INTERIM PROTOCOL FOR MEASURING HYDROLYSIS RATE CONSTANTS IN AQUEOUS SOLUTIONS

by

J. Jackson Ellington, Frank E. Stancil, Jr.,
Environmental Research Laboratory,
U.S. Environmental Protection Agency
Athens, Georgia, 30613

William D. Payne, Cheryl D. Trusty,
Technology Applications Inc.,
Environmental Research Laboratory
Athens, Georgia, 30613

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30613

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FOREWORD

As environmental controls become more expensive and penalties for judgement errors become more severe, environmental management requires more precise assessment tools based on greater knowledge of relevant phenomena. As a part of this Laboratory's research on occurrence, movement, transformation, impact, and control of chemical contaminants, the Measurements Branch determines the occurrence of unsuspected organic pollutants in the aquatic environment and develops and applies techniques to measure physical, chemical, and microbial transformation and equilibrium constants for use in assessment models and for development of property reactivity correlations.

Mathematical models are widely used in predicting the fate of organic chemicals in lakes and streams and in soil systems. Application of these models requires as input the second-order and first-order hydrolysis rate constants for chemicals containing hydrolyzable functional groups. In measuring these rate constants, some means is needed to ensure that the measurements are reliable and reproducible. This protocol specifies complete step-by-step procedures for hydrolysis measurement to produce the required level of reproducibility and reliability among measurements by different investigators.

Rosemarie C. Russo, Ph.D.
Director
Environmental Research Laboratory
Athens, Georgia

ABSTRACT

A detailed protocol was developed to measure first- and second-order hydrolysis rate constants for organic chemicals for use in predicting persistence in aquatic systems. The protocol delineates theoretical considerations, laboratory experiments, and calculation procedures. Repetitive application of the protocol to measure hydrolysis rate constants for four standard reference compounds over a period of 2 years yielded coefficients of variation of less than 12% in the measurements.

This report covers a period from October 1985 to March 1988 and work was completed as of January 1988.

INTRODUCTION

Under the Toxic Substances Control Act (PL 94-469) of 1976, the Office of Toxic Substances (OTS) screens new chemicals proposed for manufacture and reviews the safety of existing chemicals already on the market. To assess potential risk to human health and the environment OTS must evaluate both effects and exposure potential. Transport and transformation characteristics in ambient environments are major considerations in assessing potential exposure. Essential to transport and transformation assessments are physical and chemical data that permit estimation of chemical fate either by use of mathematical models or other techniques. To obtain necessary data, OTS either requests it from manufacturers or estimates values by comparing the chemical to analogous chemicals whose properties are known. In either case, reliable data are necessary. A major transformation process for many chemicals is chemical hydrolysis; therefore, measurements of hydrolysis rate constants are often required.

In the measurement of hydrolysis rate constants, some means is needed to ensure that the measurements are reliable and reproducible. Suggested laboratory protocols for measuring hydrolysis as a function of pH and temperature have been published (1-3); however, these previously published protocols fail to specify some of the step-wise procedures in sufficient detail to enhance reproducibility of measurements made by different investigators. This report, on the other hand, provides specific guidance in a protocol for deriving hydrolysis rate constants for use in mathematical models to predict the fate of a chemicals in aquatic systems. The protocol has evolved over the past several years at the Environmental Research

Laboratory, Athens GA, and has been found to provide reproducible rate constants as a function of pH and temperature.

KINETICS OF HYDROLYSIS

HYDROLYSIS MECHANISM

The importance of hydrolysis as a transformation process for chemicals in water can be determined from data on rate constants and half-lives coupled with data describing environmental conditions. Hydrolysis of organic compounds refers to reaction of the compound with water in which bonds are broken and new bonds with HO- and H- are formed. A typical example is the reaction of an alkyl halide with water resulting in the formation of halide ion (X-):

$$RX + HOH \text{ ------> } ROH + HX \text{ (or } H^+, X^-) \qquad (1)$$

The rate of hydrolysis may be promoted by the hydronium ion (H^+, H_3O^+) or the hydroxyl ion (OH^-). The former is referred to as specific acid catalysis and the latter as base mediated hydrolysis. These two processes together with the pH independent reaction with water are the only mechanisms considered in this protocol. The H_3O^+ activity is measured directly and the OH^- activity is calculated from accurate determination (calibration between secondary buffers) of solution pH.

Some chemicals undergo an elimination reaction:

$$-\overset{H}{\underset{|}{C}}-\overset{X}{\underset{|}{C}}- \xrightarrow[H_2O]{H^+,\ OH^-} >C=C< + HX \qquad (2)$$

In this protocol, only the disappearance of substrate is monitored with no attempts to identify mechanisms or reaction products. Reactions represented by equations 1 and 2 are included in a broad definition of hydrolysis.

RATE LAWS

In both processes referred to in the discussion of hydrolysis mechanisms, the rate of disappearance of the organic compound is given by the equation,

$$-\frac{d[C]}{dt} = k_h[C] = K_A[H^+][C] + k_B[OH^-][C] + k_N'[H_2O][C] \qquad (3)$$

where [C] is the concentration of organic and k_h is the observed pseudo-first-order rate constant at a specific pH and temperature; k_A and k_B are second-order rate constants; and k_N' the neutral hydrolysis rate constant for the acid, base and neutral promoted processes, respectively. The water concentration, because of the large excess, does not change during the reaction, thus $k_N'[H_2O]$ is a constant (k_N).

Equation 3 assumes each individual rate process is first-order in substrate, thus k_h can be defined as:

$$k_h = k_A[H^+] + k_B[OH^-] + k_N \qquad (4)$$

Using the autoprotolysis equilibrium expression

$$K_W = [H^+][OH^-] \qquad (5)$$

equation 4 may be rewritten as

$$k_h = k_A[H^+] + \frac{k_B k_W}{[H^+]} + k_N \qquad (6)$$

Equation 6 shows the dependence of k_h on hydronium ion concentration (pH) and on the relative values of k_A, k_B, and k_N.

When the disappearance rate constants are determined at pHs 3, 7, and 11, the second-order rate constants for acid hydrolysis and for base

hydrolysis can be calculated by dividing the pseudo-first-order rate constant obtained at the appropriate pH by the hydronium ion or hydroxyl ion activity, respectively. The neutral contribution is determined by solving equation 4 for k_N and substituting the observed rate (k_h) at pH 7 together with values for k_A and k_B. The half-life of a compound at a given pH and temperature can be calculated from equation 7, where k_h is the observed rate at the given pH and temperature and $0.693 = \ln \frac{[C_o]}{[C_t]}$ where $[C_o]$ equals concentration at time zero and $[C_t]$ equals concentration at 50% reaction.

$$t_{1/2} = \frac{0.693}{k_h} \qquad (7)$$

CONTRIBUTING FACTORS TO HYDROLYSIS RATES

Temperature

The effect of temperature on the rate constant for a specific hydrolysis process (k_A, k_N, and k_B) can be expressed in several ways. The familiar Arrenhius or absolute rate theory equation was used in this protocol development

$$k = A \exp(-Ea/RT) \text{ or } \ln k = \ln A - Ea/RT \qquad (8)$$

where A is the collision frequency term, Ea is the activation energy, and R is the gas constant (8.314 J/deg mol). To determine Ea and A for each hydrolytic process (acid, neutral, base), one must know the rate constants for the three processes k_A, k_N, and k_B at two or more temperatures (preferably separated by 20°C each). A and E are determined for each process by plotting $\ln k_X$ (X = A, N, or B) versus 1/T (T in °K) and calculating the slope of

the straight line through the data points (temperatures) or by fitting the data by regression analysis to the equation ln k = ln A - Ea/RT.

Between any two temperatures, the following relationship is applicable

$$\ln \frac{k_{T1}}{k_{T2}} = \frac{Ea}{R} \frac{(T_2 - T_1)}{T_2 T_1} \qquad (9)$$

where T_1 and T_2 are the respective temperatures and Ea is the activation energy (E_A, E_N, and E_B) for the particular process (k_A, k_N, and k_B). Equations 4 and 9 are used to calculate rate constants for hydrolysis at temperatures and pH values of interest for environmental assessment.

A second effect of temperature must be considered when using equation 5 to calculate $[OH^-]$. The equilibrium constant K_W increases with temperature and with it the activity of $[OH^-]$. When determining $[OH^-]$ activities, the K_W at the temperature of interest must be taken into account. In practice, the pH is measured at the experimental temperature and, from the measured pH, a value for $[OH^-]$ is calculated. The K_W at a given temperature is calculated by the equation given by Harned and Owen (4)

$$\log K_W = \frac{-6013.79}{T} - 23.6521 \log T + 64.7013 \qquad (10)$$

where $$T(°K) = T(°C) + 273.2$$

The $[OH^-]$ ion activity is then calculated using equation 5 and the calculated value for k_W at the experimental temperature. The $[OH^-]$ calculated by equation 5 is used to calculate k_B by dividing the measured pseudo-first-order rate constant obtained at pH 11 by the hydroxide ion activity at the measured temperature.

pH, Buffer Catalysis

The observed rate of hydrolysis (k_h) at any pH is described by equation 4, where k_A and k_B correspond to the second-order specific acid and base rate constants and k_N corresponds to the first-order neutral water hydrolysis rate constant. Because of this possible pH dependence, accurate pH data must be available for each buffer solution at each temperature. The most practical way to obtain these data is to heat a standard buffer solution to the desired reaction temperature and, then, to measure the pH of the solution using a temperature-compensating meter and probe designed to operate in the temperature ranges of interest.

The effects of ionic strength and buffer salts on hydrolysis reactions are difficult to predict: rates can be retarded or accelerated depending on the compound. Generally, buffer concentrations are 5E-3M and compound concentrations 1E-5M. Dilute buffers and the 500:1 ratio of buffer to compound minimizes buffer catalysis. As a check for catalysis, hydrolysis rates can be measured over a 100-fold concentration range. A change in k_h greater than experimental error indicates catalysis by the buffer.

Solvent Composition, Metal Ions

Cosolvents can alter the rate of hydrolysis and are to be avoided. If methanol or acetonitrile are used to prepare stock solutions, the final concentration of either solvent in samples should be less than 1%. Deionized water equivalent to ASTM Type I should be used to make all solutions to minimize introduction of metal ions.

COMPILATION AND DEVELOPMENT OF HYDROLYSIS INFORMATION

LITERATURE SEARCHES

Several things should be considered prior to laboratory measurement of the hydrolysis rate of any compound. First, the molecular structure of the compound of interest must be determined. Is the compound an optical isomer or stereoisomer? What is the correct Chemical Abstract System (CAS) number for the specific chemical compound? What are the common, trade, and IUPAC names for the compound?

A search of the literature and computerized databases should be a prerequisite to any laboratory determinations. The most optimistic result from the literature search would be to find a thorough rate study of the compound of interest enabling prediction of a hydrolysis rate constant over a wide range of pH and temperature. Unfortunately, literature values from persistence studies or laboratory mechanism studies are often obtained under conditions different from general environmental temperatures and pH. The literature values may be useful, however, in setting pH, temperature, and sampling times in the preliminary screening test.

If literature values are not available, linear free energy relationships (LFERs) can be used to estimate the hydrolysis rates of closely related compounds. With well characterized classes of compounds, LFER calculations will permit choosing experimental conditions for the preliminary screening tests that will result in reaction times appropriate for precise measurements. The literature search should be used as a source for other information about the compound such as chemical and physical properties (bp, mp, UV, IR, and solubility) and methods of chemical analysis. Examples of sources of information include:

1. Manual

   a. Merck Index

   b. CRC Handbook of Chemistry and Physics

   c. Kirk-Othmer Encyclopedia of Chemical Technology

   d. Chemical Abstracts Service (CAS)

2. Computerized

   a. CHEMFATE

      Syracuse Research Corporation
      Merrill Lane
      Syracuse, NY 13210-4080

   b. CAS On-Line (1967 to present)

      STN International
      c/o Chemical Abstracts Service
      2540 Olentangy River Road
      P.O. Box 2228
      Columbus, OH 43202

   c. CIS

      Chemical Information System, Inc.
      7215 York Road
      Baltimore, MD 21212

   d. DIALOG

      Dialog Information Service Inc.
      3460 Hillview Ave
      Palo Alto, CA 94304

STANDARD REFERENCE COMPOUNDS

Chemical standards of known concentration have long been used for assuring reliability of quantitative chemical analyses, calibrating instruments, and measuring recoveries of analytes from various matrices. Analogous to using chemicals of known concentration as standards for concentration measurement, chemicals whose hydrolysis constants have been measured with established precision by one experimenter or group can be

used as standard reference compounds (SRCs) by other experimenters in establishing and maintaining quality control in rate measurements. Precise measurement of established hydrolysis rate constants for SRCs interspersed with other rate constant measurements will help assure reliability and comparability of the measured constants.

Standard reference compounds are used as quality assurance standards and as references in inter-laboratory generation of hydrolysis data. Repetition of rate constant measurements in our laboratory for these compounds over the course of 2 years has established baseline information for evaluating experimental techniques and for all aspects of quality assurance (5,6). Four compounds were selected, one each for acid and neutral hydrolysis, and two for basic hydrolysis (Table 1).

Reproduction of the hydrolysis rate constants of the SRCs at the established concentrations, pHs, and temperatures ensured that the experimental condidtions were reproducible and helped evaluate the accuracy and precision of measurements for other compounds. Tables 2 through 5 contain SRC rate constant data generated during laboratory determinations of hydrolysis rate constants of other cheimcals of interest. Pseudo-first-order hydrolysis rate constants for all SRCs at various temperatures and pH and second-order rate constants for the acidic and basic reference compound were established from these determinations.

Table 1. Standard Reference Compounds

Name	pH Range	Ea(kJ/mol)[a]	ln A
DL-trans-4-chlorostilbene oxide (CSO)	2 - 5	84.7±13.2	37.1±5.30
Benzyl chloride	Neutral[b]	84.1±5.8	45.3±2.21
Methyl-2, 4-dichlorophenoxy acetate (2,4-DME)	8 - 9.5	40.1±4.9	22.7±1.91
Lindane	9.5 - 11	65.3±1.9	27.5±0.75

a. The activation energy (Ea) and collision frequency (ln A) were determined by plotting the mean values of either k_A, k_N, or k_B at each temperature in Tables 2-5 versus 1/T (as illustrated for lindane in Appendix J).

b. Hydrolysis of benzyl chloride is independent of pH in the pH range 2-12.

Table 2. Hydrolysis Data for DL-trans-4-chlorostilbene Oxide

Temp. (°C)	pH	10^3 k_h[a] (min $^{-1}$)	k_A[b] (M $^{-1}$ min $^{-1}$)
23.0[c]	3.13[d]	10.1[e]	13.6[f]
	3.12	15.5	20.4
	2.99	12.9	12.6
24.3	3.03	11.1	11.9
25.0	2.89	29.5	22.9
	2.89	35.8	27.8
	3.05	24.4	27.4
	3.05	22.8	25.6
	3.05	22.8	25.6
	3.02	17.4	18.2
	3.02	16.1	16.8
	3.02	19.0	19.9
	3.10	16.9	21.2
	2.96	18.5	16.9
25.3	2.95	24.2	21.6
	2.95	19.4	17.3
28.0	3.06	14.4	16.5
	3.06	16.9	19.4
	3.06	14.3	16.4
	3.06	16.9	19.4
	3.01	21.1	21.6
	3.01	23.7	24.2
	3.01	20.8	21.3
	3.07	14.6	17.1
	3.07	14.3	16.8
	3.10	14.9	18.8
	3.10	14.4	18.2
	3.13	17.4	23.5
38.2	3.63	17.0	72.3
	3.59	23.5	91.4
	3.59	24.5	95.3

a. Pseudo-first-order rate constant (k_h): the slope of the line from plot of ln % chemical remaining versus time.

b. Second-order rate constant (k_A).

c. Error of temperatures: $\leq$75°C±0.1°C (water bath); >75°C±1°C (oil bath).

d. Error ±0.02 units all measurements.

e. Standard deviation of the slope (k_h) <10% for each determination.

f. The averages of the k_A values at each temperature were used to calculate the Ea and ln A in Table 1 (as illustrated for lindane in Appendix J).

Table 3. Hydrolysis Data for Benzyl Chloride (pH 7.00)[a]

Temp. (°C)	$10^4 k_h$[b] (min^{-1})
28.0[c]	10.4[d,e]
	12.2
	11.1
	9.8
36.4	31.9
	33.9
42.7	70.0
45.0	72.7
	72.2
	65.8
	55.5
	63.9
	60.6
	69.0
	69.0
	78.0
	66.9
	69.4
46.0	67.0
49.0	98.9
	98.6
52.9	203.0
	191.0
	211.0
	216.0
53.4	140.0
	136.0
53.5	154.0

a. Error ±0.02 units all measurements.

b. Pseudo-first-order rate constant (k_h): the slope of the line from plot of ln % chemical remaining versus time.

c. Error of temperatures: $\leq$75°C±0.1°C (water bath); >75°C±1°C (oil bath).

d. Standard deviation of the slope (k_h) <10% for each determination.

e. The averages of the values at each temperature were used to calculate the Ea and ln A in Table 1 (as illustrated for lindane in Appendix J).

Table 4. Hydrolysis Data for Methyl Ester of 2,4-D

Temp (°C)	pH	10^4 k_h[a] (min^{-1})	k_B[b] (M^{-1} min^{-1})
23.0[c]	8.81[d]	29.9[e]	541[f]
	8.81	33.0	596
	8.81	36.0	650
25.0	8.87	57.1	770
	8.87	41.8	563
	9.10	95.0	754
	9.10	79.0	627
	9.38	230.0	958
	9.45	249.0	883
	9.38	224.0	933
	9.45	224.0	794
28.0	9.06	80.7	560
	9.06	70.4	489
	9.65	262.0	467
	9.65	278.0	495
	9.10	118.0	681
	9.14	103.0	593
31.0	8.72	79.2	966
	8.75	91.2	1038
45.0	8.54	350.0	2520
	8.74	340.0	1547
45.3	8.55	412.0	2847
	8.55	415.0	2867
48.5	8.00	103.0	2079
	8.00	86.0	1731
70.3	7.11	100.0	4775
	7.11	114.0	5451

a. Pseudo-first-order rate constant (k_h): the slope of the line from plot of ln % chemical remaining versus time.

b. Second-order rate constant (k_B)

c. Error of temperatures: $\leq$75°C±0.1°C (water bath); >75°C±1°C (oil bath).

d. Error ±0.02 units all measurements.

e. Standard deviation of the slope (k_h) <10% for each determination.

f. The averages of the k_B values at each temperature were used to calculate the Ea and ln A in Table 1 (as illustrated for lindane in Appendix J).

Table 5. Hydrolysis Data for Lindane

Temp (°C)	pH	10^3 k_h[a] (min^{-1})	k_B[b] (M^{-1} min^{-1})
22.8[c]	11.60[d]	9.9[e]	2.9[f]
37.0	11.29	40.2	8.7
40.0	10.92	23.6	9.8
	10.01	22.2	7.4
	11.20	40.2	8.7
45.0	10.31	18.4	22.5
	10.45	20.0	17.7
	10.37	14.5	15.4
	10.37	14.6	15.6
	10.37	14.8	15.8
45.3	10.71	33.1	15.8
	10.71	36.0	17.2
46.0	11.08	83.4	16.3
	10.98	78.0	19.2
	10.98	71.5	17.6
85.0	8.73	51.6	272.0

a. Pseudo-first-order rate constant (k_h): the slope of the line from plot of ln % chemical remaining versus time.

b. Second-order rate constant (k_B)

c. Error of temperatures: $\leq$75°C±0.1°C (water bath); >75°C±1°C (oil bath).

d. Error ±0.02 units all measurements.

e. Standard deviation of the slope (k_h) <10% for each determination.

f. The averages of the k_B values at each temperature were used to calculate the Ea and ln A (Appendix J, Table 1).

TECHNIQUES AND REAGENTS

STANDARD REFERENCE COMPOUNDS (SRCs)

The SRC hydrolysis rate constants should be determined before analysis of any compounds of interest in order to establish operator proficiency. Determinations of SRC values should then be interspersed with rate constant measurements of the chemicals of interest. Details of chemical analysis and data workup are presented in subsequent sections.

TEMPERATURE CONTROL

Reaction rates are strongly dependent on temperature; therefore, the temperature should be minimized during a run and be accurately measured. A rule of thumb is that rates double for each 10°C rise in temperature, increase by 7% for a 1°C rise, and by 0.5% for a 0.1°C rise. This corresponds to an activation energy of approximately 20 kcal/mol. Constant temperature baths should be used that can maintain the temperature within ±0.02°C. A refrigerated bath is necessary for 0 to 30°C measurements. An oil bath is required for temperatures greater than 75°C. Temperatures should be measured with American Society for Testing and Materials (ASTM) thermometers, calibrated by NBS procedures and NBS certified masters, or equivalents.

pH MEASUREMENT

Because hydrolytic reactions can be catalyzed by hydronium or hydroxide ions, accurate pH data must be available for each buffer solution at each temperature. The most practical way to accomplish this is to heat the standard buffer solution to the desired reaction temperature, and then to measure the pH of the solution. This requires temperature compensation, either automatic or manual, and a pH probe stable to varying temperatures and accurate to ±0.02 units in the pH range 3 to 11. The pH of the reaction

buffer solution should be remeasured upon completion of the hydrolysis experiment. If the pH has changed more than 0.03 pH units the run should be discarded. When the observed hydrolysis rate (k_h) of a chemical is due solely to acid catalyzed or base mediated reaction (pseudo-first-order kinetics), the value of k_h will decrease by a factor of ten for every change of one pH unit toward neutrality. An excellent treatise on pH is found in Determination of pH Theory and Practice by Roger G. Bates (7).

BUFFERS

Autoclaved (CO_2-free) water is used to prepare stock solutions of buffer and subsequent dilutions. In sealed containers, the 0.005 M buffers hold the pH within (±0.03 pH units) for up to 25 days. Standard buffers (0.005M) for pH 3, pH 7, and pH 9 to 11 are prepared in the following manner.

pH 3.00 Dilute 5-ml of 0.1 M potassium hydrogen phthalate to 100 ml with water, adjust to pH 3 with 0.1 M sodium hydroxide or 0.1 M hydrochloric acid.

pH 7.00 Dilute 5-ml of 0.1 M potassium dihydrogen phosphate to 100-ml with water, adjust to pH 7 with 0.1 M sodium hydroxide or 0.1 N phosphoric acid.

pH 9.00/pH 11.00 Dilute 5-ml of 0.1 M dipotassium hydrogen phosphate to 100-ml with water, adjust to pH 9 or pH 11 with 0.1 M sodium hydroxide.

Water used in preparing buffers is deionized and autoclaved as described in the following section. pH is measured with a pH meter accurate to ±0.02 units and equipped with a probe capable of operating accurately to 85°C.

WATER

Water must be sterile and of known electrical conductivity ($\leq$ 0.06 μmho or less) or electrical resistivity ($\geq$ 16.67 mega ohms), corresponding to ASTM Type I water described in ASTM D-1193-77, "Standard Specification for Reagent Water." Water meeting these requirements can usually be attained by passing previously deionized water through ion exchange resins to remove metal ions and a 0.2 μm membrane filter to remove bacteria. The water is Autoclaved for 30 min/liter and allowed to cool before use. Sterile water is stored in a sterile-cotton-plugged container until used. Sterility of water and buffer solutions before and after hydrolysis experiments is determined from pour bacterial plate counts on TGE agar (8).

SAMPLING CONSIDERATIONS

Two methods are preferred for conducting the hydrolysis rate constant measurements.

Removal of Aliquots

In this method, the reaction mixture is contained in one reaction vessel and aliquots are withdrawn at timed intervals for analysis by a predetermined method. It often is convenient to stop the reaction in the aliquot removed by some quenching technique (pH adjustment, or cooling to 2°C). The quenched aliquot may be analyzed at the convenience of the analyst. If the analysis must be performed immediately, it is necessary that the time required for sampling and analysis be the same for all samples. This is especially true for reactions with half-lives and analysis times of only a few minutes. Care must be taken to ensure that the removal of aliquots does not contaminate the reaction mixture or cause losses of the reactant.

Ampule or Test Tube Technique

In this method, aliquots of the reaction mixture are placed in separate vessels. The vessels can be sealed ampules or test tubes with Teflon-lined screw caps. Hydrolysis studies of volatile chemicals, long term studies ($\geq$1 week), and elevated temperature studies ($\geq$45°C) should always be performed with flame-sealed ampuls. Screw-capped tubes are adequate for less volatile chemicals with a run duration of $\leq$1 week. The head space in the sealed ampule should be kept to a minimum, and all ampules should be added to the thermostated bath simultaneously. The time zero tube is removed after thermal equilibrium is established ($\leq$1 hr).

METHODS OF ANALYSIS

In monitoring the concentration of the chemical of interest, any suitable analytical method may be employed. Chromatographic methods are recommended because of their compound specificity in analyzing the parent chemical without interferences from impurities or products of hydrolysis. In some instances, chromatographic methods allow monitoring of the parent compound and product(s) simultaneously. Chromatographic methods are helpful when products of hydrolysis are to be identified. The chosen method should have an established precision within ±5%.

TEST CHEMICAL SOLUTION

Stock solutions are prepared by dissolving the substrate in acetonitrile, methanol, or water based on consideration of the water solubility limitations of the substrate and a desired final concentration of 1E-5M. The desired concentration is such that 0.1 ml diluted to 100 ml with buffer yields a final concentration of 1E-5M. The desired

final concentration of 1E-5M is 500-fold less than the 5E-3M buffer solution. The final concentration must not exceed 50% of its water solubility. In the case of compounds soluble only in the low parts-per-billion (ppb) range (10^{-6} to 10^{-7} M), methanol or acetonitrile, not to exceed 1% of the final solution, can be added to the buffer to enhance solubility. If the concentration of substrate must be increased to greater than 1E-5M to achieve an analyzable concentration, the concentration of the buffer is increased accordingly to maintain the 500-fold ratio. The chemicals must not be heated when they are being dissolved.

HYDROLYSIS EXPERIMENTS

SCREENING TEST (LEVEL I)

Laboratory experiments should be divided into three levels. In Level I experiments, the hydrolysis protocol is tested in the laboratory by selecting and determining the hydrolysis rate constants established for the SRCs in Tables 2-5. Data generated in hydrolysis experiments on the SRCs are converted to rate constants using the computation techniques discussed below in the "Data and Reporting" section and the Appendices. The SRC rate constant measurements are repeated until the desired precision is attained in all phases of rate constant measurement.

SCREENING TEST (LEVEL II)

Level II experiments are screening tests to determine the approximate half-life and dependence of hydrolysis on pH, of chemicals of interest at pHs 3, 7, and 11 at a selected temperature. Results from Level II experiments are then used to set pH and temperature for Level III experiments. Level II experiments are intended to quantify the effects of temperature and pH on the hydrolysis rate of the chemical of interest.

Buffer solutions at pHs 3, 7, and 11 are prepared by following instructions in the section above entitled "Buffers." For each chemical, reaction mixtures should be prepared in each of the three buffer solutions without the use of heat. The chemical concentration should be less than one-half its water solubility and at less than 1E-5M (see section on "Test Chemical Solution"). The test chemical solution is transferred to sealed ampules or test tubes with Teflon-lined screw caps (15-ml tubes, 10-ml solution). A minimum of six tubes are prepared at each pH. Then based on a "best guess" half-life, the tubes are placed in either a 25, 45, 65, or 85°C constant temperature bath. After sufficient time to equilibrate (30 to 60 min), one sample is taken to determine the time zero concentration. The screening test decision tree (Figure 1) is then used to determine sampling times for subsequent tubes. Data are calculated according to procedures given in the "Data and Reporting" section.

DETAILED TESTS (LEVEL III)

The objective of this set of experiments is to determine k_A, k_N, and k_B (if all three processes are operative) at two or more temperatures (separated by 20°C or more) such that activation energies for each process can be calculated and used to predict hydrolysis rate constants at other temperatures and pHs. The preliminary values of k_A, k_N, and k_B calculated according to instruction in the "Treatment of Results" section and the screening test are used to design the Level III detailed tests. Assuming the k_h values at pH 3 and 11 are due solely to acid- and base-catalyzed reactions (pseudo-first-order kinetics), the value of k_h will decrease by a factor of 10 for every change of pH unit toward neutrality. If the k_h values at pH 3 and 11 are the same as the pH 7 value, then hydrolysis is independent of pH and controlled only by temperature. Level III experiments

for processes independent of pH are conducted at pH 7 and at temperatures that result in 50-80% hydrolysis between one day and two weeks. If either or both acid catalysis or hydroxide ion mediated hydrolysis is indicated in the screening test, then Level III experiments are conducted at pHs and temperatures (including pH 7) such that rate constants (first- and second-order) and activation energies can be calculated for the processes (k_A, k_N, K_B). Ideally rate constants would be determined at a constant temperature and at two or more pHs on each side of neutrality. Establishment of a pH versus first-order disappearance rate constant curve would allow prediction of rates at other pHs.

To quantify the effect of temperature and pH on the disappearance rate constant for hydrolysis, two objectives must be attained: (1) expertise must be established in rate constant measurements by reproducibly measuring values for the SRCs and (2) rate constants must be replicated (minimum of three) for the compounds of interest at two or more temperatures separated by 20°C. Level III experiments are set up and conducted similar to Level II measurements. Water, buffers, and test solutions are prepared in the same manner as for the screening tests (6 to 8 tubes or ampules). The temperatures and pHs are adjusted after consideration of the screening test results to yield experimental conditions that allow 50-80% reduction in concentration of the chemical in two weeks or less. The tubes are removed at regular intervals and the percent remaining of the test chemical is determined by a method of established accuracy and precision (±5% acceptable).

Two concentrations of chemical, differing by a factor of ten, can be used as a test to support the first-order kinetics hydrolytic mechanism. If plots of ln % chemical remaining versus time are linear and have the same slope within experimental error, then first-order kinetics are assumed.

Rate constants and activation energies are calculated by methods outlined in the following section.

DATA AND REPORTING

TREATMENT OF RESULTS

Screening Tests

An observed rate constant (k_h) for the pH 3, 7, and 11 runs is calculated by the appropriate method described in the Appendices (G-1, G-2, H, or I). At constant temperature, the k_h values for pHs 3 and 11 are compared to the value calculated at pH 7 for evidence of H^+ or OH^- enhancement of rates. If the observed rates are within experimental error, hydrolysis is considered to be independent of pH in the range 3 to 11 and Level III experiments are conducted at pH 7. The rate studies at pH 7 should be conducted at two or more temperatures to allow calculation of the activation energy (Ea) and collision frequency (A) by the method outlined in Appendix J.

If k_h at pHs 3 and 11 are greater than experimental error from the pH 7 rate, then second-order rate constants (k_A and k_B) can be calculated from each first-order rate constant at each temperature by dividing the measured k_h at the particular pH by the hydronium ion activity (k_A, pH 3) or hydroxide ion activity (k_B, pH 11). This calculation assumes hydrolysis at pHs 3 and 11 are second-order reactions (first-order in compound and first-order in hydronium or hydroxide activity). The effect of temperature on K_W is taken into account when using equation 5 to calculate hydroxide ion activity.

The results of the screening tests are used to estimate the hydroysis rate at other pH values, and are used to determine the pHs and temperatures

for the Level III hydrolysis rate determinations. Assuming that the k_h values at pH 3 and 11 are due solely to acid catalyzed and base mediated reactions the value of k_h will decrease by a factor of 10 for every change of pH unit toward neutrality, and vary approximately by a factor of 10 for each 20°C change in temperature (Ea = 84 kJ/mol).

Calculation of Rate Constants and Activation Energies from Level III Experiments

Conditions of the Level III determinations are set (huge excess of water, constant pH) such that the rate constant at a given pH and temperature should be pseudo-first-order. From equation 11 a concentration-time profile can be expressed where $[C_0]$ is the concentration of the test substrate at time zero and [C] is the concentration at a given time, k is the first-order rate constant and t is time.

$$\ln[C] = \ln[C_0] - kt \qquad (11)$$

Several methods can be used to calculate the pseudo-first-order disappearance rate constant for a compound at a set pH and temperature. The first two (Appendicies G-1 and G-2) consist of plotting either log % chemical remaining or ln % chemical remaining on the Y-axis versus time of sampling (time) on the X-axis. The slope of the best straight line drawn through the data points is used to derive the pseudo-first-order rate constant also called k_h (observed rate constant). A third method used to calculate k_h is illustrated in Appendix H using the rearranged log form of equation 11.

The last method (Appendix I) consists of processing data using the Lotus 1-2-3 software with the IBM PC/XT or equivalent. Software programs for slope are based on the linear least squares analysis of the ln of % chemical remaining versus time. Outputs from the linear regression program

are (1) slope (pseudo-first-order rate constant), (2) Y-intercept, (3) variance, (4) standard deviation (SD) of Y-intercept, (5) standard deviation (SD) of the slope, and (6) the correlation coefficient (r^2).

Marked curvature in a plot of ln % chemical remaining versus time or a low correlation coefficient indicates that a non-first-order process is influencing results and that additional experiments are needed. When acid catalyzed or base mediated hydrolysis is indicated by the screening test, the k_A and/or k_B are calculated by dividing the measured k_h at the particular pH by the hydronium ion activity (k_A, pH 3) or hydroxide ion activity (k_B, pH 11). This assumes that, at pHs 3 and 11, hydrolysis is 100% dependent on the hydronium and hydroxide ions, respectively. The value for k_N is calculated using equation 4 and the k_h value measured for the pH 7 hydrolysis.

Under ideal conditions, the Level III hydrolysis studies are conducted at three temperatures (separated by 20°C each) and the three pHs 3, 7, and 11. Values of k_A, k_N, and k_B are calculated at each temperature. Regression analysis using equation 8 on the three sets of three constants at three temperatures yields values for E_A, E_N, E_B, ln A_A, Ln A_N and Ln A_B. These values of E and A can be used to calculate values of k_X (X = A, N, B) at temperatures of interest. The calculated k_X values at a particular temperature are used in equation 4 to calculate k_h at a chosen pH (K_w at the particular temperature is used when calculating $[OH^-]$ by equation 5). Values of A and E for each process can be estimated by plotting log k_X versus 1/T (Arrhenius plot) and taking the best straight line through the data points. The slope will equal -Ea/R with intercept of ln A. A two point Arrhenius plot can be used when kinetic data are available at two temperatures only.

Extrapolation of k_h, k_A, k_N, and k_B Using Approximate Activation Energies

In instances where k_h, k_A, k_N, and k_B are known/measured at only one temperature and E and A are not known, the rates can be approximated at other temperatures using equation 9 and assuming a value for the activation energy (Ea). Activation energies for the majority of compounds fall in the range of 62.7 - 104.5 kJ/mol. Using an Ea value of 83.6 kJ/mol in equation 9 to extrapolate a measured k to a new temperature is a reasonable approximation. The k value obtained using 83.6 kJ/mol will be within a factor of three of the values obtained using 62.7 kJ/mol or 104.5 kJ/mol (1 kcal = 4.18 kJ). The above values correspond to 15, 20, and 25 kcal/mol.

ACKNOWLEDGMENTS

This work was conducted at the Athens Environmental Research Laboratory through the combined efforts of EPA, Technology Applications, Inc. (TAI), and University of Georgia (UGA) personnel. The technical assistance of Miss Sarah Patman (UGA) is gratefully acknowledged. The assistance of Drs. Lee Wolfe and William Steen throughout the project and including review of this report is also gratefully acknowledged. Discussions with Mr. William Donaldson were always fruitful and so acknowledged. Mrs. Karin Blankenship's effort in typing the draft and subsequent revisions was exemplary.

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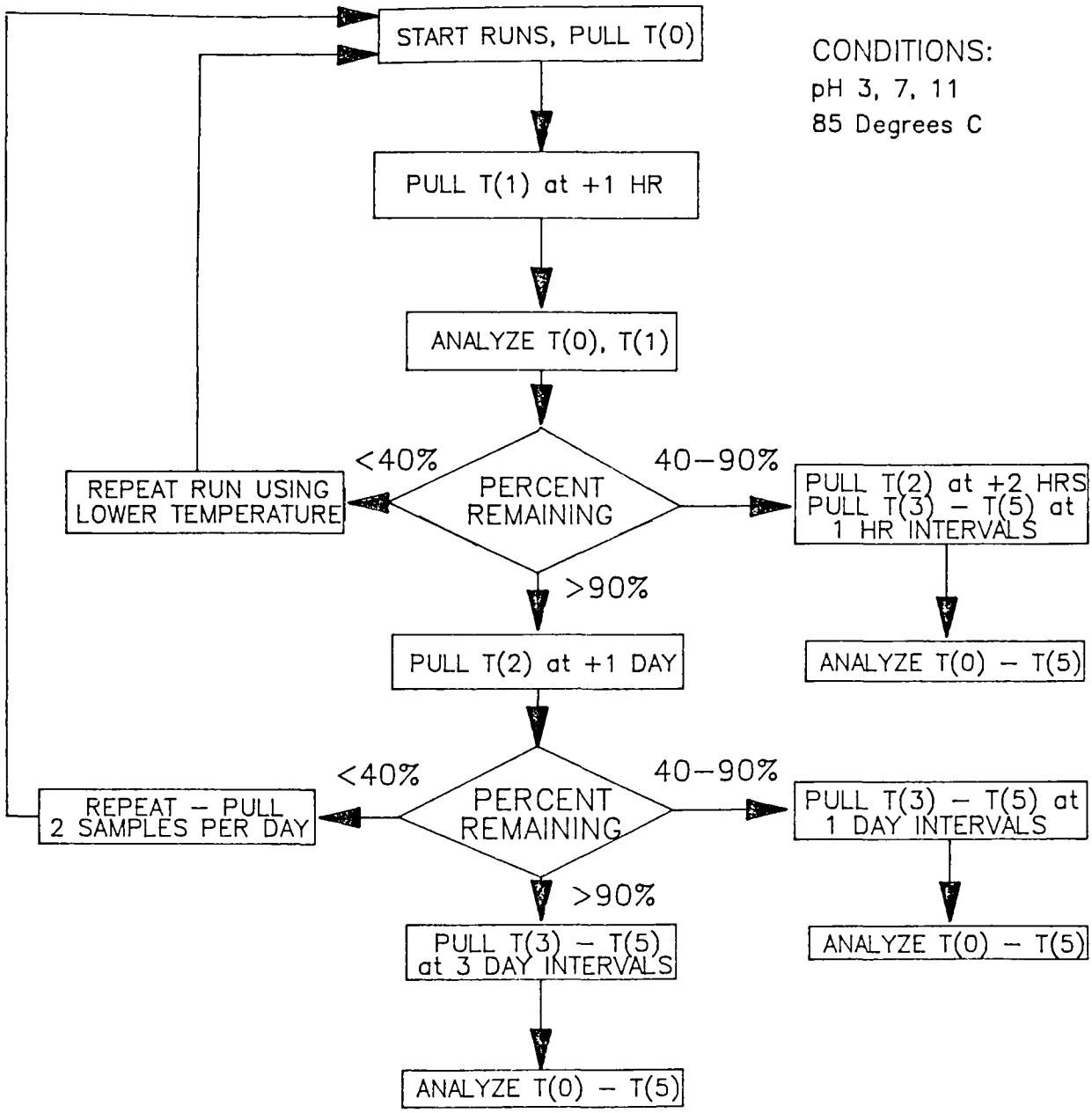
SCREENING TEST DECISION TREE



CONTENTS

Summary of Appendices

APPENDICES

Example Sheets for Generation of Data and Calculation of Rate Constants and Activation Energies.

SUMMARY OF APPENDICES

The exhibits are intended as aids in obtaining, calculating, and compiling data. They are not intended as replacements for laboratory notebooks, but yield quick access to the status of the particular chemical under study. The Chemical Information Sheet (Appendix A) should be completed before any laboratory work begins. This particularly includes the CAS number-structure match and the purity-identity analysis of the analytical standard used in subsequent hydrolysis studies. The Linearity Data Sheet (Appendix B) is completed after the method of analysis is determined. Hydrolysis studies are then performed in the range of concentrations where response versus amount of compound is linear. Appendices C, D, and E are used to record and summarize data for the three major methods of analysis used in hydrolysis rate constant measurements. In Appendix F the observed rate constants and calculated second-order rate constants are compiled according to temperature.

Actual determinations of rate constants from disappearance data is detailed in appendices G, H, and I.

In Appendicies G-1 and G-2, either log or ln % chemical remaining versus time is plotted and the negative of the slope of the "best fit" line is used to generate the rate constant. If there is random scatter in the points, a good method for estimating the best slope is to first adopt an intentional bias toward a high slope and to draw the steepest line that could legitimately be fitted to the points. Second, one should adopt the opposite bias, and draw the line with the least steep slope that could be fitted to the points. Finally, one should take the average of the two extreme slopes as the best slope of the plotted points.

By means of equation A-1 (integrated rate equation), the value of k is computed between t_0 and t_1, between t_1 and t_2, and so on, and the average of the resulting k values is taken as the rate constant (Appendix H). The interval between t_0

$$\log (a-x_i) - \log(a-x_j) = \frac{k}{2.303} (t_j - t_i) \qquad (A.1)$$

and t_1 can be neglected if there is any doubt about the accuracy of the initial concentration (a) or determination of zero time (t_0). In many instances, the k value calculated between t_0 and t_1 is much higher than succeeding k values.

In Appendix I, the % chemical remaining versus time data were processed on a Lotus 1-2-3/IBM PC-XT using a data entry/linear regression program. Values obtained from the linear regression program include the slope (pseudo-first-order rate constant), Y-intercept, variance, SD of Y-intercept, SD of slope and the correlation coefficient (r^2). The second-order alkaline elimination rate constant was calculated by dividing the pseudo-first-order rate constants by the calculated hydroxide ion activity at 40°C and pH 11.2.

Appendix J is the output from a computer program to fit temperature dependent rate constant data to a line and obtain the Arrhenius parameters [collision frequency (A) and activation energy (Ea)]. The Arrhenius plot requires plotting ln k versus the reciprocal of the corresponding temperature in degrees Kelvin. The slope of the line is equal to Ea/(R) and ln A is the Y-intercept.

APPENDIX A

Chemical Information Sheet

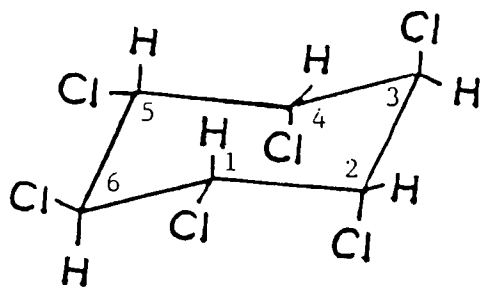
Chemical Abstract Service (CAS) Number: 58-89-9

CAS Name: Cyclohexane, 1,2,3,4,5,6-hexachloro-,(1α, 2α, 3β, 4α, 5α, 6β)

Common and Trade Names: Lindane, Hexachlorocyclohexane, gamma-BHC

Source: Research Triangle Park Repository

Structure:



Property:	Value	Source
Molecular Weight	291	
Melting Point, °C	112.5-113	
Boiling Point, °C, torr		
Water Solubility, °C	6.8-7.8 ppm at 25°C	
Vapor Pressure	20°C, 0.03mm Hg	
Octanol-water, K_{OW}	5,250	

Purity Determination: GC, GC-MS

APPENDIX B

Linearity Data Sheet

RS: _____ Date: _____

Analyst: _____

Compound (A_i) _____

_____

1. Stock Solution

Weight of A_1: _____mg dissolved in _____ml of

_____(solvent)

Purity of A_1: _____ Supplier: _____

Adjusted conc. of stock: _____pp_

Date Prepared: _____

2. Standard for Retention Check

_____pp_ of _____(A_i)

_____pp_ of _____(IS)

Solvent: _____ Date Prepared: _____

Method of Analysis: GC _____ HPLC _____ UV_____

Other _____

Attach copy of method to data sheet.

RS = Run series number
Ai = Analyte of interest
pp_ = parts per thousand, million, etc.

(Appendix B cont.)

3. Linearity Standards RS: _____

Solvent: _____ Extraction performed? Yes___ No___

Standard	Conc.	Dilution Required
1		
2		
3		
4		
5		
6		
7		
8		

(Appendix B cont.)

Data

RS: _____

Std. No.	Conc.	Response
1		
2		
3		
4		
5		
6		
7		
8		

Conclusions:

APPENDIX C

Hydrolysis Data Sheet - GC

RS: 2126 Date: 11-3-87

Analyst: WDP

Compound (A_i): Lindane

1. Stock Solution

Weight of A_i: 10.3 mg dissolved in 10 ml

of acetonitrile (solvent)

Purity of A_i: 99.4% Supplier: RTP

Adjusted conc. of stock: 1024 ppm

Date prepared: 10-6-87

2. Standard For Retention Check

205 ppb of Lindane (A_i)

206 ppb of 1,2,3- and 1,2,4-Trichlorobenzene (IS)

Solvent: Isooctane Date prepared: 10-7-87

GC = Gas Chromatography
RS = Run Series Number
A_i = Analyte of interest
pp_ = parts per thousand, million, etc.
IS = Internal Standard

(Appendix C cont.)

3. Buffer Solution RS: 2126

Buffer strength (conc): 0.005 M

Type of buffer used: K_2HPO_4

Conc. of buffer stock: 0.10 M. Date prepared/Autoclaved: 10-26-87

Date-water Autoclaved: 10-26-87

pH of buffer before addition of A_i: 11.71 @ 22.5 °C

pH of buffer after addition of A_i: 11.20 @ 40.0 °C

_____ @ _____ °C

pH meter standardized at pH: 7 and 11

Conc. of A_i in buffer 205 ppb

Dilutions required:

0.02 ml (stock) -------> 100 ml (buffer)

(Appendix C cont.)

4. Extraction RS: 1019-1023

Extraction solvent: Isooctane

Internal Std.(IS) 2,4-D methyl ester

Conc. of IS in extraction solvent: 5.25 ppm

Extract 5 ml of buffer with 1 ml of extraction solvent

concentration in extraction solvent before dilution

1.0 ppm A_i

5.25 ppm IS

Is buffer neutralized before extraction? Yes X No

Dilutions: 0.1 ml ------> 5

Final Conc.: 20 ppb A_i

105 ppb IS

(Appendix C cont.)

5. GC Procedure GC No. 1 RS: 2126

A. Column (capillary): J and W, DB-5
(Supplier and code name)

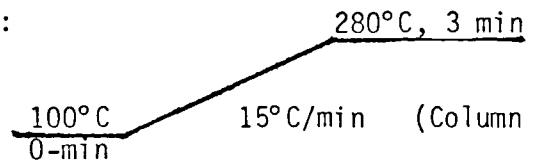
Length: 15M Dia: 0.53mm Coating: DB-5

Film thickness: 5.0 micron

B. Oven

Isothermal - oven temp: _____ °C

Program - Draw program below:



(Column compensation was used)

C. Injector

Split/Splitless: X On Column: _____

Injector temp.: 260 °C

Injection volume (size) 1.0 microliter

Septum purge: N_2 Flow = ~1.2 ml/min

D. Detector

ECD: X NPD: _____ FID: _____ :_____

Detector temp: 325 °C

Make-up gas: N_2 Flow: 27 ml/min

Attenuation: 2↑2---->2↑4

Hydrogen flow: _____ ml/min

Air flow: _____ ml/min

Filter: _____

E. Carrier Gas

Head pressure: 3 psi

Linear velocity: ~45 cm/sec

Flow: ~5 ml/min

Comments on GC technique:

(Appendix C cont.)

6. Sample Data* RS: 2126 pH: 11.20 @ 40.0 °C

Is A_i/IS ratio used for responses? Yes___ No X

Method of measurement: Area X Height _____ Both _____

Raw Data: Run Temperature = 40.0 °C

Samp. No.	Time(min)	Response	$\bar{X}$	%Remaining
1.	10:40,11-3 0 min	44181 40662 38384	41,076	100.0
2.	10.2 min	27041 30242	28,641	69.7
3.	34.8	10218 9807	10,012	24.3
4.	49.2	5111 5032	5,072	12.3
5.	60.0	3195 3270	3,232	7.9
6.	73.8	2546 2141	2,344	5.7
7.	100.2	746	749	1.8
8.				

Were any points discarded Yes ____ No X

List discarded points: _____

Give reason for discarding points:

If run was aborted, give reason for aborting the run:

Analyst: WDP Date: 11-3-87

Attach computer printout

K_1 = 0.0402 min^{-1}

K_2= 8.75 min^{-1}

T 1/2 = 17 min

*Data used in Appendices G-1, G-2, H, and I

APPENDIX D

Hydrolysis Data Sheet - HPLC

RS: _____ Date: _____

Analyst: _____

Compound (A_i): _____

_____

1. Stock Solution

Weight of A_i: _____mg dissolved in _____ml of

_____(solvent)

Purity of A_i: _____ Supplier: _____

Adjusted conc. of stock: _____pp_

Date prepared: _____

2. Standard for Retention Check

_____pp_ of _____(A_i)

_____pp_ of _____ (IS)

Solvent: _____ Date Prepared:_____

HPLC = High Performance Liquid Chromatography
RS = Run Series Number
A_i = Analyte of interest
pp_ = Parts per thousand, million, etc.
IS = Internal Standard

(Appendix D cont.)

3. Buffer Solution RS:_____

Buffer Strength (conc.): _____M

Type of Buffer used: _____

Conc. of Buffer Stock: _____M. Date Prepared/Autoclaved: _____

Date-Water Autoclaved: _____

pH of buffer before addition of A_i: _____ @ _____ °C

pH of buffer after addition of A_i: _____ @ _____ °C

_____ @ _____ °C

pH meter standardized at pH: _____ and _____

Conc. of A_i in buffer: _____pp_

Dilutions required:

Final Conc. of A_i for injection _____pp_

Is sample neutralized before injection? Yes____ No____

(Appendix D cont.)

4. LC Procedure RS: _____ LC No. _____

A. Column: _____
(Supplier and code name)

Length: _____cm Dia: _____ mm

Packing: _____ Particle size: _____m

B. Detector UV: _____ Fluorescence: _____ Electrochemical: _____

Wavelength: _____nm Attenuation: _____

Potential: _____V Range: _____

Excitation Wavelength: _____ nm

Emission Wavelength: _____ nm

Inj. Vol. _____ml Flow Rate: _____

Pressure: _____

Mobile Phase: _____

_____

Gradient: Yes _____ No _____

Draw Gradient:

Comments on LC Procedure:

(Appendix D cont.)

Sample Data RS: _____ pH: _____ @ _____ °C

Is A_i/IS ratio used for responses: Yes_____ No_____

Method of Measurement: Area _____ Height _____ Both _____

Raw Data: Run Temperature = _____ °C

Samp. No.	Time	Response	X	%Remaining
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				

Were any points discarded: Yes____ No____

List discarded points: _____

Give reason for discarding points:

If run was aborted, give reason for aborting the run:

Analyst: _____ Date: _____

Attach Computer Printout K_1 = _____ hr^{-1} T 1/2 = _____ days

K_2 = _____ $M^{-1}hr^{-1}$

APPENDIX E

Hydrolysis Data Sheet - UV

RS: _____ Date: _____

Analyst: _____

Compound (A_i): _____

_____

1. Stock Solution

Weight of A_i: _____mg dissolved in _____ml

of _____(solvent)

Purity of A_i: _____ Supplier: _____

Adjusted conc. of stock: _____pp_

Date Prepared: _____

2. Standard For Retention Check

_____pp_ of _____(A_i)

Solvent: _____ Date Prepared: _____

UV = Ultraviolet
RS = Run Series Number
Ai = Analyte of interest

(Appendix E cont.)

3. Buffer Solution RS: _____

Buffer strength (conc): _____M

Type of buffer used: _____

Conc. of buffer stock: _____M. Date prepared/Autoclaved: _____

Date-water Autoclaved: _____

pH of buffer before addition of A_i: _____ @ _____ °C

pH of buffer after addition of A_i: _____ @ _____ °C

_____ @ _____ °C

pH meter standardized at pH: _____ and _____

Conc. of A_i in buffer _____pp_

Dilutions required:

Final conc. of A_1 for measurement: _____pp_

Is sample neutralized before measuring? Yes ____ No ____

(Appendix E cont.)

4. UV Procedure RS: _____

Reference: _____

Attach spectrophotometer readout and absorption curve:

Maxima found: _____

Comments:

(Appendix E cont.)

Data

Run Temp. _____ °C RS: _____

Absorbance Readings at Maxima

Run No.	Time
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	

pH _____ @ _____ °C

(Appendix E cont.)

Were any points discarded? Yes ____ No ____

List discarded points: _____

Give reason for discarding points:

If run was aborted, give reason for aborting the run:

Analyst: _____ Date: _____

Attach computer printout

K_1 = _____ hr^{-1}

K_2 = _____ $M^{-1}hr^{-1}$

T 1/2 = _____ days

Summary of Data:

Compound: _____

RS	T	pH	$k_h(hr^{-1})$	$k_A(M^{-1}hr^{-1})$	$k_B(M^{-1}hr^{-1})$	T1/2(d)	r^2

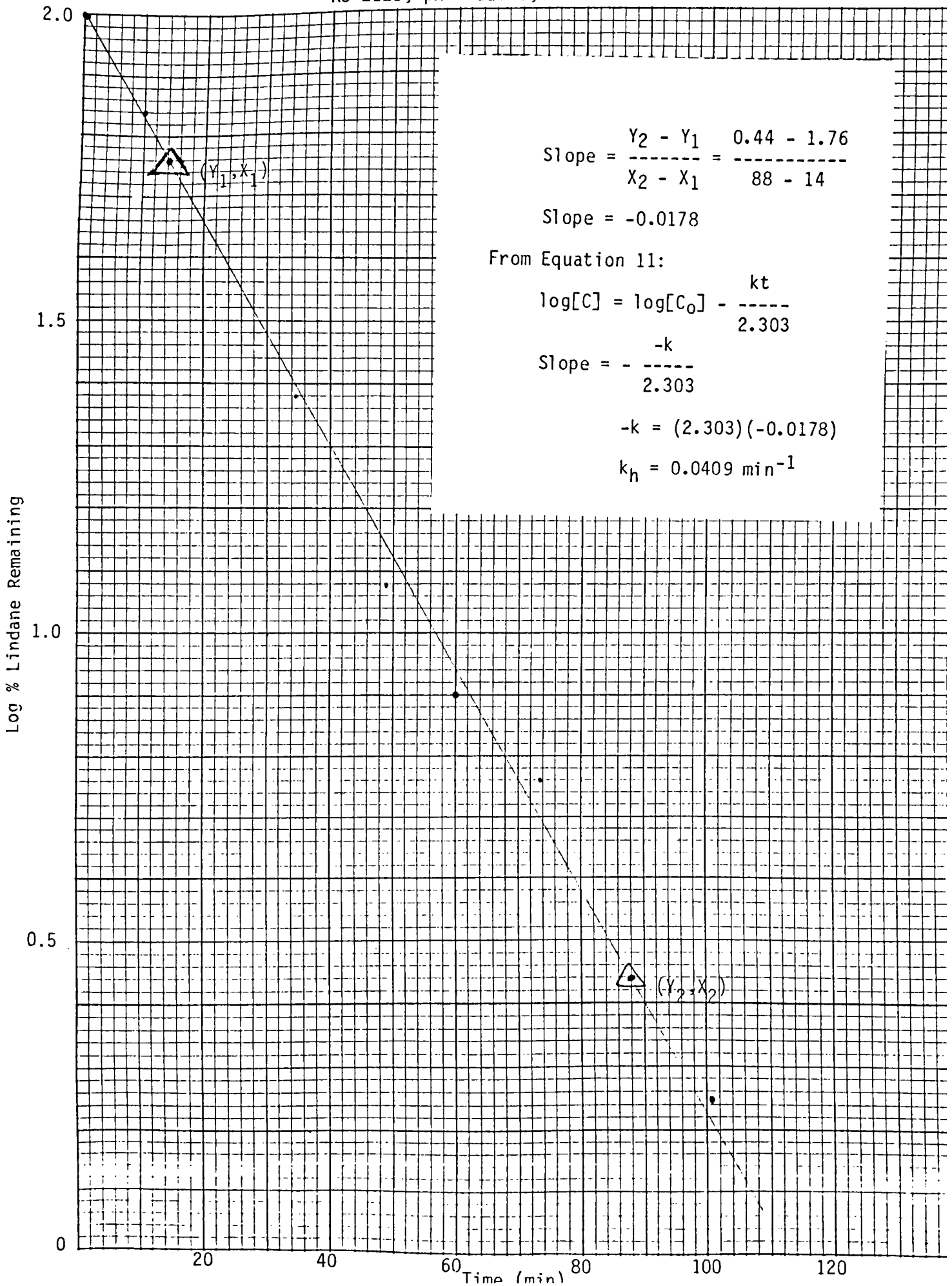
Ea = _____

Method: GC ____ LC ____ UV ____ Other ____

k_h is the pseudo-first-order rate constant measured at a particular pH and temperature.

APPENDIX G-1

RS 2126, pH = 11.20, 40°C



$$\text{Slope} = \frac{Y_2 - Y_1}{X_2 - X_1} = \frac{0.44 - 1.76}{88 - 14}$$

$$\text{Slope} = -0.0178$$

From Equation 11:

$$\log[C] = \log[C_o] - \frac{kt}{2.303}$$

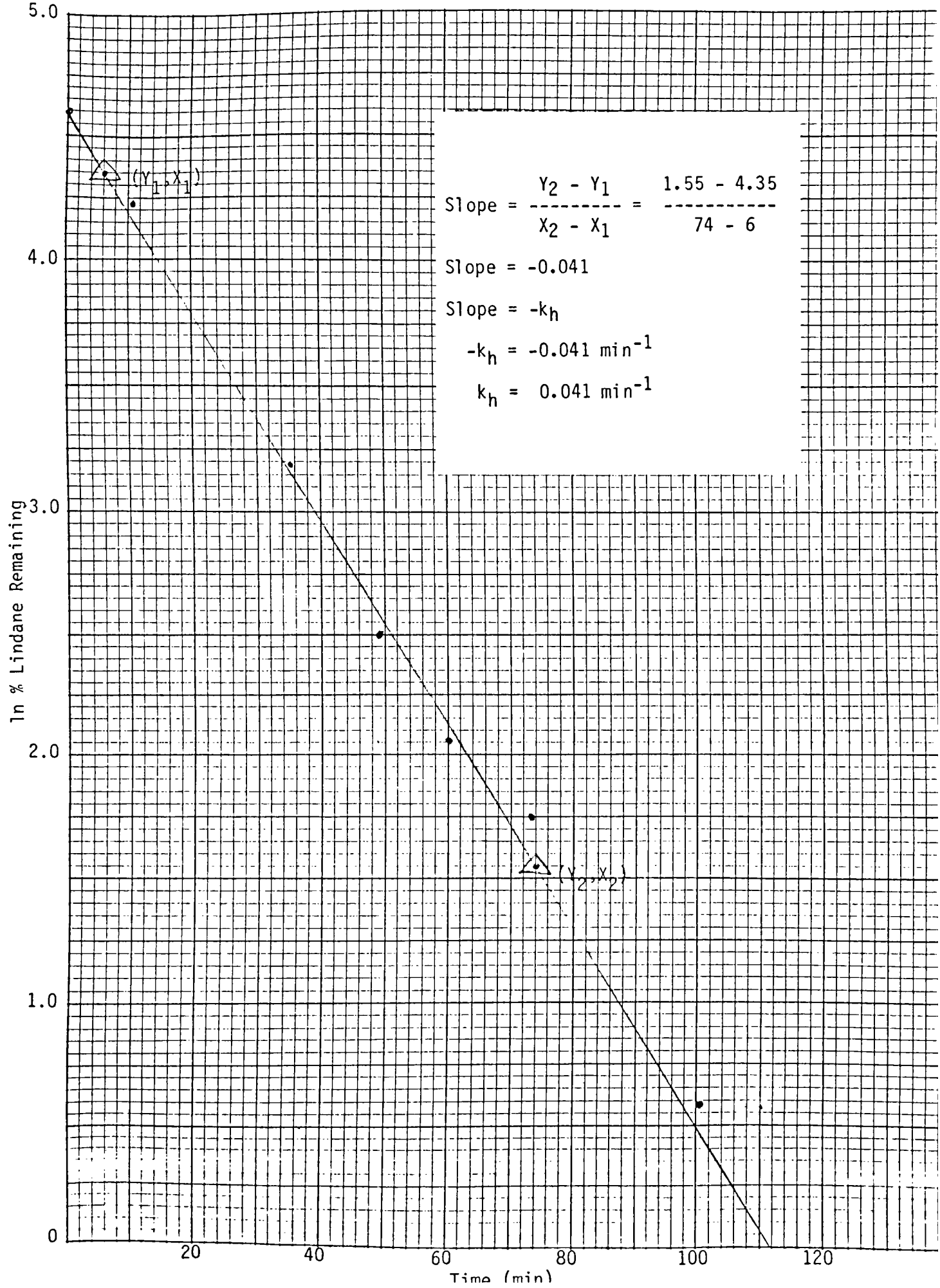
$$\text{Slope} = -\frac{-k}{2.303}$$

$$-k = (2.303)(-0.0178)$$

$$k_h = 0.0409 \text{ min}^{-1}$$

APPENDIX G-2

RS 2126, pH = 11.20, 40°C



Equation 11 can be rearranged to yeild:

$$k = \frac{2.303}{t} (\log[C_0] - \log[C])$$

This equation is often written as:

$$k = \frac{2.303}{\Delta t} [\log a - \log(a-x)]$$

Where the symbol a is used to replace C_0, x is the decrease of concentration in time t, and a-x = C the concentration at time t.

Time(min)	Log % Chemical Remaining	$k(min^{-1})$
0.0	2.00	
10.2	1.84	0.036
34.8	1.38	0.043
49.2	1.09	0.046
60.0	0.90	0.040
73.8	0.76	0.027
100.2	0.25	0.044
		0.039±0.007

$$k = \frac{2.303}{10.2} [2.00 - 1.84] = 0.036$$

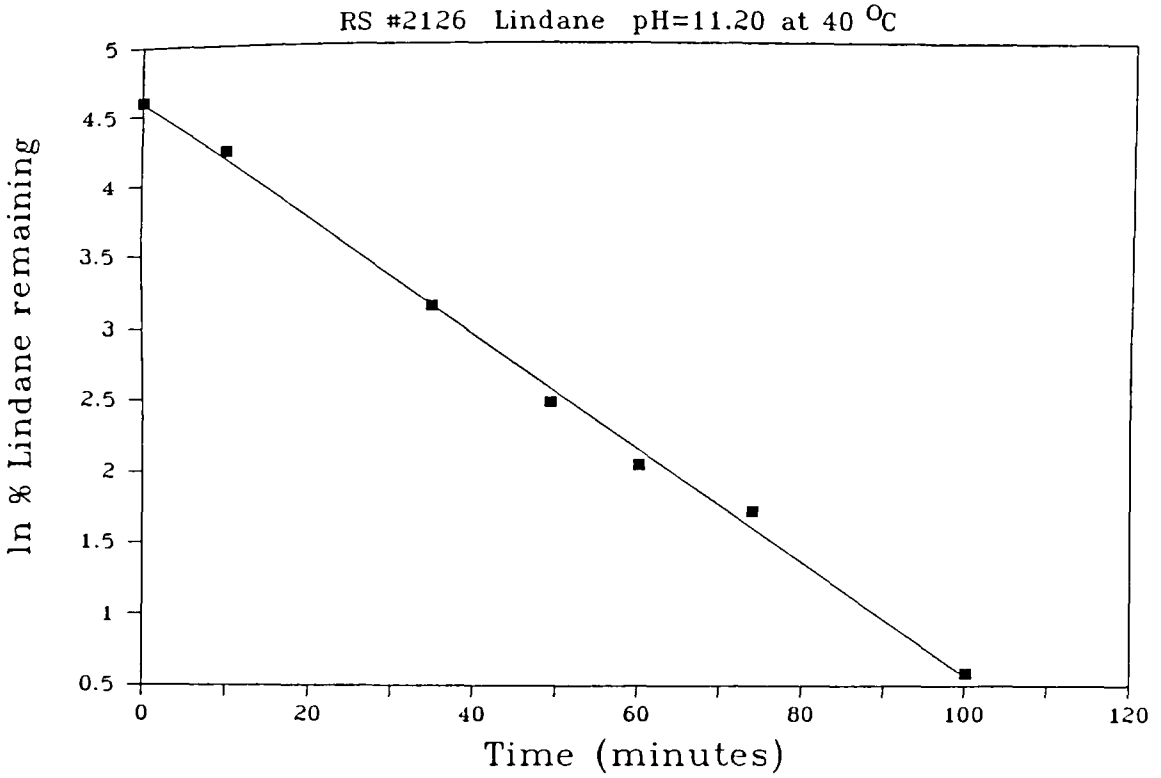
$$k = \frac{2.303}{24.6} [1.84 - 1.38] = 0.043$$

$$k = \frac{2.303}{14.4} [1.38 - 1.09] = 0.046$$

The remaining three k values are calculated in like manner.

Appendix I

Plot of ln % Lindane Remaining vs Time



RS 2126 Lindane pH = 11.20 at 40°C

Time Minutes	ln % Remaining
0.0	4.605
10.2	4.244
34.8	3.190
60.0	2.509
73.8	2.066
100.2	0.587

Number of observations	7
SD of Slope	0.00106
Slope of Regression Line	-0.04023
Regression Coefficient	-0.99827
SD of Y-intercept	0.060419
Y-intercept of Regr. Line	4.59417
R SQUARED	0.99685
Psuedo-K1	0.04023 min^{-1}

$$K_W = [OH^-][H^+],\ K_W \text{ at } 40°C = 2.919 \times 10^{-14}$$

$$[OH^-] = \frac{K_W}{[H^+]} = \frac{2.919 \times 10^{-14}}{6.30 \times 10^{-12}} = 0.0046$$

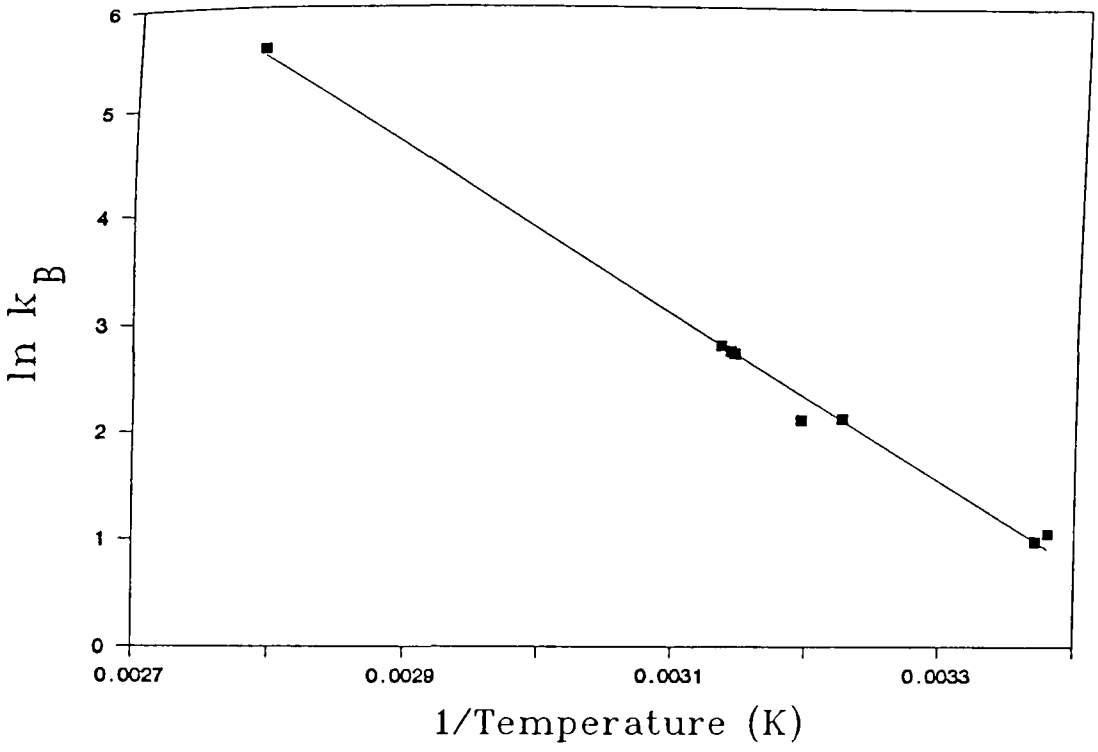
$$k_B = \frac{k_1}{[OH^-]} = \frac{0.04023}{0.0046} = 8.745\ (M^{-1}min^{-1})$$

The second-order alkaline elimination rate constant was determined at other temperatures and used in Appendix J to determine the activation energy and collision frequency.

Appendix J

Arrhenius Plot

Lindane



Temperature (°C)	k_B($M^{-1}min^{-1}$) $\bar{X}$ from Table 5
22.8	2.90
23.6	2.70
37.0	8.70
40.0	8.60
45.0	16.10
45.3	16.50
46.0	17.40
85.0	272.00

Number of points	8
Avg. of Y values	2.5521
Std. Dev. of Y	
Intercept	0.75
Coef. of Var. of Y	52.725
SSYY1	14.486
Std. Dev. of Slope	237
Slope	-7859.65
Regression Coef.	-0.997
Y-Intercept (ln A)	27.494
R Squared	0.9945
****Energy of Activation****	
65.279 ± 1.968	kJ/mol
15.617 ± 0.471	kcal/mol
for Arrhenius Equation only	

The above plot is a graphical representation of Equation 8

$$\ln k = \ln A - Ea/RT$$