticase-Soy broth at 25°C before they were used for sorption studies. Sixteen-hour cultures were either in the late logarithmic or early stationary growth phases. At this stage, each culture was harvested by centrifuging, washed with 0.05% potassium phosphate buffer (pH 7.0), resuspended and diluted with this buffer until the suspension had an optical density of 2 to 4.

Appropriate aliquots of suspensions of each of the four organisms were combined and diluted with buffer to form a mixture containing equal optical densities of each organism and a mixture that, when mixed with the solution of the substrate, resulted in the desired concentration and organisms. With substrates having a low solubility, the density of the bacterial mixture was lower than with more soluble substrates.

In some instances, biosorption studies were also conducted with heat-killed cells. Consequently an aliquot of the above mixture of organisms was heated at 100°C for 15 minutes, cooled, and centrifuged. The resulting pellet was resuspended in fresh buffer to the original volume, and an appropriate volume of this suspension was diluted as above with a solution of the substrate under study to yield the corresponding cell densities and substrate concentrations.

Biosorption studies were conducted by incubating the viable and heat-killed cell mixtures in Corex centrifuge tubes or bottles for 1 hour at 25°C. Cells were maintained in suspension by placing the containers in roller drums or on a rotary shaker. The tubes or bottles were centrifuged for 10 minutes at 12,000 or 16,000 G, respectively, and the supernatants were carefully decanted. The supernatants were extracted with an organic solvent (usually ethyl acetate). The solvent extract was dried and then assayed directly or concentrated before assay. To assay sorbed substrate in the pellets, water was added and this suspension was solvent extracted as above. With some substrates that were tenaciously retained by the cell pellets, the water-suspended cells in the presence of some solvent were slowly frozen and thawed three times before extraction.

Desorptions were conducted only if the sorption partition coefficients were 10,000 or more. The cell pellets from replicate sorption studies were suspended in volumes of buffer or buffer and solvent equivalent to those used for sorptions, incubated with shaking at 25°C for 3 hours, and centrifuged. Both supernatants and pellets were analyzed by the procedures used for sorption determinations.

In some cases, corrections were made for adsorption on glassware of substrate and cultures containing sorbed substrate. Separate controls consisted of extraction of tubes from which the incubated suspensions in test substrate solutions were decanted in lieu of separation of cells by centrifuging.

Dry weights of cells used in sorption studies were determined by weighing the pellet obtained after cells from aliquots of mixed viable or heat-killed bacterial suspensions were centrifuged, washed with distilled water, and dried for 16 hours at 90-95°C.

The biosorption partition coefficients of chemicals between bacteria and buffer were determined as

$$K_p = \frac{\mu g \text{ substrate per g dry wt of cells}}{\mu g \text{ substrate per ml in supernatant}} \quad (5.33)$$

The results obtained in these tests had good consistencies and may be regarded as indicators of sorption of the compounds on the bacteria. It would be interesting to compare these results with data that would be obtained with algae and protozoa or with biomass from natural reservoirs. The latter would present problems in separation of biomass from inorganic or humic materials that would also be centrifuged.

5.4.4 Discussion

The major problem in extrapolating the sorption partition coefficients obtained by these laboratory procedures to environmental conditions is that the composition of sediments and bacterial mixtures that would exist in the natural system change dramatically with the time of the year. Therefore, the composition of sediment and bacterial samples collected at one location are likely to be different even if they are collected only several days apart. In an attempt to overcome this problem, we have measured the sorption partition coefficient on several sediments and on a mixture of bacteria. On the basis of these and other studies, we estimate that the value of K_p for a specific sediment or group of organisms should not vary by more than a factor of 3 in the environment. Also, there is considerable evidence, both from our studies and from studies reported in the literature, that there is often good correlation between the magnitude of K_p and the total organic content of the sorbent.

6. CHEMICAL TRANSFORMATION

6.1 BACKGROUND

The chemical processes of photolysis, free radical oxidation, and hydrolysis described in this section can be important transformation processes for some chemicals in aquatic environments. Emphasis in this work has been on obtaining reliable kinetic data for use in estimating how rapidly these processes will occur in freshwater systems. Laboratory studies were conducted in pure water and in natural waters from three natural sources in California: Lake Tahoe (oligotrophic), Coyote Creek (eutrophic), and a pond near Searsville Lake (eutrophic). Chemical properties of these waters are given in Appendix A of Part II. All natural water samples were filtered through a 0.45-μm filter to minimize sorption problems and to ensure homogeneous solutions for kinetic studies. Kinetic data for reactions in these natural waters were compared with data for reactions in pure water to determine what effect, if any, the dissolved natural substances have on the rates of specific chemical processes.

When comparison with other processes under study indicated that a chemical transformation was an important pathway, the primary reaction products were identified or characterized by several procedures, including isolation by chromatography followed by spectrometric analyses. Knowledge of the reaction products is essential to understanding the chemical process and to any subsequent hazard evaluation that might be based on these data. Whenever possible, quantitative material balances were obtained to ensure that other unsuspected physical, chemical, or biological processes were not occurring simultaneously.

As a further check on the validity of the kinetic measurements, control or blank experiments were carried out along with the kinetic experiments. Where preliminary studies indicated the importance of biological transformations, sterile conditions were maintained during chemical experiments. Precautions were taken to exclude losses through volatilization, and glassware was continually checked to identify any losses through adsorption. In a very few cases, competing processes could not be excluded entirely, and corrections for these processes were made in the kinetic data obtained. These problems emphasize the need for good material balances and control experiments in order to obtain reliable chemical kinetic data and relationships for environmental processes.

It is important to recognize that the procedures described below are intended to describe experimental laboratory procedures for reliably evaluating chemical transformation processes in the solution phase of aquatic systems.

Although the immediate application of this methodology is for environmental exposure assessment, the data and procedures also serve as basis for investigations of the more complex features of aquatic environments, including the effects of suspended solids and sediments and dissolved natural organics in some waters on these environmental transformation processes.

6.2 PHOTOCHEMISTRY

The cutoff for the solar spectrum by the upper atmosphere is at about 290 nm, and in aquatic systems only absorption of photons of this or longer wavelengths can result in photochemical transformations. These transformations may occur through direct photolysis of compounds that absorb light above 290 nm or through photosensitized reactions involving other light absorbing organic substances found in natural waters. Although the kinetics and mechanism of direct photolysis of compounds can usually be evaluated using present theory and experience, the details of sensitized photolyses in which organics in natural waters act as sensitizers are largely undefined. An excellent discussion of environmental photochemistry has recently been published (Wolfe et al., 1976).

The rate of absorption of light, I_A (rate constant k_a), by a chemical at one wavelength is determined by: the molar absorbtivity ε (also called the molar extinction coefficient), a term I_λ proportional to the intensity of the incident light, and the concentration of substrate [S] at concentrations of S where only a small percentage of the light is absorbed (Zepp and Cline, 1977)

$$I_A = \varepsilon I_\lambda [S] = k_a [S] \tag{6.1}$$

where $k_a = \varepsilon I_\lambda$. The rate of direct photolysis of a chemical (rate constant k_p) is then obtained by multiplying I_a by the quantum yield ϕ, which is the efficiency for converting the adsorbed light into chemical reaction, measured as the ratio of moles of substrate transformed to einsteins of photons absorbed.

$$-\frac{dS}{dt} = k_a \phi [S] = k_p [S] \tag{6.2}$$

and

$$k_p = k_a \phi \tag{6.3}$$

The simplest and most direct method of using laboratory experiments (as contrasted to field studies) to estimate environmental photolysis rates is to expose an aqueous solution of a chemical to outdoor sunlight and monitor its rate of disappearance. However, the data obtained are of limited use because

sunlight intensity varies with the time of day, season, latitude, weather conditions, and light scattering. Thus, any outdoor experiment has questionable value in application to other conditions of sunlight irradiation.

Another method for estimating environmental photolysis rates has been described by Wolfe and coworkers (1976) and by Zepp and Cline (1977). This procedure calculates the rate constant k_p from values of ϵ and ϕ measured in laboratory experiments; the sunlight intensity (I_λ) data as a function of time of day, season, and latitude are available in the literature. Thus photolysis rates can be estimated for different environmental conditions.

Both the molar extinction coefficient (ϵ) of a chemical and the sunlight intensity (I) vary as functions of wavelength. The average value of the absorption coefficient, ϵ_λ, for a specified wavelength interval centered at wavelength λ (see Table 5.1 for wavelengths λ and the wavelength intervals) is determined from the absorption spectrum of the compound (Section 5.2). The absorption rate constant, k_a, for a compound absorbing in the solar spectrum is then obtained by summing the product of ϵ_λ and I_λ over all wavelengths, where $\epsilon > 1\ M^{-1}\ cm^{-1}$ and I_λ is the solar intensity over the wavelength interval centered at λ for a selected latitude and season or time of day.

$$k_a \propto \Sigma I_\lambda \epsilon_\lambda \tag{6.4}$$

When ϵ_λ is expressed as the molar absorption coefficient ($M^{-1}\ cm^{-1}$),

$$k_a = \frac{2.303}{J} \Sigma I_\lambda \epsilon_\lambda \tag{6.5}$$

where $J = 6.02 \times 10^{20}$ is a conversion constant that makes the units of I and ϵ compatible.

The rate constant for photolysis (k_p) is equal to the product of k_a and the quantum yield ϕ; generally ϕ does not vary significantly with wavelength.

$$k_p = \frac{2.303}{J} \phi \Sigma I_\lambda \epsilon_\lambda \tag{6.6}$$

Assuming the reaction is first order in the chemical, the half-life for photolysis is given by

$$\left(t_{1/2}\right)_p = \frac{\ln 2}{k_p} \tag{6.7}$$

The computer program used to make these calculations gives a plot of the half-life of the chemical toward photolysis as a function of the month of the year. These half-lives are based on the average values of k_p for a full day's photolysis. For half-lives of less than several days, variations in sunlight intensity during the day must also be considered. For these shorter half-lives, the computer program provides data for half-lives as a function of the time of day.

Laboratory measurements of ϕ were carried out using a merry-go-round apparatus (Moses et al., 1969) to achieve even irradiation of all reaction tubes.

We used a 450-watt medium pressure Hg lamp because it provides intense lines at 313 and 366 nm that are easily isolated by filters (Calvert and Pitts, 1966).

Samples of chemicals dissolved in water were placed in borosilicate tubes in the merry-go-round and irradiated for periods from several hours to days at each wavelength. Tubes were withdrawn at various intervals and analyzed for starting chemical. Light intensity at each wavelength was measured periodically using an o-nitrobenzaldehyde actinometer (Pitts et al., 1964) in tubes similar to those used for reaction mixtures. In most cases these photolyzed solutions of chemicals were also used for product analyses (Section 6.1).

In cases where the laboratory photolyses proceeded slowly under monochromatic light, photolyses were also carried out using only the borosilicate glassware as a filter. Although borosilicate cuts off wavelengths below 280 nm, it does not isolate a single wavelength and quantum yield measurements cannot be made. However, the greater light intensity transmitted by the borosilicate filter allowed the photolyses to be carried out rapidly to beyond two half-lives to establish that the reaction was first order in substrate. This information was needed for extrapolation of the rate data in the environmental assessment.

Outdoor photolyses using sunlight were also carried out with each chemical to validate the computer calculation of half-life in sunlight based on measured values of ε_λ and ϕ. Sunlight photolyses require some attention to placement of apparatus. Ideally, the photolysed solutions should be in a location free of excessive reflections from walls and windows and without morning and afternoon shadows. Although large diameter dishes with flat transparent tops (petri dishes, for example) are preferred, we used 11-mm-O.D. borosilicate tubes held in a rack at a 60° angle to the horizon. Test tubes are much more readily sealed for long exposure and, judging from the good agreement between computed and measured values for $t_{1/2}$ in sunlight for most chemicals, are quite satisfactory for this purpose.

When possible, photolyses were carried out in pure water or in filtered natural waters with no cosolvent. In many cases, however, it was necessary to include up to 1% acetonitrile by volume as cosolvent because the solubility of the chemical in water alone was very low. Acetonitrile was chosen because it would not act as sensitizer at wavelengths in the solar spectrum or take part in any free radical reactions that might occur in photolyses. Initial concentrations of chemicals in the photolyzed solutions were usually 1 $\mu g\ ml^{-1}$ or less, and for each chemical some photolyses were carried out to at least two half-lives to verify that the reactions were first order in substrate as predicted by equation (6.1).

For systems that absorb less than 5% of the incident light (< 0.02 absorbance) at wavelength λ, ϕ may be calculated from the photolysis rate constant k_p obtained from the slope of the first-order plot of the photolyses data using the integrated form of equation (6.1).

$$\ln (S_o/S) = k_p t \tag{6.8}$$

$$\phi_\lambda = k_p / 2.3\varepsilon_\lambda I_\lambda \ell \tag{6.9}$$

where ℓ is the pathlength, S and S_o are substrate concentrations at times t and t_o, and I_λ and ε_λ are as defined above. The value of I_λ may change from time to time and requires periodic calibration using the actinometer to correct for such changes.

When comparisons were made between measured and calculated half-lives for chemicals in sunlight at 40°N. latitude (about that of Menlo Park, California) using procedures described above, we found excellent agreement, usually within a factor of two. These results support our assertion that laboratory measurements of this kind can provide reliable estimates of half-lives toward photolysis in sunlight.

In some aquatic environments, however, rates of photolysis may differ significantly from those measured in pure water owing to the presence of naturally occurring light absorbers, quenchers, or sensitizers. In water, naturally occurring materials such as humic or fulvic acids, which have high optical densities, may absorb sunlight and effectively screen the chemical from being photolyzed. The presence of particulate materials in water may also result in light scattering. In both cases the photolysis rate of the chemical would be slower than in pure water because the physical processes reduce the light available for reaction.

Materials present in natural waters may also either accelerate or retard photolyses of substrates through chemical processes. Acceleration of photolysis rates for some pesticides in natural waters has been demonstrated (Wolfe et al., 1976). In most experiments it was not determined whether the rate acceleration was due to a photosensitized reaction or to a photoinitiated free radical process.

Both processes occur through absorption of light by the natural substance, which then interacts with the chemical. In the photosensitized reaction, the excited-state energy from the sensitizer is transferred to the chemical, which then undergoes reaction; the identity of the sensitizer is maintained. In the photoinitiated reaction, the natural substance that absorbed the light reacts with the chemical and both materials are transformed. If either process is more rapid than direct photolysis of the chemical, the rate of photolysis will be accelerated. However, in natural waters with significant optical densities, an acceleration of photolysis due to either mechanism may be somewhat offset by the screening capability of the water.

Results obtained in this project also indicate that in some cases the presence of natural substances in water may make the photolysis rate slower than it would be in pure water. The reason for this effect is not known, but it is not due to a screening effect, since the natural waters in which the observations were made had absorbances of < 0.02 at 366 nm where the photolyses were carried out.

Since the presence of natural water can either accelerate or retard the photolysis of a chemical, half-lives based on pure water photolyses must be interpreted with some caution. If experiments in natural waters give faster photolysis rates than in pure water, the photolysis rate in pure water is useful as a conservative value (i.e., maximum half-life). When the photolyses in

natural waters are slower than in pure water, the half-life estimate obtained for the pure water photolysis should be used with an appropriate qualification that in some cases longer half-lives may occur and that more experiments may be needed to determine how much slower the photolysis is likely to be.

6.3 FREE RADICAL OXIDATION

Oxidation of organic compounds by free radical processes may be important under some environmental conditions. The most general reaction scheme for radical oxidation with an azo initiator is

$$R_2N_2 \xrightarrow{k_d} 2R\cdot + N_2 \qquad \text{initiation} \qquad (6.10)$$

$$R\cdot + O_2 \xrightarrow{\text{fast}} RO_2\cdot \qquad (6.11)$$

$$RO_2\cdot + XH \xrightarrow{k_{ox}} RO_2H + X\cdot \qquad \text{oxidation} \qquad (6.12)$$

$$2RO_2\cdot \longrightarrow (2\ RO\cdot) + O_2 \qquad (6.13)$$

$$(2\ RO\cdot) \longrightarrow 2RO\cdot$$

$$(2\ RO\cdot) \xrightarrow{k_x} \text{termination products}$$

$$RO\cdot + XH \xrightarrow{k_{AB}} ROH + X\cdot \qquad \text{oxidation} \qquad (6.14)$$

$$RO\cdot \xrightarrow{k_d} \text{cleavage products} \qquad (6.15)$$

The rate of oxidation of compound XH is then

$$r_{ox} = -d[XH]/dt = k_{ox}[RO_2\cdot][XH] + k_{AB}[RO\cdot][XH] \qquad (6.16)$$

To evaluate the potential importance of oxidation under environmental conditions, we need to be able to evaluate equation (6.16) for specific compounds in specific environments. Values for rate constants k_{ox} and k_{AB} are known reliably for many organic compounds in organic solvents (Hendry et al., 1974) but have rarely been measured in water or for most of the organic compounds studied here. For these reasons we developed a simple screening experiment that provides a reasonably reliable method for evaluating k_{ox} in water for compounds of interest and a reliable method for evaluating relative reactivities toward $RO_2\cdot$ for a series of compounds.

As a source of $RO_2\cdot$ we have chosen a commercially available azo initiator, 4,4′-azobis(4-cyanovaleric acid) (AA)

$$[HO_2CCH_2CH_2C(CN)(Me)]_2N_2$$

AA is slightly soluble in water and decomposes at 50°C with a rate constant k_d of 1.9×10^{-6} sec^{-1} ($t_{½}$ = 100 hours) to give two carbon radicals, a fraction of which is rapidly converted to peroxy radicals in the presence of oxygen.

$$RN_2R \xrightarrow{k_d} 2R\cdot + N_2 \qquad (6.17)$$

$$2R\cdot \xrightarrow{(1-e)} \text{products} \qquad (6.18)$$

$$2R\cdot \xrightarrow{e,\ O_2} 2RO_2\cdot$$

The rate of production of RO_2 is $d[RO_2\cdot]/dt = 2ek^d[R_2N_2]$ where e is the fraction of radicals R· that are available for oxidation. In a separate study (Mill et al., 1977) e has been evaluated as 0.6, which is very similar to values of e found for other azo compounds in organic solvents (Denisov, 1974). Under conditions where only a small concentration of [XH] is oxidized compared with the total concentration of $RO_2\cdot$ generated, the instantaneous concentration of $RO_2\cdot$ (steady-state) depends only on the rates of initiation and termination. Moreover, under these conditions the only fate of RO· is to cleave (reaction 6.15); therefore, equation (6.16) simplifies to:

$$r_{ox} = k_{ox}[RO_2\cdot][XH] \qquad (6.19)$$

Experiments were carried out by heating an air-saturated aqueous solution containing about 10^{-4} M AA and the chemical below its solubility limit for up to 100 hours at 50°C, a half-life for AA. In some cases analyses for the chemicals were made at periodic intervals, and in other cases replicate analyses were made at 100 hours. A first-order plot of the data (log concentration versus time) at two or more times gave a straight line corresponding the relation:

$$\ln([XH]_t/[XH]_o) = -k_{ox}[RO_2\cdot]t \qquad (6.20)$$

with a slope equal to $-k_{ox}[RO_2\cdot]$.

With these concentrations of AA and XH used in most experiments, the value of $[RO_2\cdot]$ may be calculated from the steady-state assumption that the rates of initiation and termination are equal:

$$[RO_2\cdot] = (2ek_d[AA]_{av}/2k_x)^{\frac{1}{2}} \qquad (6.21)$$

where $k_d = 1.9 \times 10^{-6}$ sec^{-1}, e = 0.6, and $2k_x = 2 \times 10^7$ M^{-1} sec^{-1}.*

* A. Howard, National Research Council of Canada, private communication, 1977.

With [AA] = 7.5×10^{-5} M, [$RO_2\cdot$] is then 2.9×10^{-9} M. From the slope of the line described by equation (6.20):

$$k_{ox}^{50°} = \text{slope}/(2.9 \times 10^{-9}) \tag{6.22}$$

The value of $k_{ox}^{25°}$ may be calculated from the value of $k_{ox}^{50°}$ by assuming that the activation energy for the reaction of $RO_2\cdot$ with XH has an average value of 10 kcal $mole^{-1}$(41.8 kJ $mole)^{-1}$, which corresponds to a factor of nine in rate. Thus:

$$k_{ox}^{25°} = 0.11k_{ox}^{50°} \tag{6.23}$$

The rate of oxidation and half-life at 25°C may then be calculated from this value of k_{ox} and an estimate of the concentration of $RO_2\cdot$ present in aquatic environments.

For purposes of this study, we have assumed that [$RO_2\cdot$] in aquatic environments is 10^{-9} M. This assumption is untested, but when combined with an experimental value of k_{ox}, it places a probable upper limit on the rate of oxidation and thus on the importance of oxidation under environmental conditions when compared with competing physical, chemical, and biological transformations.

6.4 HYDROLYSIS

Hydrolysis of organic compounds usually results in introduction of a hydroxyl function (-OH) into a chemical, most commonly with the loss of a leaving group (-X). These reactions

$$RX + H_2O \longrightarrow ROH + HX \tag{6.24}$$

$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}X + H_2O \longrightarrow R\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH + HX \tag{6.25}$$

may be catalyzed by acids or bases (rate constants k_A or k_B, respectively) or both. The kinetics of hydrolysis can be expressed as

$$R_h = k_h[S] = k_B[OH^-][S] + k_A[H^+][S] + k_N'[H_2O][S] \tag{6.26}$$

where k_h is the measured first-order rate constant at a given pH. The last term is the neutral reaction with water (second-order rate constant k_N'), and

in water it can be expressed as a pseudo-first-order rate constant k_N. Since with few exceptions, hydrolysis reactions are first order in chemical, the half-life of a chemical toward hydrolysis may be expressed as

$$t_{1/2} = \ln 2/(k_B[OH^-] + k_A[H^-] + k_N) = \ln 2/k_h \qquad (6.27)$$

From equations (6.26) and (6.27), it is clear that when k_B and/or $k_A \neq 0$, k_h will depend on pH. From the autoprotolysis water equilibrium,

$$[H^+][OH^-] = K_w = 10^{-14} \qquad (6.28)$$

equation (6.26) may be rewritten

$$k_h = \frac{k_B K_w}{[H^+]} + k_A[H^+] + k_N \qquad (6.29)$$

The contribution of each term to k_h will depend on the acidity (or pH) of the solution. Three regions may be defined:

Acid $$k_A[H^+] > k_N + \frac{k_B K_W}{[H^+]}, \log k_h = \log k_A + \log[H^+] = \log k_A - pH \qquad (6.30)$$

Base $$\frac{k_B K_w}{[H^+]} > k_N + k_A[H^+], \log k_h = \log k_B K_W - \log [H^+] = \log k_B K_W + pH \qquad (6.31)$$

Neutral $$k_N > k_A[H^+] + \frac{k_B K_W}{[H^+]}, \log k_h = \log k_N \qquad (6.32)$$

These expressions assume that the catalyzed processes are first order in $[H^+]$ or $[OH^-]$. Such behavior is almost always the case in the range of pH 2 to 12 and frequently extends to greater extremes.

The dependence of k_h on the pH of the solution is conveniently shown by a plot of log k_h as a function of pH (Figure 6.1). From expressions (6.30), (6.31), and (6.32), it is seen that in the pH range where the base-catalyzed process is dominant, a slope of +1 is found; a slope of -1 is found in the acid-catalyzed region. The neutral hydrolysis is pH independent and shows a slope of zero.

The present knowledge of the theoretical and experimental aspects of hydrolysis reactions makes laboratory studies of hydrolysis rates useful for en-

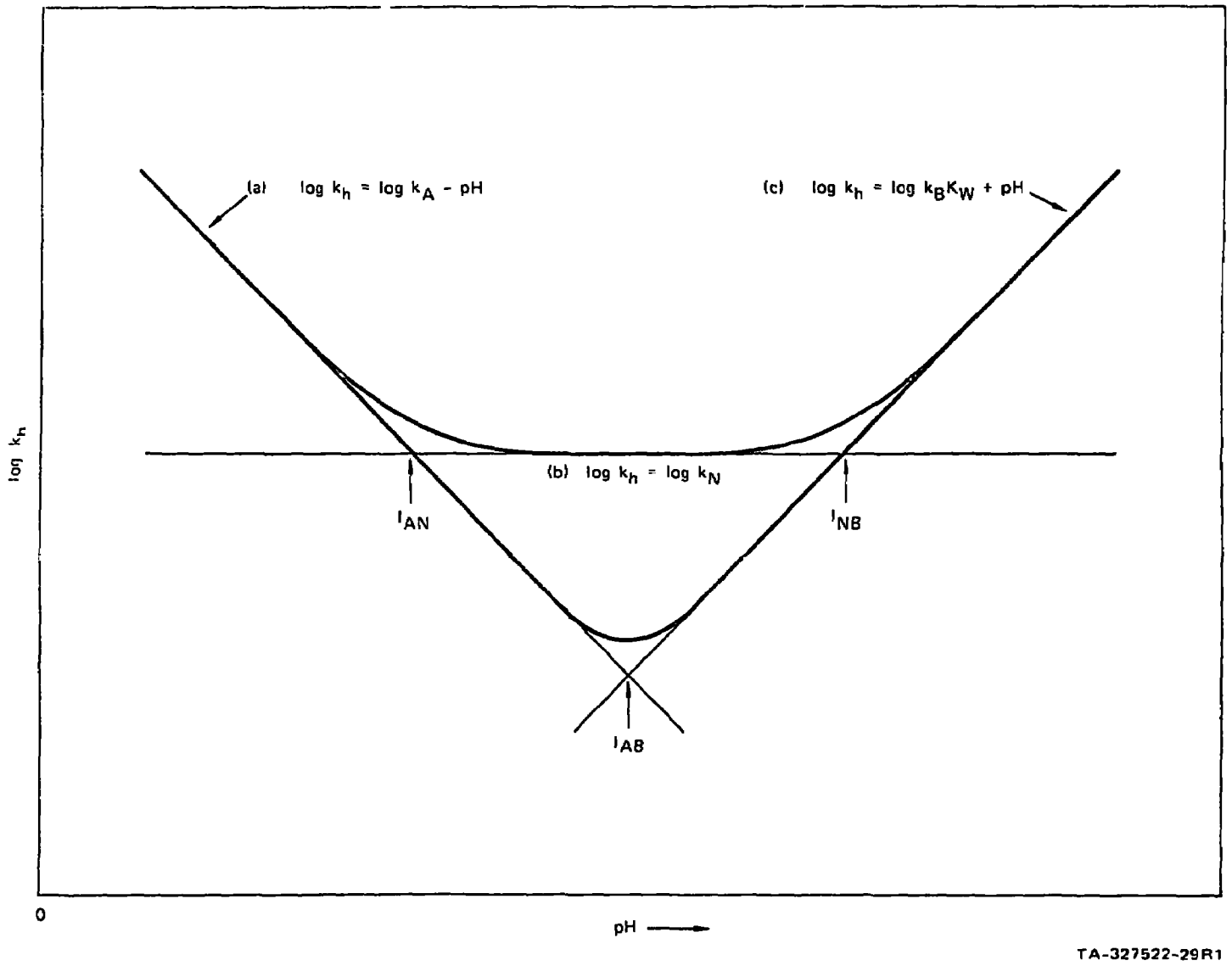

FIGURE 6.1. *p*H DEPENDENCE OF k_h FOR HYDROLYSIS BY ACID, WATER, AND BASE-PROMOTED PROCESSES

vironmental assessments. Precautions must be taken, however, to ensure that experimental artifacts are not introduced into the kinetic data. For example, the use of buffer salts to maintain constant pH is a necessary and acceptable practice, but some precautions should be taken to preclude effects of buffer catalysis and/or ionic strength.

Another problem may arise in the use of organic cosolvents in water to increase solubility. Large amounts of organic cosolvent will usually make the hydrolysis rate slower than it would be in pure water.

For chemicals that are moderately stable toward hydrolysis, it will expedite laboratory procedures to work at higher reaction temperatures (30° to 100°C). From data obtained at several temperatures, the rate constant at any environmentally relevant temperature can be calculated, using temperature relationships such as the Arrhenius equation

$$k = A \exp(-E/RT) \tag{6.33}$$

where E is the activation energy and A is the preexponential factor. However, such kinetic relationships hold only for rate constants for individual processes (k_A or k_B or k_N). If the temperature dependence of an observed rate constant k_h is measured at a pH where two processes contribute to the overall rate (e.g., k_N and k_B), k_h is then the sum of the rate expressions:

$$k_h = k_N + k_B[OH^-] \tag{6.34}$$

$$= A_N \exp(-E_N/RT) + A_B \exp(-E_B/RT)$$

Thus the measured temperature dependence of k_h is a complex function of T and useful only at the one pH. The pH profile indicates where each rate process is dominant and thus where temperature dependence measurements are most usefully made. Arrhenius parameters obtained in the pH regions of the pH profile where only one process is important (slope of -1, 0, or +1 for acid, neutral, and base processes, respectively, in Figure 6.1) then allow for calculation of rate constants for individual processes any any temperature, and the constants may then be combined in equation (6.29) to estimate the rate constant k_h that will be relevant to any particular environmental pH and temperature.

For compounds that hydrolyze extremely slowly around 25°C and thus have very long half-lives, a semiquantitative estimate of k_h or $t_{\frac{1}{2}}$ may suffice for assessment purposes if other environmental processes are relatively fast. In those cases, the rate constant for hydrolysis at some high temperature where the rate is reasonably fast may be measured and then extrapolated to 20°-25°C using the rule of thumb that the value of k_h will change by a factor of two for each 10° change in temperature. This rule of thumb assumes that the energy of activation for hydrolysis is about 18 kcal $mole^{-1}$ (75.3 kJ $mole^{-1}$).

Since the actual values of E_h range from 15 to 28 kcal $mole^{-1}$ (factors of 1.8 to 3), the extrapolated rate constants are only semiquantitatively correct.

Hydrolysis data can be used in environmental assessments at selected pHs and temperatures with considerable confidence, provided the chemical is dissolved in the water rather than suspended or emulsified. Although catalyses of hydrolysis by metal ions and nucleophiles are known, the concentration of such catalytic substances in the water column are so low that the rates of these catalyzed hydrolyses are insignificant compared with rates of the neutral and H^+ and OH^- catalyzed processes. Moreover, the concentrations of the active metal ions or nucleophiles available for reaction may be lower yet, due to complexation and association with natural substances present in natural waters. It is the availability of the catalytically active form and not the mere presence of the species that would result in any contribution to the hydrolysis rate of a substrate.

7. BIODEGRADATION

7.1 BACKGROUND

The techniques used in evaluating the biodegradability of organic substrates have varied extensively, and it is doubtful that any one procedure can be used to indicate susceptibilities to biodegradation in aquatic or soil environments. The phenomena are too complex and varied with some of the substrates that are difficult to degrade. Alexander (1965) introduced the term "recalcitrance" to define the characteristic of a compound that resists microbial biodegradation and presented some explanations of this microbial fallibility. There has been much research, elucidation of metabolic pathways, and theorizing on characteristics that are involved in biodegradation or recalcitrance of organic products. An additional complexity is that some readily biodegradable substrates can resist biodegradation when small quantities are strongly sorbed on soil or clay particles, particularly if these substrates are deposited or sorbed in locales or microenvironments into which microorganisms cannot penetrate.

Microorganisms are highly susceptible to frequent enzymatic reorientation in response to environmental change or alteration in substrate availability. Since the discovery of plasmids, many microbial degradations have been attributed to enzyme systems synthesized by these DNA particles. The phenomena of repression, derepression, induction or enrichment by analogs, and availability or lack of availability of other substrates and nutrients play important and differing roles with various culture-substrate combinations.

The phenomenon of cooxidation or cometabolism can also be very important in biodegradations. This involves the metabolism of a nongrowth-promoting substrate only when it is present with a growth-promoting substrate. Interpretations of these terms have been broadened to include growth-promoting substrates that do not necessarily have chemical structures very close to the substrate under study. In nature, organisms are exposed to a large variety of chemicals, and cometabolism can be very important.

Techniques frequently used in biodegradation studies involve pure cultures obtained from random isolations, culture collections, or enrichment cultures. Enrichment cultures are frequently mixtures of organisms that are developed by adding and incubating a water, soil, compost, or other natural substance in a medium initially or finally containing the substrate under study as the sole carbon source. In nature, of course, constantly changing mixed culture systems are invariably involved.

Analog enrichment or induction refinements involve the addition of more readily metabolizable compounds, chemically related to the enrichment substrates. If metabolism of the substrate depends on the presence of the analog, a cometabolic process is generally involved. However, in some cases the added chemical functions as a hydrogen or oxygen donor, or as an organic carbon substrate. Examples of the former are some dehalogenations or reductions of nitro groups. Under anaerobic conditions, it is not unusual to isolate systems that can reduce nitro groups or halogenate organic compounds. Another type of complex biodegradation is the metabolism of hydrocarbons by sulfate-reducing organisms. These conditions are not analog enrichment processes, but depend on the presence of a biologically reducible inorganic substrate and hydrogen donor organic compounds. These organic compounds are eventually converted to products that may be assimilated for microbial growth.

Not to be overlooked are the phytoplankton and protozoa that may be involved in environmental metabolism, but have received less research attention because of their complexities.

Most of our current knowledge of metabolic mechanisms has been derived with pure cultures and their mutants under growth conditions, as resting cells, or with their enzyme preparations. Mixed culture systems present complications in maintenance of their component character because of varying growth rates and a host of antagonistic and synergistic relationships. Included in these complex phenomena are high biosorptive characteristics for some substrates. Under these conditions, the "available" concentrations of substrates may be reduced to such a degree that organisms capable of metabolizing a substrate do not have sufficient organic carbon available for growth before they die.

In natural waters, there are normally many types of microorganisms and these may vary with the water body, season, and organic substrates being introduced. The studies described in this report were designed to reflect many of these factors. In the screening studies, we attempted to obtain biodegrading systems by enrichment procedures. If we obtained one or more biodegrading systems, detailed studies were conducted to determine the biodegradation rate characteristics of one of these systems. The specific procedures used are described in Sections 7.2 and 7.3. Isolation and identification of major biodegradation metabolites are described in Section 7.4.

7.2 DEVELOPMENT OF ENRICHMENT CULTURES

The objective of the biodegradation studies was to develop a rapid inexpensive experimental approach that would approximate natural conditions if selected compounds were introduced into freshwater environments. Under the provisions of the contract, the enrichment studies included:

- Enrichment techniques under aerobic conditions.
- Enrichment studies to be completed within six weeks. Within this period, subtransfers were made to develop enrichment culture systems that could utilize selected substrates as the sole carbon sources.

- The use of a biodegrading enrichment system in kinetic and metabolite studies without isolation of a pure culture that utilizes the substrate.
- Identification of major metabolites.

If an enrichment culture that could degrade the substrate as a sole carbon source was not developed within six weeks, no metabolite or kinetic studies were carried out.

It is understandable that any isolation procedure, whether it is an enrichment procedure or the isolation of single colonies from natural habitats, favors isolation of certain types of organisms and does not express the total microbial potential or populations in the natural habitat. Enrichment procedures such as those that were used in this work favor specific types of microbial populations, and many types of organisms that are present in the environmental sample cannot survive the competitive aspects of the process.

The principal natural aquatic reservoirs used as representative sources for cultures were:

- A eutrophic pond near Searsville Lake in Woodside, California.
- Coyote Creek, a eutrophic stream in San Jose, California.
- Aeration effluent from the Palo Alto, California, sewage treatment facility. The organic matter treated is primarily of domestic origin.
- Aeration effluent from the South San Francisco treatment plant. Approximately 45% of the biological loading in this facility is of miscellaneous industrial origin.
- Aeration effluent from the treatment plant in the Shell Oil Refinery, Martinez, California. This plant treats wastes that could have a great similarity to those that might be expected from a coal liquefaction plant.
- Aeration effluent from the sewage plant of the Monsanto Chemical Company installation in Anniston, Alabama, where parathion and methyl parathion are produced.
- Lake Tahoe, California, a large, deep, cold oligotrophic lake between California and Nevada.

Water samples were settled for approximately one hour and the supernatants were screened through fine-mesh polyester cloth. Four volumes of water sample were added to one volume of sterile 0.05% NH_4NO_3 or $(NH_4)_2SO_4$ and 1.0% KH_2PO_4/K_2HPO_4 solution. This salt solution was added to provide adequate nitrogen and to buffer the fermentation at pH 7.0.

In our enrichment procedures we used 4-liter water samples in a final 5-liter volume in the 9-liter bottle fermentors because we felt that large samplings could facilitate the development of biodegrading systems. The advantages of large volume samplings over traditional small samples became apparent in four experiments when 50-ml aliquots were also incubated in 250-ml shaker flasks in rotary shakers. This concept was supported by one experiment with p-cresol and three experiments with methyl parathion as substrates. In

these experiments, biodegrading systems were more rapidly developed in the 9-liter bottle fermentors. Dagley (1976) expressed his view that, in enrichment studies, samples may be generally too small.

The 9-liter bottles with the 1-liter of additive solutions were sterilized by autoclaving, 4-liter water samples were added, and then sterilized fittings were introduced into the 9-liter fermentors. These fittings included facilities for the introduction of sterile air through ceramic diffusers at the bottoms of the bottles, sampling ports, addition and pressure relief ports, and air exhausts through sterilizing filters. Inlet air was first humidified and partially sterilized by bubbling through 1% H_3PO_4 and then sterilized by passage through pyrex glass wool packed filters. Incubation was at 25°C. Lake Tahoe water samples were transported in ice-water baths and incubated at 15°C. Fermentors were shaken several times daily because the equivalent of 0.1 volume of air per minute was not adequate to maintain total suspension of some samples.

Occasionally, when it was apparent from previous studies with other water samples that enrichment cultures could be readily obtained, the first step of the enrichment process was conducted in cotton-plugged 2.6-liter Fernbach flasks containing 1.2 liters of 4:5 diluted water sample with proportional amounts of buffer and NH_4^+ salts. Adding the substrate as a powder might have introduced microorganisms and presented problems in obtaining fine suspensions. If the substrate had been added in a solution of a metabolizable solvent, additional carbon source would have been added. Preliminary studies indicated that dimethyl sulfoxide (DMSO) was not digested or inhibitory under aerobic conditions and that the concentrated DMSO solutions were self-sterilizing. Consequently, it was frequently convenient to add the substrate in a DMSO solution to obtain either a fine suspension or a solution of substrate in water.

In some of our enrichments, compounds with structures similar to the test chemicals were used in anticipation that they may be inducers of desired enzymes.

When possible, a rapid uv absorption assay, verified by gc or hplc, was used to monitor the breakdown of the test compound. In other cases, gc or hplc was used alone. When degradation was apparent, 2.5-ml aliquots from the 9-liter bottle fermentors or the Fernbach flasks were transferred to 250-ml Erlenmeyer shaker flasks containing 50 ml of basal salts medium at the original level, a twofold or threefold increased level of compound, with or without other nutrients including 50 μg ml^{-1} glucose with 10 μg ml^{-1} Difco Bacto yeast extract or with peptone. When degradation of a test substrate was nearly complete, successive transfers (1% to 2% by vol) were made to basal salts media with the same and higher (i.e., two levels) concentrations of substrate and lower amounts of other carbon nutrients. Eventually, no other added carbon source was used.

Each liter of basal salts medium contained: 1.4 g K_2HPO_4, 0.6 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.1 g NaCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.005 g $FeSO_4 \cdot 7H_2O$, and 1 ml of trace elements solution. The trace elements solution contained 0.1 g H_3BO_3, 0.05 g each of $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, Na_2MoO_4, and $CoCl_2 \cdot 6H_2O$ $liter^{-1}$.

Even after 10 to 15 serial transfers, the enrichment culture systems were usually mixtures of organisms. They were centrifuged and then suspended in sterile 5% DMSO-H_2O for 30 minutes; aliquots were preserved by freezing and storing them in the vapor phase of a liquid nitrogen storage tank.

7.3 BIODEGRADATION RATES

Once a biodegrading system was developed for a specific substrate, kinetic rate constants were determined so that a quantitative comparison might be made between the different pathways governing loss of a specific pollutant in aquatic environments. Four procedures for measuring kinetics were investigated and used with these mixed culture systems and with the substrate serving as the sole carbon source:

- Batch fermentations with low-level inocula of washed biodegrading cells
- Continous chemostat fermentations
- Cascade batch fermentation
- Batch fermentations with large microbial populations and low substrate levels.

Classical kinetic expressions were applied to the laboratory data to describe the rate of growth of an organism and utilization of a substrate when it was the growth-rate-limiting carbon source. These procedures were used successfully to obtain biodegradation rate constants, which were used in our environmental assessment models to compare the importance of biodegradation with the other transport and transformation pathways.

The Monod kinetic equations (Monod, 1949; Stumm-Zollinger and Harris, 1971), can be expressed as

$$\mu = \frac{\mu_m S}{(K_s + S)} \tag{7.1}$$

$$-\frac{dS}{dt} = \frac{\mu}{Y}X = \frac{\mu_m}{Y}\frac{SX}{(K_s + S)} = k_b \frac{SX}{(K_s + S)} \tag{7.2}$$

$$\frac{dX}{dt} = \mu X \tag{7.3}$$

where S is the concentration of substrate, μ is the specific growth rate, μ_m is the maximum growth rate, Y is the cell yield, X is the biomass per unit

volume, and K_s is the concentration of substrate supporting a half-maximum growth rate ($0.5\mu_m$). The utilization rate constant, k_b, is conventionally defined as

$$k_b = \frac{\mu_m}{Y} \tag{7.4}$$

It is implicit in these kinetic analyses that μ_m, K_s, and Y are constants.

The similarity of equation (7.2) to the Michaelis-Menton (1913) equation

$$-\frac{dS}{dt} = kE_o \frac{S}{(K_m + S)} \tag{7.5}$$

for the enzymatic decomposition of a substrate is apparent. In this equation, E_o is the maximum concentration of available enzyme, and K_m is the substrate concentration that produces half the maximum enzymatic velocity. In cellular metabolism, a much more complex situation exists.

Although Monod kinetics are based on the use of a pure culture and the rate of disappearance of a growth-rate limiting single substrate, these kinetic expressions can be used to obtain useful rate constants with mixed culture systems. These rate constants can be derived by various procedures in which there are specific limitations regarding relative values of X, Y, S, and K_s. In the following paragraphs, the various limits of the Monod expressions that must be built into the experimental plan will be examined in order to obtain simple relationships between the experimental variables X, S, and t.

For many of the more common substrates, K_s is on the order of 10^{-1} $\mu g\ ml^{-1}$ (Pirt, 1975), and this, in general, is considerably higher than the concentration that would be expected for chemicals in natural waters. If

$$S_o << K_s \tag{7.6}$$

equation (7.2) reduces to

$$-\frac{dS}{dt} = \frac{\mu_m}{Y K_s} XS = k_{b2} XS \tag{7.7}$$

where k_{b2} is a second-order rate constant equal to

$$k_{b2} = \frac{\mu_m}{Y K_s} = \frac{k_b}{K_s} \tag{7.8}$$

and the disappearance in substrate is first order in both X and S.

In batch fermentations with low-levels of inocula, the initial conditions were chosen so that

$$X_o << YS \tag{7.9}$$

With a small inoculum, there is generally a lag phase of growth to a biomass concentration that may be designated as X_a, and then a logarithmic or exponential phase of growth develops. During this rapid growth phase, S_o does not change significantly and the biomass concentration X at time t in this phase may be expressed as

$$\ln X = \mu t + \ln X_o \tag{7.10}$$

If ln X data obtained during this period are plotted against t, then μ is the slope of the line. A small inoculum facilitates a longer exponential phase of growth and facilitates a more accurate calculation of μ. This is particularly the situation during the early exponential phase when S has not changed significantly and there is less complication due to metabolites. These batch fermentations were conducted with different S_o values, and μ was determined for each value of S_o.

These μ values and the corresponding S_o values were used to calculate K_s and μ_m. Inverting equation (7.1) and multiplying by S_o results in the following equation:

$$\frac{S_o}{\mu} = \frac{K_s}{\mu_m} + \frac{S_o}{\mu_m} \tag{7.11}$$

It becomes apparent that when S_o/μ is plotted versus S_o, the slope of the line is $1/\mu_m$ and the intercept on the S axis is $-K_s$. This procedure was used by Lineweaver and Burk (1934) to determine K_m and E_o in the Michaelis-Menton equation (7.5). In most instances, by using the S and μ data from batch fermentations with low-level inocula, it was possible to obtain K_s and μ_m values, and then to calculate k_b and k_{b2} using equations (7.4) and (7.8).

In some cases, there were significant increases in biomass concentrations (X) before utilization of substrate was initiated and other kinetic analyses were used. Equation (7.2) was integrated by Stratton and McCarty (1967) and it can be written as

$$-\left[\frac{K_s}{X_o + YS_o} + \frac{1}{Y}\right] \ln (X_o + Y\Delta S) + \frac{1}{Y} \ln X_o$$

$$+ \frac{K_s}{X_o + YS_o} \ln \frac{SX_o}{S_o} = -k_b t = -\frac{\mu_m}{Y} t \tag{7.12}$$

If the initial experimental conditions were

$$X_o \ll YS_o \tag{7.9}$$

then

$$X_o \ll Y\Delta S \tag{7.13}$$

$$\frac{K_s}{X_o + YS_o} \approx 0 \tag{7.14}$$

and equation (7.12) reduced to

$$-\frac{1}{Y} \ln Y\Delta S + \frac{1}{Y} \ln X_o = -\frac{\mu_m}{Y} t \tag{7.15}$$

or

$$\ln \Delta S = \mu_m t + \ln\left(\frac{X_o}{Y}\right) \tag{7.16}$$

A plot of $\ln\Delta S$ versus t should be a straight line with a slope μ_m and an intercept (at $t = 0$) of $\ln(X_o/Y)$. This behavior was observed in the batch fermentations with low-level inocula.

The value of Y can be calculated since

$$Y = \frac{\Delta X}{\Delta S} = \frac{X - X_o}{S_o - S} \tag{7.17}$$

However, this procedure did not provide a value for K_s to calculate k_{b2} by equation (7.8). Stratton and McCarty (1969) developed a graphical procedure that can be used to determine K_s in batch degradations. This procedure depends on determining the time periods when there are equal utilizations or degradations of substrate (ΔS), with different S_o levels. Their equation (6) is

$$K_s = \frac{dS_n/dt - dS_m/dt}{[(dS_m/dt)/S_m] - [(dS_n/dt)/S_n]} \tag{7.18}$$

where S_m and S_n are the concentrations of substrate present at times when ΔS values were equal for two fermentations with different levels of substrate. The slopes of the S versus t curves at these times correspond to the dS_n/dt and dS_m/dt values. This procedure was used to determine K_s in some batch degradations

with low-level inocula and when it was more convenient to determine the time periods necessary for equal utilizations of substrates. By these methods, values for μm, Y, and K_s were calculated from batch fermentations using low-level inocula.

In continuous fermentations conducted in chemostats, when equilibrium was established, the dilution rate or $(\text{residence time})^{-1}$ was equivalent to μ at the concentrations of substrates present in the chemostats or in the overflow from the chemostats. If the dilution rates were changed and equilibria were established, new μ and S values were obtained. The values of K_s and μ_m were calculated by the Lineweaver-Burk plot procedure using equation (7.11). Then k_b and k_{b2} were calculated using equations (7.4) and (7.8).

In the cascade fermentations, low levels of inocula and relatively high S_o levels were used. Equations (7.16) and (7.17) were used to calculate k_b values, and K_s values from other procedures to convert k_b to k_{b2}. In this procedure, there was no culture selection as occurs in sequential transfers of enrichment systems on substrate/basal salts media.

Batch fermentations with large microbial populations and low substrate levels were observed to be pseudo-first-order reactions with respect to S (plots of lns versus t were linear for each case tested). In these fermentations, the microbial populations would not change significantly if all the substrate were utilized for growth purposes. This is a particularly useful procedure with substrates that have low solubilities and/or critical limitations in analyses.

The experimental data obtained under these conditions can be described by the equation (7.19)

$$\frac{dS}{dt} = k_b' S \tag{7.19}$$

where k_b' is a pseudo-first-order rate constant. A choice of different X_o would give a different value of k_b. Based on equation (7.7), which is

$$\frac{dS}{dt} = k_{b2} XS \tag{7.7}$$

k_{b2} can be calculated from k_b' and X_o by equation (7.20) (assuming X_o is a constant)

$$k_{b2} = k_b'/X_o \tag{7.20}$$

Note that the values of μ_m, Y, and K_s cannot be determined by this procedure, but in fact they are not required since equation (7.7) is a satisfactory rate expressions for biodegradation in natural waters.

The procedures used in the laboratory techniques were carried out in the following ways.

Batch fermentations with low-level inocula were conducted in shaker flasks incubated at 25°C in rotary shakers. The enrichment culture systems were grown on substrate/basal salts media or substrate in 0.1 strength nutrient broth (Difco). The cells were removed by centrifugation, washed three times with 0.05% potassium phosphate buffer at pH 7, and rested in buffer for 2 to 18 hours at room temperature. These cells were then added at appropriate levels to sterile substrate/basal salts medium. In some experiments, several lots of inoculated media were prepared with different concentrations of substrate; 800-ml volumes of inoculated media were incubated in 2-liter Erlenmeyer flasks. During the fermentation, duplicate samples were removed from these shaker-incubated flasks for analyses.

Continuous chemostat fermentations were conducted in 350-ml working volume New Brunswick chemostats. Inocula in the exponential phase, grown in shaker flasks containing substrate/basal salts media, were transferred to sterile chemostats containing substrate/basal salts media to bring the liquid volumes to capacity. Fermentations in the chemostats were initiated with aeration (350 ml air min^{-1}), stirring, temperature control, and continuous feed of substrate/basal salts medium. Initially, feed rates were very slow to prevent washout of cells. The feed media were at higher concentrations of substrates in basal salts medium than the concentrations anticipated in the chemostat. These feed media were introduced into the chemostats at different rates until equilibria were established. At equilibrium, the samples from the overflow had reached a steady state with respect to substrate and biomass concentrations. Because there were, at times, attachments of microbial cells to the wall and other parts of the chemostats (aerator, sampling tube, temperature control units, and stirrers), chemostats were thoroughly shaken and contents were transferred once or twice daily to other similar chemostats.

Cascade batch fermentations were initiated with freshly developed degrading systems from eutrophic waters. When the substrates were almost totally degraded in the original 9-liter bottle fermentors, small aliquots from these fermentors were transferred to 250- or 500-ml Erlenmeyer flasks containing fresh water samples (from the same sources), NH_4^+ salt, buffer, and substrates. These flasks were incubated at 25°C in shakers. Cell counts and substrate levels were monitored. Sequential transfers were made daily to new flasks containing fresh water samples, salts, and substrate. In this procedure it was difficult to determine the volume of inoculum needed for the sequential transfers to develop essentially total decomposition of substrate in 24 hours, and it was difficult to follow cell counts at critical times in the fermentations.

Batch fermentations with large microbial populations and low substrate levels were conducted with cells grown on substrate/basal salts media. The fermentations producing the inocula were monitored for substrates to be certain that the substrates were consumed. In each case, several shaker flasks had to be used to produce sufficient cells, and these organisms were undoubtedly in the late exponential or early stationary phase. Cells were separated by centrifuging at room temperature and high speeds, resuspended in basal salts medium, and centrifuged. They were resuspended in basal salts medium and incubated at 25°C in a shaker flask for 4 hours, centrifuged, and again resuspended in basal salts

medium. The optical densities of these cell suspensions were used as guides for the dilutions to be used in the kinetic studies. Appropriate aliquots were added to substrate/basal salts media. The relative quantities of cells and substrate were such that if all the substrate was utilized, there would be insignificant increases in cell mass. The inoculated media were vigorously stirred at 25°C in siliconized tissue culture spinner flasks. Cell counts and substrate levels were determined at short time intervals.

Each of the kinetic evaluations by the above four procedures has its particular advantages and shortcomings when mixed culture systems are used to develop rate constants. The batch fermentations with large microbial populations require the least time and for this reason avoid the relative changes of individual microbial components from the initiation of the experiment. However, they cannot reflect on the character of the culture mixture several transfers prior to the kinetic study. This is also the procedure most adaptable to fermentations with very low substrate levels in which changes in biomass concentrations may be difficult to follow by other methods.

The cascade batch fermentation represents a procedure most similar to that existing in nature. In this procedure, the biodegrading cultures were returned to an environment that contained all types of predator-prey relationships originally present in the water source (assuming that daily water samplings were similar). There is a problem in maintaining a supply of unchanged inflow water sample and selecting inoculum levels. If realistic low levels of substrate are used, it would be virtually impossible to determine the biomass attributed to the metabolism of the compound.

Even if the enrichment mixed culture system consisted of only primary utilizers of the pollutant being evaluated, the batch fermentations with low-level inoculations or continuous fermentations in chemostats could suffer from changes in relative components in the culture mixture during the course of the experiments. Also, in both these procedures, with realistic low levels of substrate, the biomass determinations as discussed by Pirt (1975) would be subject to considerable error. Cell counts appeared to be the best alternative. In the continuous culture procedure, the solubility of the compound in the feed stock must be sufficient for the dilution it is subject to on addition to the fermentation vessel.

In continuous fermentations at high feed-rates, some metabolites that are utilized at slower feed-rates may be lost from the fermentors and thus not contribute to cell growth, but the analyses for substrate consumed would not indicate this. If substrate levels were high enough for accurate carbon analyses or if uniformly labelled ^{14}C- substrates were used, corrections could be made or at least a better understanding would be obtained from carbon balance studies.

The batch fermentations with low-level inocula and the continous fermentations in chemostats are excellent and indeed are the procedures of choice if a pure culture is used with a substrate that provides normal cell growth and when the level of substrate is adequate to produce sufficient biomass to be measured with precision. With the compounds tested in these studies, we invariably developed organisms that were much smaller than those that would develop in good microbiological media or as they are normally found in eutrophic milieu

containing a mixture of digestible carbon substrates, and this was another reason for using cell counts as indices of biomass.

7.4 ISOLATION AND IDENTIFICATION OF MAJOR BIODEGRADATION METABOLITES

During the course of uv, gc, or hplc analyses of extracts from biodegradation studies, there was constant surveillance for evidence of metabolites. Most extractions were conducted under acidic or neutral conditions, and the types of major metabolites expected would have been acidic or neutral and extracted with our solvents. When nitrogen heterocyclic substrates were used, some extractions were made under slightly alkaline and neutral conditions.

If there was evidence for metabolites, mass spectometric analyses were applied to the gc or hplc fractions containing the products. When possible, these spectra were compared with those of authentic reference samples of the anticipated metabolites to positively establish their structures.

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Appendix A

FLOW OF WATER AND SEDIMENTS BETWEEN COMPARTMENTS IN THE COMPUTER MODEL

TABLE A.1. FLOW OF WATER AND SOLIDS BETWEEN COMPARTMENTS IN THE POND MODEL

From compartment \ To	Water compartment 1		Solids compartment 7	
	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)
1	-	-	0.1	3.75
7	0.1	3.75	-	-

TABLE A.2. FLOW OF WATER AND SOLIDS BETWEEN COMPARTMENTS IN THE RIVER SYSTEM

From \ To	Water compartments						Solids compartment					
	1		2		3		7		8		9	
compartment	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)
1	-	-	1.01×10^6	1.01×10^5	0	0	4.0	1.08×10^4	0	0	0	0
2	0	0	-	-	1.01×10^6	1.01×10^5	0	0	4.0	1.08×10^4	0	0
3	0	0	0	0	-	-	0	0	0	0	4.0	1.08×10^4
7	4.0	1.08×10^4	0	0	0	0	-	-	0	0	0	0
8	0	0	4.0	1.08×10^4	0	0	0	0	-	-	0	0
9	0	0	0	0	4.0	1.08×10^4	0	0	0	0	-	-

TABLE A.3. FLOW OF WATER AND SOLIDS BETWEEN COMPARTMENTS IN THE EUTROPHIC AND OLIGOTROPHIC LAKE SYSTEMS

From \ To	Water compartments								Solids compartment					
	1		2		3		5		7		8		9	
compartment	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)	Water ($m^3 hr^{-1}$)	Solids ($kg\ hr^{-1}$)
1	-	-	1.1×10^4	5.46×10^2	0	0	0	0	0.46	375	0	0	0	0
2	1.1×10^4	498	-	-	1.1×10^4	546	800	40	0	0	0	0	0	0
3	0	0	1.1×10^4	4.98×10^2	-	-	0	0	0	0	0	0	0.46	375
5	0	0	800	40	0	0	-	-	0	0	4.63	3750	0	0
7	0.46	375	0	0	0	0	0	0	-	-	0	0	0	0
8	0	0	0	0	0	0	4.63	3750	0	0	-	-	0	0
9	0	0	0	0	0.46	375	0	0	0	0	0	0	-	-

Appendix B

THEORY OF VOLATILIZATION OF ORGANIC SUBSTRATES FROM WATER

The theory of volatilization of slightly soluble organic substances from aqueous solutions and oxygen reaeration in water has been developed by several authors.* They assumed a two-film model in which the rates of diffusion in air and in water control the rate of transfer of both oxygen and the substrate across the interface between air and water. (Oxygen and the substrate are represented by the superscripts O and S, respectively, in the equations in this appendix.)

Figure B.1 illustrates the major features of the two-film model of mass transfer. The water phase is assumed to be well-mixed so that any volatile component is at a uniform concentration C_S, except in the vicinity of the interface. A stagnant liquid film or concentration boundary layer of thickness δ_L separates the bulk of the water phase from the actual interface. Since turbulence levels in this film are low, any movement of a volatile component through this film is due to diffusion alone. The concentration of a volatilizing component decreases across this film from the bulk concentration C_S to the interface concentration C_{Si}. This concentration decrease is the driving force for mass transport.

On the air side of the interface is a stagnant gas film or concentration boundary layer of thickness of δ_G, where diffusion is again the only mass transport mechanism. The partial pressure P_{Si} on the air side of the interface is related to the molar concentration $[S_i]$ on the water side of the interface by Henry's law:

$$P_{Si} = H_c[S_i] = Hx_{Si} \tag{B.1}$$

where H_c and H are the Henry's law constant expressed in concentration (M) or mole fraction units, respectively, and

$$H_c \simeq H \frac{18}{1000} = 1.8 \times 10^{-3} H \tag{B.2}$$

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*P. S. Liss and P. G. Slater, "Flux of Gases Across the Air-Sea Interface," Nature 247:181-184 (1974); D. Mackay and Y. Cohen, "Prediction of Volatilization Rate of Pollutants in Aqueous Systems," Symposium on Nonbiological Transport and Transformation of Pollutants on Land and Water, May 11-13, 1976, National Bureau of Standards, Gaithersburg, Maryland.

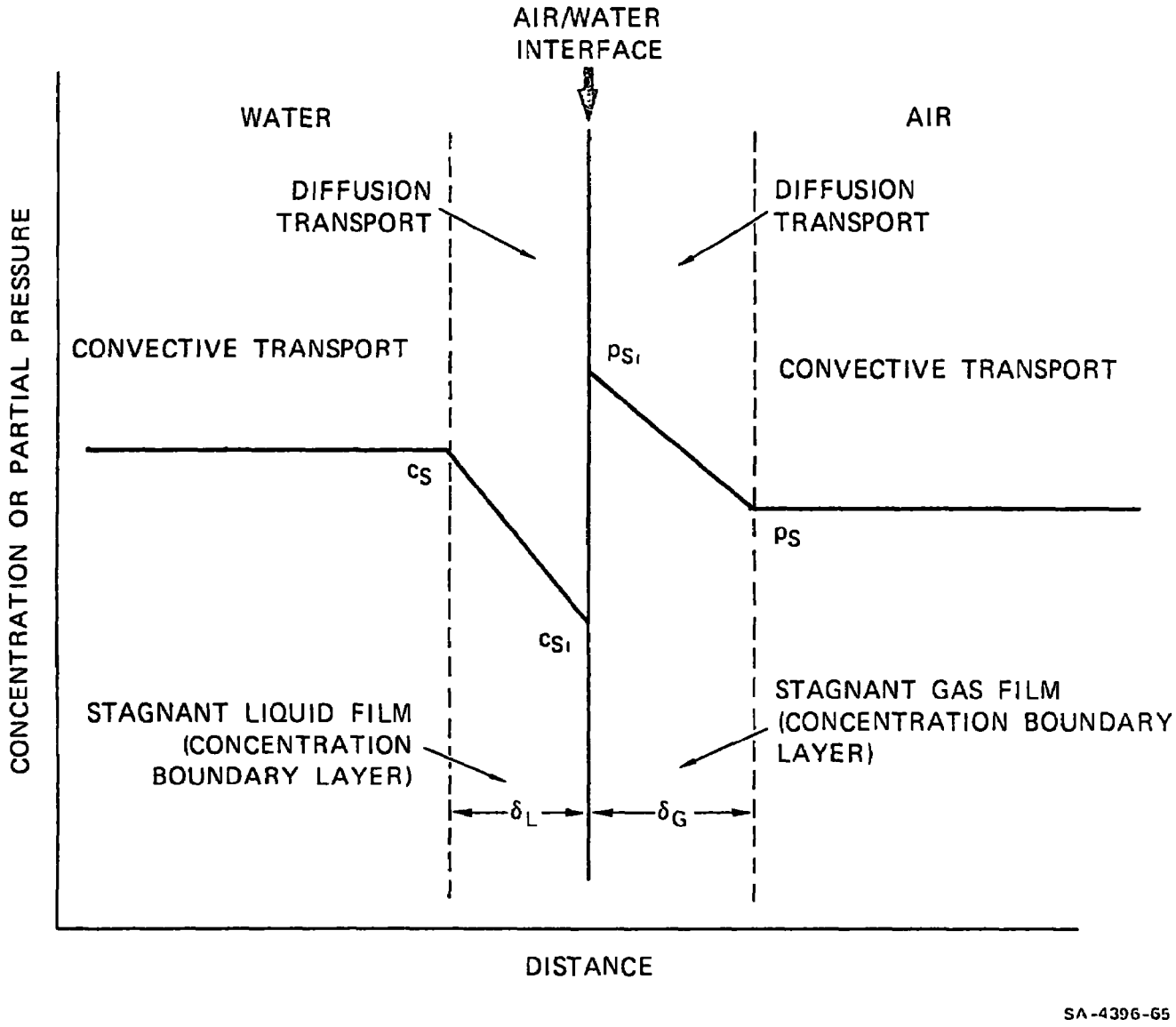

FIGURE B.1. SCHEMATIC OF THE TWO-FILM MODEL OF VOLATILIZATION FROM THE SURFACE OF WATER BODIES

If we denote the rate at which substrate is being transported across these films by N_S, in moles dm^{-2} hr^{-1}, then

$$N_S = K_L^S([S] - [S_i]) \text{ liquid film} \tag{B.3}$$

$$N_S = \frac{K_G^S}{RT}(p_{Si} - p_S) \text{ gas film} \tag{B.4}$$

Combining equations (B.1), (B.3), and (B.4), we obtain:

$$k_v^S = \frac{A}{V}\left(\frac{1}{K_L^S} + \frac{RT}{H_c^S K_G^S}\right)^{-1} \tag{B.5}$$

where:

k_v	Overall mass transfer coefficient (hr^{-1})
A	Interfacial area (dm^2)
V	Liquid volume (dm^3)
D	Molecular diffusion coefficient (dm^2 hr^{-1})
H_c	Henry's law constant (torr M^{-1})
K_L	Liquid film mass transfer coefficient (dm hr^{-1})
K_G	Gas film mass transfer coefficient (dm hr^{-1})
R	Gas constant
T	Temperature (°K)

A similar equation can be written for oxygen transport.

$$k_v^O = \frac{A}{V}\left(\frac{1}{K_L^O} + \frac{RT}{H_c^O K_G^O}\right)^{-1} \tag{B.6}$$

In a liquid with dilute concentration of oxygen or substrate and when the amount of material being transferred across the interface into air is small, the two-film model assumes that

$$K_L = \frac{D}{\delta_L} \tag{B.7}$$

where D is the diffusion coefficient of oxygen or substrate in water and δ_L is the thickness of the mass transfer film or boundary layer on the liquid side

of the interface. This relation develops as a simplification of Fick's law of diffusion. In a similar manner, it can be shown that

$$K_G = \frac{D}{\delta_G} \tag{B.8}$$

where δ_G is the thickness of a mass transfer film on the gas side of the gas-liquid interface and D is the diffusion coefficient of oxygen or substrate in air. High turbulence in the liquid causes δ_L to be thin, and similarly, high turbulence in the gas phase causes δ_G to be thin.

The k_v^0 data cited in Table 5.2 for most natural water bodies show that the k_v^0 values are less than about 0.03 hr^{-1}. Therefore, the mixing levels are such that liquid film resistance controls the volatilization rate (K_G is very large). Under these conditions, equations (B.5) and (B.6) reduce to the form

$$k_v = \frac{A}{V}\left(\frac{1}{K_L}\right)^{-1} = \frac{AK_L}{V} \tag{B.9}$$

and, therefore

$$\frac{k_v^S}{k_v^0} = \frac{K_L^S}{K_L^0} = \frac{D^S}{D^0} \tag{B.10}$$

It has been shown* that, if the molecules are spherical, molecular diffusion coefficients in solution are inversely proportional to molecular diameters, so that

$$\frac{k_v^S}{k_v^0} = \frac{D^S}{D^0} = \frac{d^0}{d^S} \tag{B.11}$$

where d^0 is the molecular diameter of O_2, and d^S is the molecular diameter of the substrate. Equation (B.11) has been tested and validated for mixtures of

*E. C. Tsivoglou, "Tracer Measurements of Atmospheric Reaeration-1. Laboratory Studies," J. Water Pollution Control Federation 37:1343-1362 (1965).

radon/oxygen, krypton/oxygen, CO_2/oxygen, and N_2/oxygen. For example, Tsivoglou showed that

$$\frac{k_v^{Kr}}{k_v^0} = 1.22 \pm 0.06 \text{ experimentally} \qquad (B.12)$$

$$= 1.25 \qquad \text{theoretically}$$

over a range of k_v^0 from 0 to 0.6 hr^{-1}.

At high levels of liquid turbulence, δ_L becomes very small and as a consequence K_L becomes very large and the gas phase resistance becomes the rate-controlling step. Based on the findings of Tsivoglou, this occurs when $k_v^0 >> 0.6\ hr^{-1}$, although the actual point where the transition occurs is unknown. When gas phase resistance is rate controlling, equation (B.9) becomes

$$k_v = \frac{A}{V}\left(\frac{RT}{H_c K_G}\right)^{-1} = \frac{A H_c K_G}{VRT} \qquad (B.13)$$

and

$$\frac{k_v^S}{k_v^0} = \frac{H_c^S K_G^S}{H_c^0 K_G^0} = \frac{H^S K_G^S}{H^0 K_G^0} = \frac{H^S D_S}{H^0 D_0} \qquad (B.14)$$

where here D refers to the diffusion coefficient in air. If it is assumed that the diffusion coefficients are still inversely proportional to the molecular diameters

$$\frac{k_v^S}{k_v^0} = \frac{H^S d^0}{H^0 d^S} \qquad (B.15)$$

there is also a transition region where both liquid phase resistance and gas phase resistance control the transport rate. In this transition region, the ratio k_v^S/k_v^0 must be expressed as the ratio of equations (B.5) and (B.6), and does not reduce to a simple form.

If data on the diffusion coefficients or molecular diameter for the substrate are not available, molecular diameters can be estimated from the

critical volume (V_c), which is a commonly tabulated physical constant.* If the critical volume cannot be found, a volume for a closely related compound can be used. The critical volume is two or three times the molecular volume. From the molecular volume, the molecular diameter can be calculated by assuming that the molecule is spherical:

$$\frac{\pi d^3}{6} = \frac{V_c}{2N} \text{ or } \frac{V_c}{3N} \tag{B.16}$$

where N is Avogadro's number. A widely accepted value for d^O is 2.98 Å.

Values for the Henry's law constant can be estimated from solubility and vapor pressure following the procedure of Mackay and Wolkoff.‡ Based on thermodynamic principles, they determined that

$$H_c^S = \frac{P^S}{S_{wo}} \tag{B.17}$$

where P^S is the substrate vapor pressure in pure form and S_{wo} is its solubility in water. If data for the substrate are not available, data for a related compound can be used.

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*One source is the American Chemical Society Advances in Chemistry Series, Vol. 15 (1955).

†R. D. Present, Kinetic Theory of Gases (McGraw-Hill, New York, 1958).

‡D. Mackay and A. W. Wolkoff, "Rate of Evaporation of Low Solubility Contaminates from Water Bodies to the Atmosphere," Environ. Sci. Tech. 7:611-614 (1973).

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

This research was initiated to develop environmental exposure assessment procedures that can be used to predict the pathways of potentially harmful chemicals in freshwater environments. The approach is based on three premises: (1) the overall rate of disappearance of a chemical from the aquatic environment is controlled only by the dominant transformation and transport processes, (2) these processes can be studied independently in the laboratory, and (3) the laboratory data can be extrapolated to environmental conditions.

Laboratory procedures have been developed for measuring the rates of volatilization, photolysis, oxidation, hydrolysis, and biotransformation as well as the sorption partition coefficients on natural sediments and on a mixture of four bacteria. Two models have been used to extrapolate the laboratory results to the environment. The one-compartment model assumes that the aquatic system is a single well-mixed reactor from which chemicals are transformed, degraded, and/or transported. It can be used to analyze acute discharges such as spills and to establish priorities for in-depth laboratory studies. The nine-compartment computer model is used to study the effect of transport and transformation processes studied in the laboratory on the distribution of a chemical in ponds, streams, and eutrophic and oligotrophic lakes. Part II of this report describes the application of these procedures to environmental assessment of the distribution and fate of eleven organic compounds.

17. KEY WORDS AND DOCUMENT ANALYSIS

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