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EVALUATION AND OPTIMIZATION OF HYDROLYSIS SCREENING PROTOCOLS

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ABSTRACT

We have evaluated two protocols that provide hydrolysis rate constants k_A, k_B, and k_N for acid-catalyzed, base-catalyzed, and neutral hydrolysis processes, respectively. The protocols have been performed at 25.0°C using ethyl acetate, cyclohexene oxide, and isopropyl bromide as test compounds. One major conclusion is that rate constant data obtained from experiments of short duration generally have higher precision than data from longer experiments. Other factors related to obtaining reliable hydrolysis data are discussed.

We have developed a collaborative test design for evaluating the precision and accuracy of the test protocol among several laboratories. We discuss the several factors including the number of laboratories, replication and use of regression analysis and have developed appropriate statistical methods for analyzing the test data. We also developed the detailed methodology, including initial planning, evaluation of chemicals, preparation of the collaborative test and evaluation of the reported results.

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SUMMARY AND CONCLUSIONS

This report describes a two part study to evaluate and optimize screening tests for hydrolysis kinetics and to design a collaborative test program for interlaboratory comparison of screening test results using standard chemicals.

In part one we selected three chemicals for the laboratory study, to provide a variety of physical properties and different absolute rates and rate dependence on pH:

(1) ethyl acetate (EA), a moderately soluble and volatile ester, hydrolyzes to give ethanol and acetic acid by acid and base catalyzed and neutral processes. First-order rate constants were measured at pH values of 3, 5, 7, 9, and 11 in buffered solutions at 25°C (Table 1); the half-lives of these reactions varied from 1.7 hours (pH 11) to 114 days (pH 5 and 7). The very slow reactions at pH 5 and 7 caused problems in using the EPA protocol to obtain reliable estimates of k_h, k_A, k_B, and k_N; the use of the SRI protocol which requires measurements at pH values of 3, 7 and 11 led to more reliable estimates of all rate constants including those for k_h at pH 5 and 7. The rate measurements for k_A and k_B are in good agreement with published values but k_N is about 400 times larger (Table 2).

(2) Cyclohexene oxide (CHO) has lower solubility and vapor pressure than EA but we estimate its volatility from water is similar. CHO hydrolyzes to give the corresponding diol (with no change in pH) and exhibits only acid catalyzed and neutral processes. We measured rate constants at pH 3, 5, 7, 9, and 11 at 25°C. At pH 3 the half-life is only six minutes, increasing to about 75 hours at pH 7-11 (Table 4); small effects of both buffer salts and ionic strength were noted (Table 5). Values of k_A and k_N agreed well when estimated from the SRI and EPA protocols but k_B was estimated much more reliably from the SRI protocol because of its small contributions to k_h at pH values lower than 11. In general estimates

of k_h based on the SRI protocols were in better agreement with measured values at all pH values than were those based on the EPA protocol but at pH values below 11 the EPA procedure gave satisfactory agreement between calculated and measured values of k_h (Table 8).

(3) Isopropyl bromide (IPB) is a quite volatile, insoluble chemical, which hydrolyzes to the alcohol and bromide ion by base-catalyzed and neutral processes only. At 25°C the half-life is close to 55 hours at all pH values up to 11 where the half-life decreases to 47 hours (Table 9).

In addition to measuring k_h by loss of IPB, we also performed experiments to estimate k_h from formation of isopropyl alcohol and bromide ion (Table 11). Although several rate measurements done this way gave good agreement with rate measurements performed on loss of IPB, significant discrepancies (factors of two or three) were observed at pH 7 and 9 for formation of bromide ion. Both SRI and EPA protocols gave satisfactory results with IPB for pH values of 3 to 9; only the SRI protocol would be used to estimate k_B satisfactorily owing to the small contributions made by this process. Our measurements showed small buffer salt and ionic strength effects on the hydrolysis rate constant (Table 10).

We propose an optimized protocol for hydrolysis using pH values of 3, 7, and 11 with a minimum of 6 time points at each pH and replicate analysis at each time point. We recommend that both EA and CHO be used as standard chemicals for calibrations of the testing methodology. Additional work is necessary to decide whether IPB is suitable as a standard or whether some other alkyl halide or other chemical might be a better choice. We estimate the cost of performing the optimized protocol at about $4,000.

In part 2 we describe a collaborative test design that provides statistical and operational methods for evaluating the precision and accuracy of the hydrolysis laboratory protocol. Definitions of terms used in the literature on collaborative testing serve as background information. A review of the hydrolysis testing protocols shows that the fundamental statistical problem is to estimate the rate coefficient for a first-order chemical reaction. Our statistical approach to designing

a collaborative study is based on a linear model of the natural logarithm (ln) of concentration versus time.

We discuss this approach in terms of the required number of laboratories, experimental replication, and the appropriateness of least squares regression analysis. Statistical methods are then presented for analyzing collaborative test data for a kinetic chemical process such as hydrolysis. Computational formulas estimate rate coefficients from data for individual laboratories and from collective data for all laboratories and determine whether differences among laboratories are explained by random error or are systematic.

Within-laboratory precision and repeatability are evaluated from the residual variation of data from the regression line that represents a first-order kinetic process. Between-laboratory precision and reproducibility are evaluated by subtracting the within-laboratory variance from the total variance of rate coefficients for laboratories in a collaborative study.

Finally, a discussion of operational elements of a collaborative study provides general information about initial planning, preliminary evaluation of chemicals, preparing a collaborative test protocol, conducting interlaboratory testing, analyzing data, and reporting results.

INTRODUCTION

The Toxic Substances Control Act of 1976 (P.L. 94-469) requires that the EPA evaluate all new chemicals for their possible adverse effects on the environment before manufacture and use are permitted. The act also provides that the manufacturers of new chemicals provide the EPA with laboratory and other test data on fate and effects for specific chemicals that may constitute a possible hazard to a biological population. To be useful to the EPA, test data must be developed under conditions that allow meaningful interpretation in the context of environmental transport and transformation processes.

Laboratory test methods or protocols have now been developed by SRI for EPA/ORD and by OPTS* for a variety of kinetic and equilibrium fate processes believed to be important in aquatic, atmospheric, and soil systems; other protocols are still being developed. These protocols describe in detail screening and detailed laboratory tests, from which the investigator can determine first an approximate rate constant for a process and, if needed, a set of detailed rate constants to cover a wider range of environmental conditions.

The screening protocols for hydrolysis proposed by EPA and SRI are designed to provide approximate rate constants for hydrolysis over a range of pH commonly found in aquatic systems, at one temperature. Although the procedures described in the protocol are standard practices in environmental research laboratories and most of the procedures have been performed satisfactorily at SRI, the complete procedure has not

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*A set of interim test protocols for transformation and transport processes has been prepared by the Office of Pesticides and Toxic Substances (OPTS); SRI has prepared another set of protocols under the EPA contract 68-03-2227 for the Office of Research and Development. These two sets of protocols are very similar in most respects, and we have examined both sets of protocols during this study.

been optimized and systematically evaluated with several chemicals having widely different physical properties and chemical reactivities. Nor have any standard chemicals suitable for calibration and checking procedures been recommended or tested. We seek to remedy these deficiencies in the existing EPA and SRI protocols and to develop a carefully designed collaborative test to be performed by several laboratories.

The results of this study will be broadly applicable to environmental fate testing programs in industry and government in the United States.

OBJECTIVES

The objectives of this study are

(1) Evaluate and optimize the accuracy and efficiency of proposed EPA and SRI screening test protocols for hydrolysis by performing screening tests using selected chemicals to examine the effect of pH, volatility, reactivity, and solubility on the accuracy of the procedure.

(2) Recommend and test standard chemicals for calibration of laboratory procedures for hydrolysis.

(3) Design but not implement a collaborative test program to be performed in several different laboratories to objectively evaluate the optimized protocol.

DESIGN OF TEST PROTOCOLS

The foundation for use of laboratory data for environmental assessment is based on the following assumptions:

- The rate of transformation or transport of a chemical in or from an environmental system is the sum of the rates of known individual chemical, physical, and biological processes.
- The rate or equilibrium constants for these processes can be measured independently in the laboratory.
- The laboratory data for individual processes can be integrated and extrapolated to the appropriate set of environmental conditions using simple or computer models.

The second factor refers specifically to extrapolation or scaling methods that accurately combine environmental variables, such as pH, wind velocity, or microbial cell count, with the process affected by the variable. Since a specific equilibrium or rate process can be measured quite accurately usually to within ± 10%, whereas values of environmental variables can vary dramatically and are rarely known to within more than a factor of two, the accuracy of fate estimates is usually limited by the accuracy of the environmental descriptors, not the laboratory data.

Criteria for Selecting Test Methods

Although many experimental procedures have been described to measure rate and equilibrium constants for processes analogous to those found in the environment, for several reasons most procedures are inapplicable for developing data useful for fate assessment. One reason is that some procedures give only qualitative information about the process and thus can be used only to judge whether the reaction occurs or not.

Another reason is faulty design of the experimental procedure, which prevents control of some important variables and hence gives data that are affected by some other, unsuspected and more rapid process. An example of this situation is found in measurements of loss of a highly insoluble chemical from water at elevated temperature, where the loss is thought to be caused by hydrolysis but actually is caused by volatilization. A third reason is that some procedures are used in the laboratory under conditions for which no satisfactory extrapolation is possible to a specific environmental situation.

Thus, the scientific criteria for judging the suitability of a test procedure for environmental assessment are the quantitative character of the data, the use of proper controls to ensure the applicability of the data for the intended process, and the availability of reliable scaling or extrapolation procedures.

Apart from the purely scientific validity of specific laboratory tests, the generality and complexity or sophistication of tests must also be considered in evaluating available methodologies, especially if such tests are intended as protocols for regulatory use. Preferred test methods are those generally performed by experienced laboratory personnel with instruments commonly found in well-equipped analytical and physical chemistry laboratories. Each procedure must be evaluated and optimized for the balance between speed, accuracy, and cost.

Hydrolysis Processes and Kinetic Relations*

Hydrolysis refers to a reaction of a compound with water, usually resulting in the net exchange of some leaving group (-X) with OH at a reaction center:

$$RX + H_2O \longrightarrow ROH + HX$$

*A detailed discussion of environmental hydrolysis processes is found in the review by Mabey and Mill (1978).

The mechanism of the reaction may involve a cationic or anionic intermediate, and the hydrolysis rate may be promoted or catalyzed by acidic or basic species, including hydroxide (OH^-) and hydronium (H_3O^+ or H^+) ions. The promotion of the reaction by H_3O^+ or OH^- is referred to as specific acid or specific base catalysis, as contrasted to general acid or base catalysis encountered with other cationic or anionic species.

For the hydrolysis protocol we consider only specific acid or base catalysis together with the neutral water reaction. The concentration of H_3O^+ or OH^- is directly measured by the pH of the solution, an easily measured variable for aquatic systems. Although other chemical species can be involved in hydrolysis reactions, their concentrations in aquatic systems are usually quite low and their effects are not expected to contribute to the overall rate (Mabey and Mill, 1978).

The rate law for hydrolysis of chemical RX usually can be put in the form

$$-\frac{d(RX)}{dt} = k_h[RX] = k_B[OH^-][RX] + k_A[H^+][RX] + k_N'[H_2O][RX], \quad (2)$$

where k_B, k_A, and k_N' are the second-order rate constants for base- and acid-catalyzed and neutral processes, respectively. Since the concentration of water is nearly constant and much greater than the chemical RX, $k_N'[H_2O]$ is a constant (k_N). The pseudo-first-order rate constant k_h is the observed or estimated rate constant for hydrolysis at a specific and constant pH and temperature. Equation (2) assumes that the individual rate processes for the acid, base, and neutral hydrolyses are each first order in substrate. With only a few exceptions, this is the case, and

$$k_h = k_B[OH^-] + k_A[H^+] + k_N . \quad (3)$$

From the autoprotolysis water equilibrium [equation (4)], equation (3) may be rewritten as equation (5).

$$[H^+][OH^-] = K_w \tag{4}$$

$$k_h = \frac{k_B K_w}{[H^+]} + k_A[H^+] + k_N \tag{5}$$

From equation (5), it is evident how pH affects the overall rate: at high or low pH (high OH^- or H^+) one of the first two terms is usually dominant, whereas at pH 7 the last term can often be most important. However, the detailed relationship of pH and rate depends on the specific values of k_B, k_A, and k_N. At any fixed pH, the overall rate process is pseudo first order, and the half-life of the substrate is independent of its concentration:

$$t_{1/2} = 0.693/k_h \ . \tag{6}$$

Figure 1 shows how the log of the rate constant for hydrolysis varies as a function of pH following equation (5).

Hydrolysis Protocols*

Screening test protocols for hydrolysis of chemicals in water have been prepared to enable an investigator to estimate hydrolysis rate constants and half-lives for chemicals at any environmental pH between pHs 3 and 11 (SRI) or 5 and 9 (EPA).

The test protocol for hydrolysis is based on a large body of experimental data together with a detailed kinetic analysis of the process. In most natural waters, only pH and temperature affect rates of hydrolysis,

*The SRI and EPA protocols are similar; where they differ, we note the differences and designate the source (EPA or SRI).

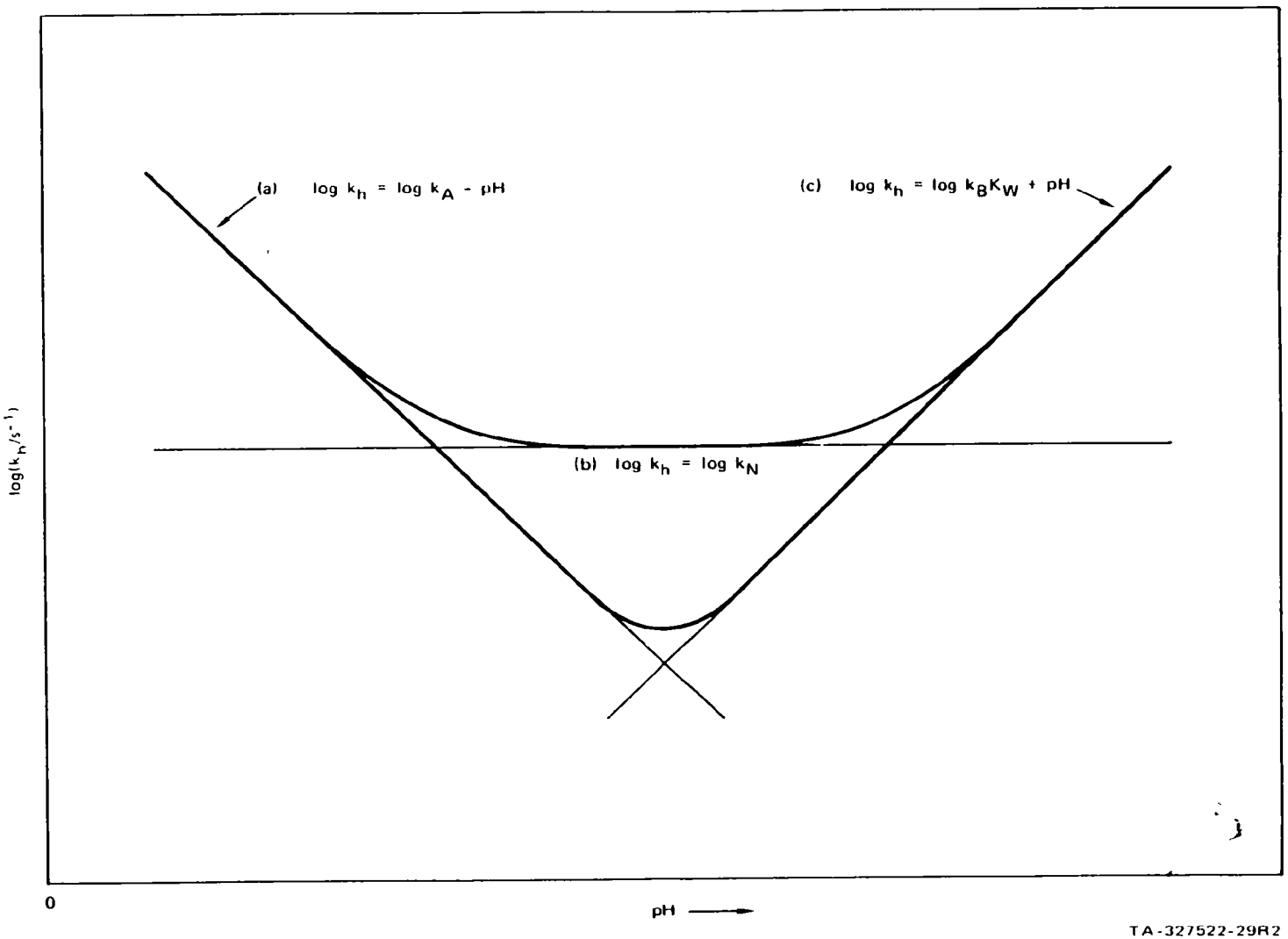


FIGURE 1 pH DEPENDENCE OF k_h FOR HYDROLYSIS BY ACID, WATER AND BASE PROMOTED PROCESSES

and all investigations to date show that hydrolysis rates at the same pH in buffered and natural waters are closely similar. Many classes of chemicals exhibit rate dependence on pH because of acid- or base-catalyzed hydrolysis. The EPA protocol recommends using pH 5, 7, and 9 to estimate the pH effect, whereas the SRI protocol recommends the wider range of pH 3, 7, and 11. Both protocols have sound rationales for their recommended pH ranges. One trade-off may be one of accuracy versus time: at pH 3 or 11 the rates are faster by a factor of 100 than at pH 5 or 9 for the acid- or base-promoted reactions, respectively, and thus are more quickly measured at useful conversions of chemical. However, the rate constants measured at pH 5 and 9 are more useful for environmental assessment purposes if accurately measured; the relative accuracy of the methods depends on several factors including the contribution of the neutral process to the total rate. If $k_N = 0$, the accuracy of the two methods is the same. Measuring k_h at pH values of 3 and 11 increases the contribution of the acid- or base-catalyzed process to the value of k_h, thereby making estimates of k_A or k_B more accurate. However, the data must be extrapolated to the more environmentally relevant region of pH 5 to 9.

Conditions under which hydrolysis experiments are conducted in the laboratory differ from those in the aquatic environment. In the environment, natural processes maintain the pH of a water body relatively constant (CO_2 absorption from air, metabolism, natural buffers); low concentrations of a chemical usually will not significantly affect the pH of the water. In laboratory measurements, however, the pH of the solution is usually kept constant by mixtures of acids or bases and their salts (buffers), which can also act as general acids or bases to catalyze the reaction under study and increase ionic strength. Therefore the challenge to the investigator is to select buffer concentrations high enough to maintain constant pH and yet avoid significant buffer catalysis or ionic strength effects.

Below we summarize the hydrolysis protocols recommended by SRI and EPA.

Screening Tests

The proposed screening protocols provide an estimate of the half-life of a chemical at pHs 3, 7, and 11 (SRI) or 5, 7, and 9 (EPA).

Solutions should be prepared using sterile, pure water and reagent grade (or purer) chemicals. Buffer solutions hsould be prepared according to the recommended procedure for pHs 3, 7, and 11 or 5, 7, and 9.

For each chemical being tested reaction mixtures should be prepared in each of the three buffer solutions without the use of heat. The chemical should be at a concentration less than one-half its solubility in water and at less than 10^{-3} M. If necessary, 1-vol% acetonitrile may be added to facilitate solubilization if the chemical is too insoluble in pure water to permit rapid dissolution.

Sealed ampoules or stoppered (no grease or polymers) volumetric flasks containing the reaction mixtures should be placed in a constant temperature bath at 25° ± 1°C. Chemicals that exhibit sensitivity to visible light should be placed in foil-covered flasks.

In the SRI test, solutions are analyzed for the concentration of chemical at t = 0, 44, and 88 hr. A measured half-life of less than 88 hr at pH 3 or 11 is equivalent to a half-life of less than 8800 hr (or 1 year) at pH 5 or 9, respectively. If more than 75% of the chemical has hydrolyzed after 2 hr, the half-life is less than an hour.

The EPA protocol recommends using detailed measurement regimens at pHs 5, 7, and 9: one for chemicals that hydrolyze rapidly, one for chemicals of moderate reactivity, and another for unreactive chemicals.

Data Treatment and Reporting

The EPA protocol uses the analytical data to calculate the rate constant and half-life at the pH of the measurements. The first order kinetic relation is

$$\ln(C_t/C_o) = -k_h t, \qquad (7)$$

where C_o and C_t are concentrations of chemical at times zero and t, and k_h is the first order rate constant in hr^{-1}. A least-squares fit of the values of C_t and t to the regression equation (7) gives the slope k_h. The half-life (in hr) is

$$t_{\frac{1}{2}} = 0.69/k_h \quad . \tag{8}$$

The EPA protocol specifies that the following data be reported: k_h and its correlation coefficient for each experiment, the mean value of k_h and its standard deviation for replicate experiments at the same pH, and $t_{\frac{1}{2}}$ from equation (8) using the mean value at pHs 5, 7, and 9. The SRI protocol uses the concentrations at 44 or 88 hr to estimate limits for $t_{\frac{1}{2}}$ thus giving approximate values of k_h.

Scope and Limitations of the Hydrolysis Protocol

Although many chemicals exhibit limited rate dependence on pH because of the relative unimportance of one or more of the hydrolysis processes in the pH region of prime interest, kinetic studies should always be performed at three pH values near 3, 7, and 11 or 5, 7, and 9 to check the consistency of the rate data. While intervention of other unsuspected processes can be partly anticipated and minimized through proper experimental design (e.g., sealed and sterile containers to eliminate volatilization and biodegradation, respectively), other chemical processes such as pyrolysis, rearrangement, or elimination may be important for some chemical structures.

Another limitation on the scope of this protocol is in the measurements of the hydrolysis rates of chemicals that reversibly ionize or protonate in the pH range of interest. The hydrolysis rates of these compounds will often have unusual pH-rate profiles because of competition between the reactions of the charged and uncharged forms:

$$H_2O + HAY \rightleftharpoons AY^- + H_3O^+$$

$$AY^- \longrightarrow A + Y^-$$

$$HAY \longrightarrow HA^+ + Y^-$$

The net effect is that the pH-rate profile for HAY will be more complicated than the typical curve and will often have a minimum or maximum, and the exact features cannot be decided a priori. Should there be any question concerning the possible importance of this effect in hydrolysis of an ionic chemical having a pK_a or pK_b in the pH region of 3 to 11, additional measurements should be made to define the pH-rate profile.

EVALUATION OF SRI AND EPA PROTOCOLS

Laboratory Studies

We selected three simple commercially available chemicals to evaluate the SRI and EPA protocols: ethyl acetate, $CH_3C(O)OC_2H_5$, (EA); cyclohexene oxide, $C_6H_{10}O$, (CHO); isopropyl bromide, $(CH_3)_2CHBr$, (IPB). Each compound was selected because we believe it represents a class of chemicals that exhibit specific hydrolytic properties common to many organic structures, e.g., only acid and neutral hydrolysis or only acid and base hydrolysis. Each chemical also has specific physical properties, such as solubility and volatility, that can lead to special problems that occur in testing many chemicals. Thus we chose these chemicals to provide a limited but reasonable cross section of hydrolysis kinetic systems expected to be encountered among many potential chemicals that might be tested in the future.

We specifically avoided chemical structures that we believed would hydrolyze by more than one chemical pathway to minimize kinetic complications in testing the screening protocols; complications may be introduced at a later time when the basic screening test is optimized.

We also tried to exclude all other loss processes by using foil wrap to avoid photolysis, sterile water and containers to avoid microorganisms, and sealed containers to avoid volatilization. Controls help check for the presence of these adventitious processes, but, despite our best efforts, some experiments were confounded by other processes and had to be repeated. In at least one case with IPB, at the lowest concentrations we could use in nearly pure water, insolubility may have been a contributing problem. Buffer catalysis and ionic strength effects, which were also encountered, are potentially serious problems that are discussed in the context of specific experiments.

Hydrolysis of Ethyl Acetate

The reaction for hydrolysis of EA is as follows:

$$CH_3C(O)OC_2H_5 + H_2O \longrightarrow CH_3C(O)OH + C_2H_5OH$$

Ethyl acetate was chosen as a representative of a class of chemicals that exhibit both acid-, and base-catalyzed and neutral hydrolysis in the environmental pH region 4 to 9. In our earlier review of hydrolysis (Mabey and Mill, 1978) we found that aromatic and aliphatic esters had no important neutral (pH independent) hydrolysis process unless a strongly electron-withdrawing group(s) was present on the α-carbon of the carboxyl or alcohol group.

The Henry's law constant for EA was calculated to be 118 torr M^{-1} at about 25°C, using a solubility of 7.44 g EA per 100 ml water (0.85 M) (The Merck Index, 1976) and a vapor pressure of 100 torr (or 0.13 atm) at 27°C (CRC Handbook, 1975). Therefore EA would not volatilize rapidly from water. The high water solubility of EA also showed that sorption to glass would not be a problem in experimental work.

Our earlier review of hydrolysis data (Mabey and Mill, 1978) reported the following rate constants for hydrolysis of EA at 25°C:

$$k_A = 1.1 \times 10^{-4}\ M^{-1}\ s^{-1}$$

$$k_B = 0.11\ M^{-1}\ s^{-1}$$

$$k_N = 1.5 \times 10^{-10}\ s^{-1}$$

These data were reported by Skrabel and Ruckert (1928), and the k_B value has been confirmed by Halonen (1956). No other data were found to corroborate the value of k_A or k_N. Further evaluation of these data has shown that the value of k_N is questionable because at pH 5.5, where the acid- and base-catalyzed process rates are equal, the neutral hydrolysis

process contributes only 18% of the total value of k_h and might not be detectable.

Using the values of k_A, k_B, and k_N listed above and using equations (5) and (6), we calculated the half-lives of EA at 25°C and at pH values used in the EPA and SRI protocols. These values, listed below, were used in planning the hydrolysis experiments:

pH	$t_{1/2}$
3	73 days
5	16 yr
5.5	26 yr (minimum)
7	720 days
9	7.3 days
11	1.8 hr

The analytical method of choice was gas chromatography; detectability by direct analysis from water solution (the preferred method) limited initial concentrations of EA to about 1×10^{-3} M. The very slow rate of hydrolysis expected at pH 5.5 also suggested that bringing each reaction mixture to pH 5.5 was an effective quencher for hydrolysis and was used throughout the study. Initial studies on EA found that adventitious process(es) (probably biodegradation or volatilization) complicated the experiments, and some hydrolysis data did not agree with literature data. Results of well-controlled experiments at pH values of 3, 5, 7, 9, and 11 are given in Table 1.

From these data, the acid, base, and neutral rate constants, k_A, k_B, and k_N, for EA were calculated using sets of simultaneous equations given in Appendix A. These second-order rate constants were obtained in accordance with the EPA and the SRI protocols (see Background). In addition, acid, base, and neutral rate constants were calculated using the overall rate constants at all four pHs. These constants are summarized in Table 2.

The k_A and k_B values from the literature and those calculated according to the SRI protocol agree exceptionally well. However, the value of k_A obtained from the EPA protocol is more than a factor of ten larger than the literature and the SRI values for k_A. The values of k_B from the literature and the EPA experiments agree more closely than the

Table 1

HYDROLYSIS OF ETHYL ACETATE AT 25°C

pH[a]	Elapsed Time (hr)	Concentration $10^3[EA]_o$, M	Number of Time Points	% Conversion	$k_h(s^{-1})$[b]	R^2
3	864	0.998	10	38	$(1.73 \pm 0.15) \times 10^{-7}$	0.97
5	234	1.05	1	5.7	7.0×10^{-8}	c
7	1008	1.03	2	25	6.98×10^{-8}	c
9	552	0.998	4	99	$(1.69 \pm 0.18) \times 10^{-6}$	0.96
11[d]	3.8	1.04	7	78	$(1.08 \pm 0.01) \times 10^{-4}$	0.999

[a] Standard pH buffer concentrations were used for all five solutions (CRC Handbook, 1975).

[b] These estimates of k_h are obtained from the regression of $\ln([C_o]/[C_t])$ versus time where C_t is the average concentration obtained from triplicate analyses.

[c] R^2 is not calculated for one or two points.

[d] See Appendix C and footnote in Table 2 for discussion of the effect of inadequate buffering during this experiment. Appendix D discusses analytical error and systems error.

Table 2

SUMMARY OF MEASURED VALUES OF ACID, BASE, AND NEUTRAL RATE CONSTANTS FOR HYDROLYSIS OF ETHYL ACETATE IN WATER AT 25°C

Experiment	k_A (M^{-1} s^{-1})	k_B (M^{-1} s^{-1})*	k_N (s^{-1})*
Literature	1.1×10^{-4}	1.1×10^{-1}	1.5×10^{-10}
SRI[a,*]	$(1.14 \pm 0.15) \times 10^{-4}$	$(1.08 \pm 0.01) \times 10^{-1}$	$(5.90 \pm 0.01) \times 10^{-8}$
EPA[b]	1.66×10^{-3}	1.64×10^{-1}	5.33×10^{-8}
ALL[c]	(-7.55×10^{-5})	1.08×10^{-1}	2.47×10^{-7}

[a] SRI denotes the set of EA experiments performed at pHs 3, 7, and 11.
[b] EPA denotes the set of EA experiments performed at pHs 5, 7, and 9; no error can be calculated since pH 5 and pH 7 rate constants are based on one data point.
[c] ALL denotes the set of EA experiments performed at pH values of 3, 5, 7, 9, and 11.
* Appendix C discusses the effect of inadequate buffering of pH during the pH 11 experiment; allowing for the drop in pH during the experiment, k_B is $(1.26 \pm 0.01) \times 10^{-2}$ M^{-1} s^{-1}, or an error of $\sim$ 14%. Using a value of $k_{(11)} = 1.26 \times 10^{-5}$ ($= k_B[10^{-3}]$), k_N is calculated to be 5.72×10^{-8} s^{-1} from the data at pH values of 3, 7, and 11; the error in k_N is then $\sim$ 3%. Further error analyses for the EA experiments is presented in Appendix D.

values of k_A. Still, the EPA value of k_B is about 50% larger than the SRI value. Finally, the k_N values for the EPA and the SRI experiments agree within 12%; however, the EPA and SRI values differ from the literature value by a factor of 400. As noted previously, the literature k_N value is highly suspect and we believe the present value to be more correct.

When the data from all five pH value experiments were used to calculate k_A, k_B, and k_N (by multiple linear regression, HP-97 program ST1-13A), k_B was identical to the SRI value. The k_N value was about a factor of five larger than either the SRI or EPA k_N value; k_A was negative and therefore invalid. The statistical section of this report discusses the difficulties associated with the multiple linear regression procedure.

As a check on the values of k_A, k_B, and k_N calculated by the several methods, the values of k_h were calculated for pH values other than those used to originally calculate the respective k_A, k_B, and k_N values (that is, the rate constants k_h at pHs 5, 7, and 9 were calculated from k_A, k_B, and k_N obtained according to the SRI protocol using data from experiments at pHs 3, 7, and 11). These k_h values are given in Table 3 and are compared with the measured values.

The rate constants calculated according to the SRI protocol agree fairly well with the measured values at pHs 5 and 9; the measured and the calculated rate constants are identical at pH 7. Comparison of the measured rate constants and those obtained using the EPA protocol shows that the k_h values at pH 3 are in poor agreement, whereas the calculated value at pH 11 is only in fair agreement with the measured rate constant . Finally, the rate constants calculated from the acid, base, and neutral rate constants obtained by multiple linear regression were compared with the measured rate constants for all five pHs. The measured and calculated rate constants at pHs 3 and 11 are identical; the rate constants at pH 9 agree fairly well; the calculated rate constants at pHs 5 and 7 are, however, much larger than the measured values.

Product analyses for ethanol and acetic acid were performed by gas chromatography (GC) but no good material balances were obtained. The reasons for this failure are not clear because ethanol appears to be well-behaved on GC analysis even though acetic acid is not.

Table 3

RATE CONSTANTS k_h (s^{-1}) CALCULATED FOR HYDROLYSIS OF ETHYL ACETATE AT 25°C AS A FUNCTION OF pH

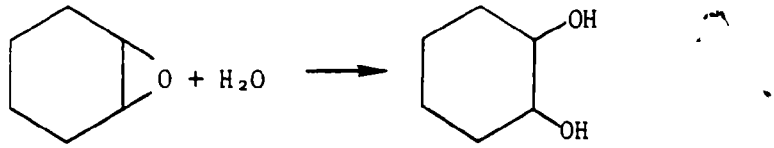
Source	pH 3	pH 5	pH 7	pH 9	pH 11
Measured	$(1.73 \pm 0.15) \times 10^{-7}$	7.0×10^{-8}[a]	6.98×10^{-8}[a]	$(1.69 \pm 0.18) \times 10^{-6}$	$(1.08 \pm 0.01) \times 10^{-4}$
Calculated[b] from pH 3, 7, 11 data/SRI	--	$(6.02 \pm 0.02) \times 10^{-8}$	$(6.98 \pm 0.14) \times 10^{-8}$	$(1.14 \pm 0.01) \times 10^{-6}$	--
Calculated[b] from pH 5, 7, 9 data/EPA	$(1.71 \pm 0.18) \times 10^{-6}$	--	$(6.98 \pm 0.19) \times 10^{-8}$	--	$(1.64 \pm 0.18) \times 10^{-4}$
Calculated[b] from pH 3, 5, 7, 9, 11 data	1.72×10^{-7}	2.46×10^{-7}	2.58×10^{-7}	1.32×10^{-6}	1.08×10^{-4}

[a] Standard deviations could not be calculated for these reactions where only one data point exists.

[b] Acid, base, and neutral rate constants obtained from the rate constants at these pH values are used in the calculations of the rate constants given on this line.

Hydrolysis of Cyclohexene Oxide

The reaction for hydrolysis of cyclohexene is shown below:



The reaction is acid catalyzed via protonation of the oxygen to give the intermediate $C_6H_{10}\overset{+}{O}H$, which is followed by reaction with water. Since alcohols are not very acidic (pK_A > 14), hydrolysis does not change the pH of the solution.

Cyclohexene oxide was chosen as a representative of a class of chemicals that exhibit neutral (pH independent) and acid-catalyzed hydrolysis processes in the environmental pH region 4 to 9. Our earlier reivew of hydrolysis (Mabey and Mill, 1978) found that base-catalyzed hydrolysis of epoxides was not important below pH 11, but that acid catalysis was important and competitive with neutral hydrolysis at pH values ranging from 4 to 8, depending on the structure of the epoxide. CHO was chosen as a model compound because it is structurally similar to 2-butene oxide [whose half-life at pH 7 and 25°C was estimated to be 4.4 days (Mabey and Mill, 1978)] but less soluble and more volatile.

No data on the physical properties of CHO were found other than the boiling point of 129° to 130°C at 760 torr. Another compound with the same molecular formula $C_6H_{10}O$, mesityl oxide (boiling point of 130°C), is reported to have a vapor pressure of 10 torr at 26°C, (CRC Handbook, 1975) and CHO probably has a similar vapor pressure. Although no water solubility data were found for CHO, a value of 9.4×10^{-2} M was calculated from a log K_{ow} value of 1.60 (Johnson, 1980) and the water solubility-log K_{ow} correlation equation of Yalkowsky and Valvani (1980). From these data the Henry's law constant was calculated as 106 torr M^{-1}. This value indicates that CHO has only moderate volatility from water at 25°C.

Sampling times for hydrolysis studies of CHO were initially selected using data for 2-butene oxide. Because initial time points showed that

CHO was hydrolyzing more rapidly than expected, samples were taken more frequently.

The results of these experiments to measure the hydrolysis rate of CHO at 25°C and at several pH values are tabulated in Table 4. In all experiments, samples were taken according to the SRI and EPA protocols. Standard buffer concentrations were used in this initial set of experiments (CRC Handbook, 1975).

The results of the initial set of experiments at pH values 7, 9, and 11 show that the rate constant k_h varied from 1.96 x 10^{-6} s^{-1} to 2.76 x 10^{-6} s^{-1} in this pH range and was not independent of pH over the pH range 7 to 11, as expected. Epoxides are known to undergo buffer catalysis (Whalen, 1973), and therefore another set of experiments at pHs 7 and 11 were performed using one-tenth the standard buffer concentrations (SBC) as well as with added sodium perchlorate ($NaClO_4$) to determine the importance of buffer catalysis on the hydrolysis of CHO. The results of these experiments are given in Table 5.

The rate constants at 0.1 SBC-pH 7.11 (Table 5), 0.1 SBC-pH 11 (Table 5), and SBC-pH 9 (Table 4) agree more closely (1.83 to 2.02) x 10^{-6} s^{-1}, as expected. The rate constant at 0.1 SBC-pH 7.11 (1.83 x 10^{-6} s^{-1}), however, is smaller than the rate constant at SBC-pH 9 (Table 4).

Another experiment was then performed at pH 9 in minimally buffered pH 9 borate solution to determine the extent of buffer catalysis on the hydrolysis of CHO. The measured rate constant in this minimally buffered (0.01 SBC) pH 9 solution is 1.81 x 10^{-6} s^{-1}. As shown in Table 5, this rate constant is in excellent agreement with the result from 0.1 SBC-pH 7.11 solution (1.83 x 10^{-6} s^{-1}) and in good agreement with the rate constant at 0.1 SBC-pH 11 (2.02 x 10^{-6} s^{-1}).

In earlier experiments at 0.1 SBC-pH 7, the measured pH was 7.11. Although the rate constant from these experiments (0.1 SBC-pH 7.11) should be unaffected by a small change in pH in this region, another experiment was performed to measure the rate of hydrolysis (at pH 7.00) in a minimally buffered system. The rate constant in this minimally buffered system (0.4 SBC) is 2.10 x 10^{-6} s^{-1}. This rate constant agrees much better with

Table 4

HYDROLYSIS OF CYCLOHEXENE OXIDE AT 25°C USING STANDARD BUFFER CONCENTRATIONS

pH,[a] Buffer Conditions[b]	Total Time Elapsed (days)	% Conversion	No. of Time Points	Initial Conc. x 10^3 (M)	Hydrolysis Rate Constant, 10^6 k_h (s^{-1})	Correlation Coef., R^2
3, SBC	14 min	80	6	1.01	1950 ± 9	0.999
5, SBC	1 03	90	9	1.01	25.5 ± 1 2	0.983
7, SBC	8 9	84	7	1 08	2.76 ± 0.11	0.992
9, SBC	9.8	80	7	1.02	1.96 ± 0.04	0.998
11, SBC	8 8	85	7	1.03	2.46 ± 0.08	0.995

[a] pH values are accurate to ± 0.02 pH.
[b] Buffer solutions made of standard buffer concentrations (SBC) listed in CRC Handbook (1975).

Table 5

HYDROLYSIS OF CYCLOHEXENE OXIDE AT 25°C USING SEVERAL BUFFER CONCENTRATIONS

pH,[a] Buffer Conditions[b]	Total Time Elapsed (days)	% Conversion	No. of Time Points	Initial Conc. x 10^3(M)	Hydrolysis Rate Constant, 10^6 $k_h(s^{-1})$	Correlation Coef., R^2
7, 0.4 SBC[b]	6.9	73	5	0.995	2.10 ± 0.09	0.994
7.11, 0.1 SBC	8.0	76	6	1.04	1.83 ± 0.12	0.988
9, 0.01 SBC	7.8	74	6	0.998	1.81 ± 0.08	0.992
9, 0.01 SBC 0.1 M $NaClO_4$	3.7	46	4	0.986	1.88 ± 0.13	0.991
11, 0.1 SBC	8.0	75	6	1.05	2.02 ± 0.10	0.990

[a] pH values are accurate to ± 0.02 pH units.
[b] Buffer solutions made of standard buffer concentrations (SBC) listed in the CRC Handbook (1975).

the other rate constants in the pH-independent region than the rate constant in the SBC-pH 7 experiments, although some buffer catalysis may have occurred.

To check for the effects of ionic strength on the rate of hydrolysis, we conducted experiments with CHO solutions in a minimally buffered 0.01 SBC-pH 9 solution with added 0.1 M $NaClO_4$. The measured rate constant k_h for this experiment was $(1.88 \pm 0.13) \times 10^{-6}$ s^{-1}, compared with $(1.81 \pm 0.08) \times 10^{-6}$ s^{-1} for the 0.01 SBC-pH 9 CHO solution without any added $NaClO_4$. These results are also in excellent agreement with the value of k_h from the experiment with 0.1 SBC-pH 7.11.

These experiments show that the hydrolysis rate constant is independent of ionic strength, but subject to moderate buffer catalysis. Examination of the data in Table 6 shows that the value of k_h in the pH-independent region (pH $\sim$ 7 to less than 11) is about 1.8×10^{-6} s^{-1} and corresponds to k_N.

Based on the foregoing discussion, we believe the values of k_h shown in Table 6 are the best values of these hydrolysis rate constants.

Table 6

BEST VALUES OF k_h FOR HYDROLYSIS OF CYLCOHEXENE OXIDE AT 25°C AT SELECTED pHs

pH, Buffer Conditions	k_h (s^{-1}) x 10^6	$t_{1/2}$
pH 3, SBC	1950 ± 9	5.92 min
pH 5, SBC	25.5 ± 1.2	7.55 hr
pH 7, 0.1 SBC	1.83 ± 0.12	105 hr
pH 9, 0.01 SBC	1.81 ± 0.08	106 hr
pH 11, 0.1 SBC	2.02 ± 0.10	95.3 hr

The acid, base, and neutral rate constants, k_A, k_B, and k_N calculated from these values of k_h are given in Table 7. The rate constants were calculated according to the SRI and EPA protocols and from multiple linear regression fit of selected k_h values (see Appendix A for equations).

Table 7

ACID, NEUTRAL AND BASE RATE CONSTANTS k_A, k_B, and k_N FOR CYCLOHEXENE OXIDE AT 25°C

	k_A (M^{-1} s^{-1})	$10^3 k_B$ (M^{-1} s^{-1})	10^6 k_N (s^{-1})
Calculated[a] from pHs 3, 7, 11/SRI	1.95 ± 0.01	0.385 ± 0.156	1.63 ± 0.12
Calculated[a] from pHs 3, 7, 9/EPA	2.38 ± 0.02	21.7 ± 14.6	1.59 ± 0.12
Calculated[b] from pHs 3, 5, 7, 9, 11	1.95 ± 0.00	-1.15 ± 2.58	3.15 ± 1.45
Calculated[b] from pHs 3, 7, 9, 11	1.95 ± 0.00	0.301 ± 0.148	1.72 ± 0.09

[a]These standard deviations represent estimation errors due to imperfect fitting of experimental "time points".

[b]These standard deviations represent estimation errors due to imperfect fitting of the k_h "data points" and should not be compared with those above.

The k_N values calculated using the SRI and EPA protocols agree very well, the k_A values agree only moderately, and the k_B values differ by two orders of magnitude. When all the values of k_h were used to calculate k_A, k_N, and k_B (by multiple linear regression, HP-97 program STI-13A), k_A was identical to the value calculated using only pH 3, 7, and 11 data (SRI), k_N was a factor of two larger than either the SRI or EPA value and k_B was negative and therefore not valid. The rate constants were then recalculated with the HP-97 program using the four k_h values from pH values 3, 7, 9, and 11; the pH 5 rate constant was excluded on the basis of its low correlation coefficient and suspected contribution resulting from buffer catalysis.

These calculated values of k_A, k_B, and k_N are in much better agreement with the values calculated using the SRI and EPA data sets: k_A

agrees extremely well with the SRI value, k_N is in good agreement with both the SRI and EPA values, and k_B is of the same magnitude as the SRI value. Since the contribution from the base-catalyzed process to the overall value of k_h is small at these pH values and therefore difficult to measure, the agreement of k_B with the SRI value is acceptable.

One method of verifying the accuracy of k_A, k_B, and k_N is to use them in calculating k_h and comparing the results with the measured values. Values of k_h for CHO were therefore calculated from the various sets of acid, base, and neutral rate constants according to equation (3),

$$k_h = k_A[H^+] + k_N + k_B[OH^-] \quad . \tag{3}$$

and the results are summarized in Table 8.

Values of k_h at pHs 5, 7, and 9 were calculated from values of k_A, k_B, and k_N derived from data at pHs 3, 7, and 11 according to the SRI protocol (Table 6). These k_h values compare well with the measured values of k_h at pHs 5, 7, and 9. The values of k_A, k_B, and k_N estimated using the EPA protocol give a value of k_h at pH 7 that agrees well with the measured value of k_h at pH 7. The calculated rate constants at pHs 3 and 11, however, differ by 22% and a factor of ten, respectively, from the measured values. In addition to these calculated values of k_h, rate constant values were calculated from the acid, base, and neutral constants obtained from using all five k_h data points. While good agreement was obtained between these calculated values and the measured values at pHs 3, 5, and 11, the rate constants at pHs 7 and 9 are a factor of two larger than their corresponding measured values.

Finally, the acid, base, and neutral rate constants obtained from the rate constants at pHs 3, 7, 9, and 11 were used to calculate k_h values at all five pH values. The k_h values at pHs 3 and 11 are identical to the measured values. The rate constants at pHs 7 and 9 are in excellent agreement with the corresponding measured values, whereas the rate constant

Table 8

COMPOSITE RATE CONSTANTS, k_h (s^{-1}) x 10^6, FOR CYCLOHEXENE OXIDE AT 25°C AND pH VALUES 3 THROUGH 11

Source	pH 3	pH 5	pH 7	pH 9	pH 11
Measured[a]	1950 ± 9	25.5 ± 1.2	1.83 ± 0.12	1.81 ± 0.08	2.02 ± 0.10
Calculated[b] from pHs 3, 7, 11 data/SRI	--	21.1 ± 0.12	1.82 ± 0.12	1.64 ± 0.12	--
Calculated[b] from pHs 5, 7, 9 data/ EPA	2387 ± 15	--	1.83 ± 0.12	--	23.3 ± 14.6
Calculated[b] from pHs 3, 5, 7, 9, 11 data[c]	1950	22.6	3.35	3.14	2.01
Calculated[b] from pHs 3, 7, 9, 11 data[c]	1950	21.2	1.91	1.72	2.02

[a] See Table 6.

[b] Acid, neutral and base rate constants obtained from the rate constants at these pH values are used in the calculation of the rate constants given on this line.

[c] Standard deviations have not been calculated for these k_h's because a valid statistical formula is not available.

at pH 5 agrees well with the measured pH 5 rate constant.* In addition, all the rate constants derived from the SRI protocol agree well with those calculated from the data obtained at pH 3, 7, 9, and 11.

Hydrolysis of Isopropyl Bromide

The equation for the hydrolysis of isopropyl bromide is as follows:

$$(CH_3)_2CHBr + H_2O \longrightarrow (CH_3)_2CHOH + HBr$$

Isopropyl bromide (IPB) was chosen as a representative of a class of chemicals that hydrolyze at rates independent of pH in the environmental pH region 4 to 9. Our critical review of hydrolysis (Mabey and Mill, 1978) found that hydrolysis rates of monohalogenated alkanes were not acid- or base-catalyzed in the region pH 3 to 11; although the base-catalyzed process is important for these alkyl halides above pH 11, there is no evidence that a specific acid-catalyzed mechanism for alkyl halide exists (i.e., reaction with protonated water H_3O^+).

The hydrolysis of isopropyl bromide produces bromide ion, hydronium ion (H_3O^+), and isopropyl alcohol (IPA) as products. Since acid is formed during hydrolysis, buffering of reaction solutions is necessary to maintain constant pH.

Isopropyl bromide has a boiling point of 59.4°C and a Henry's law constant of approximately 5700 torr M^{-1} at 25°C making IPB a highly volatile chemical. The Henry's law constant was calculated from water solubility for IPB of 3.5×10^{-2} M estimated using a logarithmic octanol/water partition coefficient (log K_{ow}) of 2.0 (Johnson, 1980) and the correlation of Yalkowsky and Valvani (1980) for water solubility and log K_{ow}; the vapor pressure of IPB used in the Henry's law constant estimation was 200 torr at about 20°C. [The CRC Handbook (1975) lists a IPB vapor pressure of 400 torr at 41°C and 100 torr at 8.0°C.]

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*The calculated value at pH 5 is slightly lower than the measured value as a result of buffer catalysis in the experiment at pH 5.

The use of isopropyl bromide as a model compound also allowed us to evaluate the IPB hydrolysis rate using the rate of product formation as an independent check on the IPB loss rate. IPA concentrations were determined concurrently with the IPB analyses using gas chromatography, and bromide ion was measured using an ion-selective electrode.

The initial set of IPB hydrolysis experiments were performed in solutions containing standard buffer concentrations (CRC Handbook, 1975). The results of these experiments based on IPB loss at several pHs are given in Table 9. The rate constants at pHs 3 to 11 appear to be independent of pH, and the averaged observed value of 3.79×10^{-6} s^{-1} ($t_{\frac{1}{2}}$ = 50.8 hours) in this pH-independent region agrees very well with the literature value of 3.77×10^{-6} s^{-1} (Koskikallio, 1967). The hydrolysis rate constant at pH 7, however, is low compared with the rate constants at other pH values in the neutral range. For this reason, a new set of experiments at pH 7 were performed to check this value of the rate constant and the effects of buffers and ionic strength on the rate constant. The results of these experiments showed that our earlier estimate of the rate constant probably was too high, but both buffer salts and ionic strength seem to influence the rate. These data are summarized in Table 10. We cannot explain the apparent increase in rate with a decrease in buffer concentration but the increase in rate constant on addition of $NaClO_4$ is consistent with Koskikallio's (1967) earlier results with IPB. It should be noted that ionic strengths of the standard buffers vary by less than a factor of 2 over the pH range of 3 - 11 as listed below

pH	10^2 Ionic Strength (μ)M
3	7.23
5	7.26
7	7.91
9	4.21
11	4.78

In addition to determining the rate constant k_h by loss of IPB, we also measured the rate constants for the appearance of IPA and bromide ion (Br^-). Rate constants for the production of IPA were obtained by linear regression of ln[IPA] versus time and are listed in Table 11 for various pH values. The rate constants for IPB loss and for IPA formation

Table 9

HYDROLYSIS OF ISOPROPYL BROMIDE AT 25°C MONITORED BY THE LOSS OF ISOPROPYL BROMIDE

pH, Buffer Conditions	Total Time Elapsed (hr)	% Conversion	Number of Time Points	Initial Concentration $10^4[IPB]_0$ (M)	Hydrolysis Rate Constant,[b] $10^4\ k_h$ (s^{-1})	Correlation Coefficient, R^2
3, SBC	100.8	78	6	8.79	3.90 ± 0.09	0.998
5, SBC	98.0	77	5	9.46	3.75 ± 0.22	0.999
7, SBC	96.8	72	6	9.52	3.28 ± 0.23	0.980
9, SBC	98.6	77	6	9.06	3.96 ± 0.13	0.996
11, SBC	95.1	74	8	10.80	4.08 ± 0.70	0.871

[a] Buffer solutions made up to standard buffer concentrations (SBC) listed in the CRC Handbook (1975).
[b] These estimates of k_h are obtained from the regression of $\ln[IPB]_t$ versus t where $[IPB]_t$ is the concentration of IPB at time t

Table 10

EFFECT OF BUFFER AND IONIC STRENGTH (μ) ON HYDROLYSIS OF IPB AT 25°C

Conditions	$k_h \times 10^6$ (s^{-1})	$[IPB]_o \times 10^4$ (M)	Number of Points
pH 7 SBC[a]	3.28 ± 0.23	9.52	6
pH 7 SBC[b]	2.13 ± 0.54	9.66	6
pH 7 MBC[c]	2.82 ± 0.33	9.35	6
pH 7 MBC/[c,d] 0.1 M $NaClO_4$	3.38 ± 0.35	9.43	6

[a]Original measurement shown in Table 9 μ = 7.91×10^{-2} M.
[b]Repeat experiment μ = 7.91×10^{-2} M.
[c]Minimally buffered solution μ = 3.16×10^{-2} M.
[d]μ = 0.131 M.

agree well for pHs 5, 7, and 11. In addition, these rate constants for IPA formation agree extremely well with the average measured value of k_h (3.79×10^{-6} s^{-1}) based on IPB loss and with the literature value of k_h (3.77×10^{-6} s^{-1}) (Koskikallio, 1967). The rate constants for IPA appearance at pHs 3 and 9, however, are both much larger than the corresponding rate constants for IPB loss.

Hydrolysis experiments were performed at pHs 3, 5, 7, and 9 to measure the rate of Br^- ion appearance. We sampled these solutions in accordance with the SRI and EPA protocols. A linear regression of $\ln[Br^-]$ versus time was used to obtain a rate constant for the production of bromide at pHs 3, 5, 7, and 9. Rate constants for these experiments are given in Table 11; they agree well with the corresponding rate constants for IPB loss at pH value of 3, 5, and 9. The rate constant for Br^- formation at pH 7 differs from the corresponding value of k_h for IPB loss by a factor of three. The rate of bromide formation at pH 11 was not monitored. The data in Table 11 show that the rate constants for loss of IPB and for production of Br^- and IPA do not always agree closely. At pH 3 the rate

Table 11

COMPARISON OF HYDROLYSIS RATE CONSTANTS FOR IPB AT 25°C OBTAINED FROM ANALYSIS FOR IPB AND FOR IPA OR Br^-

[k_h (s^{-1}) x 10^6]

Estimation Method	pH 3	pH 5	pH 7	pH 9	pH 11
From IPB[a] loss	3.90 ± 0.09	3.75 ± 0.22	3.28 ± 0.23	3.96 ± 0.13	4.08 ± 0.70
From IPA[b] production	5.58 ± 1.28	3.67 ± 0.94	3.83 ± 0.82	5.77 ± 1.21	3.51 ± 0.52
From Br^-[b] production	3.68 ± 1.42	3.90 ± 0.55	10.69 ± 3.82	4.68 ± 1.43	--

[a] Rate constant obtained from regression analysis of $\ln([IPB]_o/[IPB]_t)$ versus t where $[IPB]_t$ is the concentration of IPB at time t.

[b] Rate constant obtained from regression analysis of $\ln[C]_t$ versus t where $[C]_t$ is the concentration of IPA or Br^- at time t.

constant for IPA appearance is about 40% higher than for Br^- appearance or for loss of IPB. At pHs 5 and 7 the rate constants for IPA formation agree well with those for loss of IPB; both have large statistical errors, but this is probably due to the way in which the data were treated. Rate constants for appearance of Br^- at pHs 7 and 9 and appearance of IPA at pH 9 have significant errors and differ markedly from the value of k_h for loss of IPB.

Experimental Methods

Chemicals

Ethyl acetate, cyclohexene oxide, and isopropyl bromide were purchased from Mallinckrodt, Aldrich Chemical Company, and Matheson, Coleman, and Bell, respectively. According to infrared and gas chromatographic analyses, EA and CHO were better than 98% pure and were used without additional purification. IPB was analyzed only by GC and used as received.

Constant Temperature Bath

The constant temperature bath used in these experiments consisted of an 8-gallon glass container contained in a large wooden box and surrounded with 1 to 3 inches of vermiculite as insulation. A Plexiglass top covering the water bath had holes for the heater, stirrer, and temperature control probe ; the cover was hinged at the diameter for easy access to the samples. The shaft speed of the stirrer was set at about 2500 rpm. The 250-watt ceramic heater was controlled by a YSI Model 72 thermoregulator that maintained the temperature to within ± 0.02°C. To further ensure that the temperature in the bath remained at 25 ± 0.02°C, a heat exchanger coil of 1 m by 6.4 mm diameter copper tubing was placed into the bath. Water was pumped through the coil in a closed loop from a reservoir; this circulated water was cooled by passage through the inner loop of a 50-cm-long Allan condenser whose outer jacket was connected to the water tap. Direct circulation of tap water through the coil was unsatisfactory because of diurnal fluctuations in flow rate. The heat exchanger was essential to ensure proper temperature control when the ambient temperature exceeded 25°C, as it sometimes did in this laboratory.

Preparation of Reaction Solutions

All glassware used in these experiments was thoroughly washed and placed in an oven (560°C) overnight.

Reaction solutions were prepared according to the procedure outlined below. Reagent grade chemicals and sterile, pure water were used for all sample preparation. The concentration of chemical in the final buffered solution used in kinetic measurements was about 1×10^{-3} M or lower. Enough chemical to make a 0.1-M solution was weighed into a 100-ml volumetric flask that already contained some buffer solution or acetonitrile; acetonitrile was used to assist in the dissolution, if the chemical was not readily soluble in water. The flask was filled to the mark with either buffer solution or acetonitrile and a 1.00-ml aliquot was diluted 1:100 with buffer solution to the final concentration. Heat was not used in any stage of the preparation.

The solutions were placed in the bath according to the procedure outlined below. In addition, an aliquot was immediately quenched; this quenched mixture serves as a t = 0 sample.

Because hydrolysis of CHO at pH 3 is fast ($t_{\frac{1}{2}} \sim 6$ minutes), a special procedure was used to prepare this test solution. A known volume of pH 3 buffer in a round-bottomed flask was allowed to thermally equilibrate in a 25°C constant temperature bath. A known amount of CHO dissolved in acetonitrile was introduced into the flask; aliquots were withdrawn from the flask at known times and quenched to pH 9. After sampling for two half-lives, we analyzed the samples randomly to minimize systematic errors.

Buffer Solutions

The buffer solutions used in these experiments were prepared in the following manner, using the composition and proportions of the standard buffer concentration (denoted SBC) given below and described in the CRC Handbook (1975):

pH 3: 250 ml of 0.100 M $KHC_6H_4C_2O_4$
111.5 ml of 0.100 M HCl

pH 5: 250 ml of 0.100 M $KHC_6H_4C_2O_4$
113 ml of 0.100 M NaOH

pH 7: 250 ml of 0.100 M KH_2PO_4
145.5 ml of 0.100 M NaOH

pH 9: 250 ml of 0.025 M $Na_2B_4O_7 \cdot /OH_2O$
23 ml of 0.100 M HCl

pH 11: 250 ml of 0.0500 M $NaHCO_3$
113.5 ml of 0.100 M NaOH

All final volumes were 500 ml.

Minimally buffered aqueous systems were prepared by adding enough standard buffer to a known volume of sterile water to adjust the pH to that of the standard buffer. For pH 9, for example, 1 ml of SBC pH 9 was diluted to 100 ml with water to obtain a pH of 9.00 ± 0.02; this solution was labeled 0.01 SBC-pH 9. A minimally buffered pH 7 solution was prepared by diluting 40 ml SBC pH 7 to 100 ml with sterile water.

Minimally buffered solutions of high ionic strength were prepared by replacing half the volume of water called for in the preparation of the minimally buffered solution with 0.2 M sodium perchlorate. For example, 1 ml of SBC pH 9 was mixed with 49 ml of water and 50 ml of 0.2 M $NaClO_4$ to make a minimally buffered pH 9 solution of high ionic strength.

Kinetic Measurements

Aliquots of the reaction mixture were transferred with a pipette to 13 mm OD by 100 mm test tubes (approximately 8 ml capacity). Each tube was filled to overflowing and immediately covered with a watertight Teflon-coated Mininert valve. A test tube was filled for each time point. Each tube was covered with aluminium foil to exclude light and labeled. The tubes were suspended from wires stretched across the bath so that each tube was submerged in the bath.

At selected time points, a tube containing reaction solution was removed from the bath and quenched by either cooling in ice or adding a known volume of the reaction mixture to a known volume of acid or base solution, which had been precooled to ~ 5°C. The acid or base solution was adjusted so that at the final solution pH, the hydrolysis rate was minimized. For the chemicals studied in our laboratory, the reaction solutions were quenched by adjusting the pH as well as by cooling:

EA - The pH of all solutions was adjusted to pH 5.5.

CHO - pH 3 solutions were adjusted to pH 9.

IPB - pH 11 solutions were adjusted to ∿ pH 6.

All other samples were cooled to further quench the hydrolysis reaction.

Sampling Regimen

Sampling of the reaction mixture was performed according to EPA and SRI protocols. The EPA protocol recommends using one of three measurement regimens at pHs 5, 7, and 9:

(1) For chemicals that hydrolyze rapidly, 6 analyses should be taken between t = 0 and t = 672 hours at which time 60% to 99% of the chemical will have hydrolyzed.

(2) For chemicals with moderate reactivity, 15 to 20 analyses should be taken between t = 0 and t = 672 hours at which time 20% to 60% of the chemical will have hydrolyzed.

(3) For unreactive chemicals one analysis should be taken at t = 672 hours at which time less than 20% of the chemical will have hydrolyzed.

The SRI protocol requires that the rates of hydrolysis be measured at pHs 3, 7, and 11, according to the following regimen:

(1) For chemicals that hydrolyze rapidly, i.e., 60% to 70% of the chemical hydrolyzes in several weeks, a minimum of 6 analyses should be taken.

(2) For chemicals that hydrolyze more slowly, i.e., 20% to 30% of the chemical hydrolyzes in several weeks, 15 to 20 analyses should be made with most of the points taken between 10% and 30% conversion.

The SRI protocol also requires that a control solution be analyzed.

Table 12 shows the sampling regimens for the chemicals tested in our laboratory.

Analytical Method

All analyses for EA, IPB, and CHO were performed by directly injecting aqueous solutions onto a HP-5700 gas chromatograph coupled with a Sepctra-Physics integrator. The instrument settings and columns used for these analyses are summarized in Table 13.

Table 12

SAMPLING REGIMENS FOR EA, CHO, AND IPB AT 25°C

	pH				
	3	5	7	9	11
EA					
No. of Points	13	1	1	4	6
% Conversion	10-30	5.7	25	95	80
CHO					
No. of Points	6	9	6	6	6
% Conversion	80	90	∿ 30	∿ 80	∿ 80
IPB					
No. of Points	6	5	6	6	8
% Conversion	78	77	72	77	74

For all chemicals, GC analyses were performed in triplicate at each time point; injection sizes were usually 2.5 µl. Peak areas were determined by electronic integration. Calibration runs were performed before the reaction mixtures were analyzed. The calibration mixture was prepared at a pH where the hydrolysis rate was the slowest. The mixture was prepared according to the general procedure outlined previously. Immediately after the calibration solution was prepared, it was quenched and analyzed. The average areas from three analyses for the chemical and the internal standard were then used to estimate concentration of the chemical.

The concentration of chemical, C, in a sample was measured by comparing the ratio of the peak areas of C and the internal standard, IS, in the sample with the ratio of the IS and C peaks in the calibration solutions. The concentration of C_S was then obtained using the following relationship:

$$[C]_s = \frac{\text{Peak Area } IS_c}{\text{Peak Area } IS_s} \times \frac{\text{Peak Area } C_s}{\text{Peak Area } C_c} \times [C]_c$$

Table 13

INSTRUMENT SETTINGS AND COLUMNS USED FOR CHEMICAL ANALYSES

	EA	CHO	IPB
Column	2.75 m x 0.2 cm I.D. glass	2.75 m x 0.2 cm I.D. glass	2.75 m x 0.2 cm I.D. glass
Packing	Porapak Q Mesh 80/100; Porapak QS Mesh 80/100	--	--
GC Support	--	Chromosorb 750 Mesh 80/100	Chromosorb 750 Mesh 80/100
Stationary phase	--	Carbowax 20M	Carbowax 20M
Solvent	--	Chloroform	Chloroform
Internal standard	Acetone with Porapak Q; methyl ethyl ketone with Porapak QS	Methyl ethyl ketone	n-Butanol
Detection method	Flame ionization	Flame ionization	Flame ionization
Temperature	160°C (isothermal)	75°C at 4°C/min to 110°C	80°C for 4 min; then 4°C/m to 100°C
Flow rates			
N_2	30 ml/min	30 ml/min	30 ml/min
H_2	30 ml/min	30 ml/min	30 ml/min
Air	240 ml/min	240 ml/min	240 ml/min

where s denotes the sample and c the calibration standard where the concentrations of IS in the sample and in the calibration solutions are identical.

Data Treatment

First-order rate constants were estimated from the regression of $\ln(C_o/C_t)$ versus time using standard linear regression programs available in many hand calculators, including the HP-97 Stat Pac. The special case of $C_t = C_o$ was not used. The slope of the regression is k_h.

C_t was measured thrice by GC at each time point; the ratio C_o/C_t was calculated, transformed to $\ln (C_o/C_t)$, and averaged for the three values of $\ln (C_o/C_t)$ for use as one data set with time t in the regression analysis. Values of the standard error(s) and R_2 were given for k_h or k_p by the regression program.

Optimized Screening Protocols

The similarities and differences between the screening protocols proposed by EPA and SRI are summarized in Table 14. We evaluated both hydrolysis screening protocols for precision, accuracy, simplicity, and economy. A new screening test protocol should incorporate the best features of the existing screening tests with any changes that would improve the test without undue complications or loss of economy. Perhaps one of the most striking results of this investigation is the finding that at least for EA, rate measurements at low and high pH (3 and 11) provide a more reliable basis for estimating k_h at pHs 5 and 9 than do the direct but very slow measurements at those pH values. This paradox is explained by the relative reliability of measurements of fast versus slow reactions. Very slow reactions with half-lives of two or more weeks are much more subject to intrusion by unwanted processes, especially biodegradation, to failure of control equipment, and to intermittent power failures. Because of the long intervals between analyses in very slow reactions, discovery of these problems can lead to significant losses of time.

Table 14

SIMILARITIES AND DIFFERENCES IN SRI AND EPA PROTOCOLS

		Differences	
	Similarities	SRI	EPA
pH	Hydrolysis rate measured at 3 pHs	Rates measured at pHs 3, 7, and 11	Rates measured at pHs 5, 7, and 9
Samples	Sample prepared in sterile H_2O without heat; final conc. < 1×10^{-3} and < ½ solubility	1 sampling procedure: at t = 0, 44, and 88 hr plus 8 optional samplings	sampling procedure varies according to chemical's reactivity

Another perhaps less important factor, which may diminish the reliability of rate measurements at pHs 5 and 7, is the occurrence of buffer catalysis that results from using phosphate buffer. The importance of this effect, generally, in confounding accurate measurements is not readily assessible, but our data demonstrate the occurrence of this effect in the hydrolysis of both CHO and IPB. We believe that measurements at pH values within the range of environmental relevance and interest, e.g., pHs 5, 7, and 9, will constitute the preferred method of screening for hydrolysis; however, our results show that when half-lives exceed one to two weeks, measurements at pHs 3, 7, and 11 are preferred.

We therefore recommend that wherever possible zero-level screening by structure-activity relationships be used first to help select optimum pH values to minimize measurement times needed for reliable measurements. In the absence of clear indications of life-times we again recommend measurements at pHs 3, 7, and 11, but with a minimum of six to eight time points over 40 to 70% conversion to define more accurately the rate constant k_h. In this respect the recommended protocol is really a synthesis of the SRI and EPA methods--the SRI pH values and the EPA measurement regimen.

Estimated Cost for Optimized Screening Protocol

We used our experience in performing hydrolysis experiments in the laboratory as a basis for estimating the cost for performing such experiments for an optimized protocol in a modern, well-equipped laboratory by trained personnel. The costs used for the estimation are listed in Table 15; note that no capital costs are included for instruments such as chromatographs, electronic integrators, or temperature controllers. No cost for analytical method development is included; that would add another 25% to labor cost.

In our estimate we assume that each screening protocol requires 88 hours (Table 15) plus 25% additional time for unexpected problems, for a total of 110 hours per screening experiment. The prorated cost per protocol is estimated to be $20 for chemicals and solvent and $100 for materials, including some breakage and replacement costs. The combined cost of chemists and supervisory time is estimated to be $35 per hour.

Thus the total cost for time, materials, and chemicals would be about $3970 per protocol in 1980 dollars.

COLLABORATIVE TEST DESIGN FOR HYDROLYSIS

Background

A collaborative test program involves applying a test method at multiple, independent laboratories. Such testing provides data for evaluating the precision and accuracy of the method. Also, problems with the method may be discovered that were not apparent at the originating laboratory. Since the participating laboratories represent a diversity of environments, equipment, instrumentation, reagents, and technicians, a collaborative study provides an evaluation under circumstances that are similar to those that will occur when the test method is put into practice.

Another use of a collaborative test program is to obtain the best estimate of the property being measured. Such an estimate is based on the results from independent laboratories and is possibly more reliable. By and large, the design of a collaborative test program is the same whether the primary interest is in the test method or the test result.

There is a well-established literature on collaborative testing. This literature is concerned with measuring nonkinetic entities, such as in analytical chemistry. Collaborative testing for hydrolysis differs in that the property being measured is a kinetic rate coefficient or half-life. However, since the principles and definitions given in the collaborative testing literature are still relevant, we review them here. Two authoritative sources for information on collaborative testing are the American Society for Testing and Materials (ASTM) and the Association of Official Analytical Chemists (AOAC).*

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* See, for example, the ASTM publication "Suggested Recommended Practice for Conducting an Interlaboratory Test Program to Determine the Precision of Test Methods" or Statistical Manual of the AOAC which includes "Statistical Techniques for Collaborative Tests" by W. J. Youden and "Planning and Analysis of Results of Collaborative Tests" by E. H. Steiner.

Intralaboratory study: A principle often emphasized in the literature is that a test method should be thoroughly evaluated within a single laboratory before submitting it to a collaborative, interlaboratory study. In practice, this usually means that the originating laboratory trys the method with various materials and under various conditions. Most of the literature on intralaboratory testing is devoted to formal experimental designs that allow the effects of several factors to be evaluated simultaneously. The most prominent design is one suggested by Youden (1950). In this design, test conditions are varied by an amount similar to the range encountered when several laboratories are presumably following the procedure. Youden calls this type of intralaboratory study a test of ruggedness.

Precision: The ASTM defines the precision of a test method as the degree of agreement among individual test results obtained under prescribed similar conditions when the number of individual observations in a single test result is specified in the method of the test. Precision may be inversely characterized by the imprecision of the test results as measured by their standard deviation.*

Systematic error or bias: A systematic error or bias is the difference between the average test result and a reference value. Whether or not a reference value actually exists, it is often conceptually helpful to think of the reference value as being the idealized true value.

Accuracy: The ASTM defines accuracy as the degree of agreement of the individual test results with an accepted reference value when the number of individual observations in a single test result is specified by the test protocol. So defined, accuracy includes both the random error of precision and any accompanying systematic error or bias.

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* These definitions of precision, systematic error or bias, accuracy, repeatability, and reproducibility are taken in part from Duncan (1978).

Repeatability: Repeatability refers to the variability of test results within a single typical laboratory under conditions that have been carefully prescribed. It is often described by a 95% confidence interval for the difference between two test results from the same laboratory.

Reproducibility: Reproducibility refers to the variability of test results between laboratories under conditions that have been carefully prescribed. It is often described by a 95% confidence interval for the difference between two test results from different laboratories.

Outliers: The analysis of laboratory data will sometimes reveal results that are way out of line and suggest that something went wrong. Statistical methods are available to assess the likelihood that such deviations are due to chance, and graphical methods are available to provide a more descriptive impression. The ASTM position is that, while such techniques are useful for "flagging" suspicious data, the decision to reject such data should be the prerogative and responsibility of the committee running the study. Such decisions should be based on general scientific as well as statistical considerations.

Replication: Statements about the precision of a test method require information about random variability within a laboratory. The usual approach, and the ASTM recommendation, is to perform replicate determinations on each material in each laboratory to estimate the within-laboratory variation. The conditions of the repeat determinations need to be specified, e.g., whether replicate tests are done by the same personnel using the same reagents, equipment, and instrumentation on the same day. Another approach, suggested by Youden (1975), is to estimate the within-laboratory variability from the difference between single measurements on pairs of samples with slightly different levels of content. The advantage of this "Youden pairs" approach is that individual measurements are more likely to be independent of one another and that the paired results plotted for all laboratories provide an informative graph for interpreting results.

Hydrolysis Testing Protocols

The SRI screening and detailed test methods for hydrolysis in water were reviewed in the Background section and discussed in the subsection Optimized Screening Protocol; we repeat that information here to highlight data collection and data analysis specifications and to describe the scope of this development of collaborative testing methodology.

The screening protocol for hydrolysis is intended to identify chemicals with half-lives of less than one year and more than one hour at 25°C. Each chemical being tested is hydrolysed in pH 3, 7, and 11 buffer solutions. The pH 7 solution is analyzed after 2 hr and 88 hr; if more than 75% of the chemical has hydrolyzed after 2 hr, the hydrolysis half-life will be less than 1 hr; if no loss of chemical occurs in 88 hr, any loss in the pH 3 and pH 11 solutions is probably due to hydrolysis. The pH 3 and pH 11 solutions are analyzed at 44 hr and 88 hr; if more than half the initial concentration of chemical has hydrolyzed in 88 hr at either pH 3 or pH 11, then the chemical is expected to have a half-life of less than 1 year at pH 5 or pH 9; the loss at 44 hr should be more than 29% if the half-life is less than 1 year.

The detailed protocol for hydrolysis is designed to estimate hydrolysis rate constants and half-lives for most chemicals at any pH and temperature. Like the screening method, each chemical being tested is hydrolysed at pH 3, 7, and 11 at a fixed temperature, preferably 25°C. However, the detailed protocol requires that solutions be sampled and analyzed more frequently: at least six times if 60% to 70% conversion occurs within several weeks and even more frequently for slower reactions. The hydrolysis rate constant, k_h, is estimated from the concentration-time data for each pH experiment by (1) plotting concentration versus time on semilog graph paper or (2) calculating a linear regression analysis of ln concentration versus time. The estimates of k_h at the three experimental pH levels are used to estimate the hydrolysis rate coefficients for the acid, neutral, and base process,

meeting the assumptions required for regression analysis and on the appropriateness of the least squares criterion.

Linearity: Applying the natural log transformation to the exponential decay function results in

$$\ln C_t = \ln C_o - kt$$

or

$$\ln C_o/C_t = kt \ ,$$

where C_t denotes the concentration at time t. Regression analysis based on either of these linear functions will lead to the same estimate of the rate coefficient k. The SRI kinetic testing protocols do not force the latter form of the regression function through the origin. Instead, the regression function used is

$$\ln C_o/C_t = a + kt$$

The intercept a is interpreted as a systematic error of analytical measurements that should not affect the estimation of the rate coefficient. Whether the kinetic process being studied is, in fact, a first-order reaction can best be judged by inspecting a plot of C_t or C_o/C_t versus time on semilog graph paper. A formal statistical test of this assumption would require multiple observations of independent experiments at synchronized time points, which is probably not technically feasible in a laboratory.

Independence: The independence assumption is that the random error associated with the concentration at each time point is statistically independent from the random error for other time points. This is quite plausible for the error from the analytical method and possibly also for technical error deriving from the sampling procedure. However, the independence assumption may not be met for experimental error due to physical or chemical conditions or competing processes. Such experimental effects are likely to cause random errors to be serially correlated over time.

important implications for the subsequent data analysis and interpretation of results.

There are two basic types of collaborative test designs for selecting the number of laboratories:

(1) A few carefully chosen laboratories may participate in the collaborative tests, leading to a comparison of results for a fixed selection of laboratories with any number of laboratories down to two providing a meaningful comparison.
(2) Randomly chosen laboratories may represent a population of laboratories, leading to more general conclusions about the precision and accuracy of the test method as revealed by the random selection of laboratories; note that ten or more laboratories are needed to represent a large population of laboratories (Youden,1975).

In this report, we consider both types of collaborative test designs and the subsequent data analysis based on fixed or random effects statistical models. However, considering the status of hydrolysis testing, near-term needs for collaborative testing, and probable constraints on the number of participating laboratories, more attention is given to collaborative testing that involves relatively few (3 to 6) carefully selected laboratories.

Replication

Another key design parameter is the number of experimental replicates within each laboratory. In nonkinetic collaborative testing, such as in testing an analytical method, within-laboratory variability is ascertained from either replicate measurements on the same sample or single measurements on pairs of samples, i.e., Youden pairs. For kinetic process testing, the standard error of a rate coefficient estimate is obtained from the regression analysis of sample measurements at multiple time points. This standard error may be interpreted as the within-laboratory error for estimating a rate coefficient. In this way experimental error analysis is based on the residual variability within a single experiment without needing to replicate experiments within each laboratory.

There are advantages and limitations to basing the error analysis for a kinetic test method on residual variability, i.e., deviations from a first-order rate law. Of course, laboratory work is held to a minimum if there is no need to replicate experiments, and resources may be used to test other chemicals or to test under alternative controlled conditions. Furthermore, studying residual variability within an experiment leads to a detailed understanding of error sources and sampling regimens, which would not be possible if the test results were simply the rate coefficient or half-life estimates for replicate experiments. The primary limitation of our approach is that there are probably important sources of experimental variability within a laboratory that are not represented by residual variability within a single experiment. Within a kinetic experiment, the "random error" of the reaction corresponds to "chance events" that occur between sampling times, leading to deviations from a deterministic process and hence estimation error. Within a laboratory, there are probably additional chance events that would occur if the experiment is replicated at a different time or a different initial concentration, which may lead to results outside the confidence interval derived from a single experiment.

The statistical methods described in this report do not consider experimental replication within each laboratory. Within-laboratory variability is estimated from regression analysis techniques applied to single experiments. This approach was also used in the intralaboratory study of this project and is an extension of the statistical method and error analysis provided in the original test methods protocol (Mill and Mabey, 1980).

Least Squares Regression

It is common practice to linearize the exponential decay function that describes a first-order process by a natural log transformation, then to use least squares regression analysis to estimate the first-order rate coefficient. The validity of this approach depends on

meeting the assumptions required for regression analysis and on the appropriateness of the least squares criterion.

Linearity: Applying the natural log transformation to the exponential decay function results in

$$\ln C_t = \ln C_o - kt$$

or

$$\ln C_o/C_t = kt \ ,$$

where C_t denotes the concentration at time t. Regression analysis based on either of these linear functions will lead to the same estimate of the rate coefficient k. The SRI kinetic testing protocols do not force the latter form of the regression function through the origin. Instead, the regression function used is

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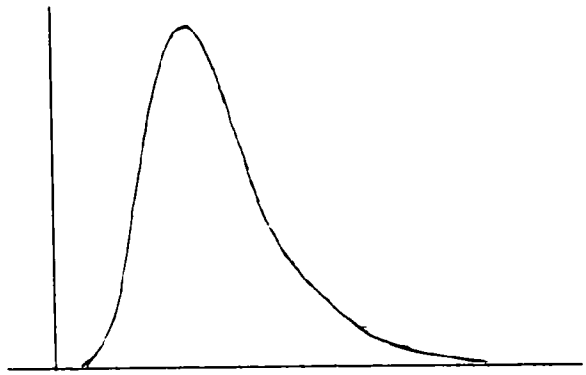
Independence: The independence assumption is that the random error associated with the concentration at each time point is statistically independent from the random error for other time points. This is quite plausible for the error from the analytical method and possibly also for technical error deriving from the sampling procedure. However, the independence assumption may not be met for experimental error due to physical or chemical conditions or competing processes. Such experimental effects are likely to cause random errors to be serially correlated over time.

Equal variance: Because of the ln transformation, the assumption of equal error variance for all data points is in terms of the relative concentration rather than the absolute concentration. Constant relative variability is a reasonable assumption for chemical data since experimental error rates are often stated in relative terms for such data, e.g., s.d. = $\pm$ 5% of true value. For kinetic testing, this assumption can be checked by the plot of C_t or C_o/C_t versus time on semilog paper; the variability of points about the fitted line should be constant across time.

Normality: The assumption of normally distributed relative error is needed for the statistical tests presented in the next section. (This assumption is not needed to merely fit a least squares regression line.) The required distribution is more specifically a lognormal distribution because the log of the error in concentration is assumed to be normally distributed. The following graph illustrates a typical lognormal distribution.



Distribution of error

A skewed distribution of this type is reasonable for chemical data.

Least squares: Applying the least squares criterion to estimate a logarithmic relationship is sometimes advocated (Hoel, 1962) and sometimes criticized (Cvetanovic and Singleton, 1977).

Let C_t denote the observed concentration at time t and $\hat{C}_t$ denote the concentration predicted at time t by estimates of a and k. Then the least squares estimates of a and k are those that minimize the sum of squared deviations between observed and predicted concentrations according to the following equation:

$$SS = \sum (\ln(C_o/C_t) - \ln(C_o/\hat{C}_t))^2 = \sum (\ln(\hat{C}_t/C_t))^2 \qquad (6.5)$$

This amounts to minimizing the sum of squares of the ln of the relative deviations about the regression line. The appropriateness of this approach depends on whether relative deviations are more relevant than absolute deviations, and if so, whether minimizing SS is a meaningful estimation criterion. The relevance of relative deviations for chemical data has been discussed. Minimizing SS provides a meaningful criterion, as can be seen by sample calculations, in that $(\ln\hat{C}/C_t)^2 = 0$ when $\hat{C} = C_t$, and $(\ln\hat{C}/C_t)^2$ increases as the error of prediction increases, e.g. if the prediction error is ± 5%, then $(\ln\hat{C}_t/C_t)^2 = (\ln 1.05/1)^2 = .0024$ or $(\ln\hat{C}_t/C_t)^2 = (\ln .95/1)^2 = .0026$; if the prediction error is ± 10%, then $(\ln\hat{C}/C_t)^2 = (\ln 1.10/1)^2 = (\ln 1.10/1)^2 = .0091$ or $(\ln\hat{C}_t/C_t)^2 = (\ln .9/1)^2 = .0111$. Therefore, the following statistical methods are based on generalizations of least squares regression techniques.

Statistical Methods

Statistical methods for analyzing the data from collaborative testing apply to two types of questions. The first type involves the comparison of results among laboratories. Are the rate coefficients the same? If not, which laboratories are different? Are the standard errors of rate estimates the same? The second type involves statements about the precision and accuracy of the test method. What is the within-laboratory variability and what are its components? How can the precision be described in terms of the repeatability of results? Is there evidence of systematic bias? What is the between-laboratory variability and how does it effect the reproducibility of results?

Comparison of Laboratories

The rate coefficient is first estimated by simple regression analysis in each laboratory. Let the subscript ℓ index the N laboratories in the collaborative study and the subscript i index the n_ℓ data time points for the ℓth laboratory. Employing the ln transformation, the regression function for the ℓth laboratory is

$$\ln(C_{\ell o}/C_{\ell i}) = a_\ell + k_\ell t_{\ell i} \tag{6.6}$$

The rate coefficient (k_ℓ) and intercept (a_ℓ) for each laboratory are estimated in the usual way:*

$$k_\ell = \frac{n_\ell \sum t_{\ell i} \ln(C_{\ell o}/C_{\ell i}) - \left[\sum t_{\ell i}\right]\left[\sum \ln(C_{\ell o}/C_{\ell i})\right]}{n_\ell \sum t_{\ell i}^2 - \left[\sum t_{\ell i}\right]^2} \tag{6.7}$$

$$a_\ell = \frac{1}{n_\ell} \sum \ln(C_{\ell o}/C_{\ell i}) - k_\ell \frac{1}{n_\ell} \sum t_{\ell i} \tag{6.8}$$

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* All Σ denote $\sum_i$ unless indicated otherwise.

The standard errors (se) of the estimates of the rate coefficient and intercept are a function of the variance of concentrations about the regression line in each laboratory. This variance is estimated in each laboratory by using the following equations:

$$S_\ell^2 = \frac{1}{n_\ell - 2}\left[\sum [\ln(C_{\ell o}/C_{\ell i})]^2 - \frac{[\sum (C_{\ell o}/C_{\ell i})]^2}{n_\ell} - k_\ell^2\left[\sum t_{\ell i}^2 - \frac{[\sum t_{\ell i}]^2}{n_\ell}\right]\right] \qquad (6.9)$$

The standard error of the estimate of the rate coefficient is

$$Se(k_\ell) = S_\ell\left[\frac{n_\ell}{n_\ell \sum t_{\ell i}^2 - [\sum t_{\ell i}]^2}\right]^{1/2} \qquad (6.10)$$

The standard error of the estimate of the intercept is

$$Se(a_\ell) = S_\ell\left[\frac{1}{n_\ell} + \frac{n_\ell [\sum t_{\ell i}^2]}{n_\ell \sum t_{\ell 1}^2 - [\sum t_{\ell i}]^2}\right]^{1/2} \qquad (6.11)$$

A statistical test is first described for comparing the rate coefficients for <u>two</u> laboratories. The difference in their coefficient estimates is statistically significant if it is unlikely that a difference of this magnitude would occur by chance if their true coefficients are equal. A pooled estimate of the variance of concentrations about the regression line is calculated as

$$S_p^2 = \frac{(n_1 - 2)S_1^2 + (n_2 - 2)S_2^2}{n_1 + n_2 - 4} \qquad (6.12)$$

The standard error of the difference between the rate coefficients is given by

$$S_d^2 = S_p^2\left[\frac{n_1}{n_1 \sum t_{1i}^2 - \left[\sum t_{1i}\right]^2} + \frac{n_2}{n_2 \sum t_{2i}^2 - \left[\sum t_{2i}\right]^2}\right] \qquad (6.13)$$

The t statistic for testing the hypothesis that the rate coefficients are equal is given by

$$t = \frac{k_1 - k_2}{S_d} \qquad (6.14)$$

The hypothesis is rejected if the absolute value of t is greater than the value in a statistical "t" table for the selected α level and $(n_1 + n_2 - 4)$ degrees of freedom.

The test given above assumes that the variance about the regression line is the same for both laboratories. This assumption may be tested by the F statistic

$$F = \frac{S_1^2}{S_2^2} \qquad (6.15)$$

The variances are significantly different if this statistic is greater than the tabled "F" value for the selected α level, $df_1 = (n_1 - 1)$ and $df_2 = (n_2 - 1)$. (The reciprocal also needs to be checked.)

A more general statistical comparison for any number (N) of laboratories involves the collective estimates of the rate coefficients (k_c) and intercept (a_c) based on the data points from all the laboratories. These estimates are calculated from the formulas for k_ℓ and a_ℓ by replacing n_ℓ by $\sum_\ell n_\ell$ and by taking all summations over both i and ℓ; i.e., Σ denotes $\sum_\ell \sum_i$. The collective estimate of the variance about the regression line (S_c^2) is calculated similarly, as are the standard errors for the estimates of k_c and a_c.

The idea behind the comparison of N laboratories is that if their true rate coefficients are equal, then the collective regression line based on all data will be nearly as good as when regression lines are fit for each laboratory individually.

A statistical test of the hypothesis that the regression functions (slope and intercept) are the same for all laboratories is based on the

difference between the sum of squared residuals for the collective regression and the sum of squared residuals for the individual regressions for all laboratories. The F statistic for this test is

$$F = \frac{\left(\sum_{\ell} n_{\ell} - 2\right) S_c^2 - \sum_{\ell} (n_{\ell} - 2)\, S_{\ell}^2}{\sum_{\ell} n_{\ell} - 2 - \sum_{\ell} (n_{\ell} - 2)} \div \frac{\sum_{\ell} (n_{\ell} - 2)\, S_{\ell}^2}{\sum_{\ell} (n_{\ell} - 2)} \qquad (6.16)$$

The hypothesis is rejected if F is greater than the tabled "F" value for the selected α level, $df_1 = \sum_{\ell} n_{\ell} - 2 - \sum_{\ell} (n_{\ell} - 2)$ and $df_2 = \sum_{\ell} (n_{\ell} - 2)$.

It is preferable to test whether the rate coefficients for laboratories are equal while allowing their intercepts to be different. This way, different systematic errors in analytical methods do not affect the comparison of rate coefficients. The t-test for comparing laboratories does this; however, the preceding F-test for comparing any number of laboratories tests whether the rate coefficients and intercepts are the same for all laboratories.

For testing the equality of rate coefficients only, a modified version of the collective rate coefficient is calculated.

$$k_{c'} = \frac{n \sum_{\ell} \sum_{i} t_{\ell i} \ln(C_{\ell o}/C_{\ell 1}) - \sum_{\ell} \left[\sum_{i} t_{\ell 1} \sum_{i} \ln (C_{\ell o}/C_{\ell 1})\right]}{n \sum_{\ell} \sum_{i} t_{\ell 1}^2 - \left[\sum_{\ell} \sum_{1} t_{\ell i}\right]^2} \qquad (6.17)$$

(For simplicity, it is assumed that each laboratory has the same number (n) of data points.) Next, $k_{c'}$ is used to calculate the associated residual variance.

$$S_{c'}^2 = \frac{1}{nN - N - 1} \left\{ \sum_{\ell} \sum_{i} \left[\ln(C_{\ell o}/C_{\ell 1})\right]^2 - \frac{\left[\sum_{\ell} \sum_{i} \ln(C_{\ell o}/C_{\ell i})\right]^2}{nN} \right.$$

$$\left. - k_{c'}^2 \left[\sum_{\ell} \sum_{1} t_{\ell 1}^2 - \frac{\left[\sum_{\ell} \sum_{1} t_{\ell 1}\right]^2}{nN} \right] \right\} \qquad (6.18)$$

The hypothesis is rejected if S is greater than

$$S > \sqrt{(N - 1)\ F_d;\ N - 1,\ \sum_{\ell} n_\ell - 2N} \qquad (6.23)$$

where $F_{\alpha;\ N - 1,\ \Sigma n_\ell - 2N}$ is the table "F" value for the selected α level, $df_1 = N - 1$ and $df_2 = \sum_{\ell} n_\ell - 2N$.

If N = 2, this test reduces to the t-test for comparing the rate coefficients for two laboratories.

A more common way to present the result of applying the S-method is by a confidence interval. Let d_T denote the true difference between the rate coefficients k_ℓ and $k_{\ell'}$ for any two laboratories. Then the $(1 - \alpha)$ confidence interval for d_T is given by

$$k_\ell - k_{\ell'} - S_d\sqrt{(N - 1)\ F_{\alpha;\ N-1\ \sum_{\ell} n_\ell - 2N}} \leq d_T \leq k_\ell - k_{\ell'} + S_d\sqrt{(N - 1)F_{\alpha;\ N - 1\ \sum_{\ell} n_\ell - 2N}} \qquad (6.24)$$

The probability that this interval covers the true difference is $(1 - \alpha)$. The difference between the coefficient estimates is statistically significant if the confidence interval does not overlap zero.

Evaluation of Precision and Accuracy

The within-laboratory precision of a kinetic test method is represented by the variance (or standard error) of the estimate of the rate coefficient. Let σ_e^2 denote the variance of observed values of $\ln(C_o/C_t)$ about the regression line for each laboratory. Then the precision of the test method for a particular set of sampling times $\{t_i\}$ is represented by

$$\text{var}(k) = \frac{\sigma_e^2}{\sum_{i} (t_i - \bar{t})^2} \qquad (6.25)$$

The residual variance σ_e^2 is estimated by S_ℓ^2 for the ℓth laboratory or by S_p^2 when the S_ℓ^2 are pooled across all laboratories.

In theory, var (k) can be reduced by sampling more frequently at the beginning and end of an experiment since this will increase $\Sigma(t_1 - \bar{t})^2$. However, this practice is not recommended because it is more important to have a better check on whether the reaction is first-order by spacing sampling times evenly.

The residual variance σ_e^2 has two components: one due to the analytical method and the other due to the experimental reaction. The analytical component σ_a^2 can be estimated by a preliminary test of the analytical method. Its contribution to the residual variance can be reduced by basing the regression analysis on the average of $\ln(C_o/C_t)$ for multiple chemical analyses of the concentration for each time point.*

The contribution of the experimental reaction component σ_r^2 cannot be reduced by multiple samples at each time point, since the solution being tested is assumed to be homogenous. (There would be reason for multiple samples at each time point if the sampling procedure itself contributes substantially to the residual variance.)

The variance of the estimate of the rate coefficient reflects the error components due to the analytical method and the experimental reaction. For a single chemical analysis of concentration at each time point,

$$\sigma_e^2 = \sigma_a^2 + \sigma_r^2 \tag{6.26}$$

For the average of the ln of (C_o/C_t) for m chemical analyses at each time point,

---

* The average of the $\ln(C_o/C_t)$, rather than the ln of the average (C_o/C_t), should be used in the regression analysis. These two quantities are nearly the same when analytical measurement are within 5% of one another.

$$\sigma_{\bar{e}}^2 = \sigma_a^2/m + \sigma_r^2 \qquad (6.27)$$

The number of chemical analyses for each time point should be the same to satisfy the regression analysis assumption of equal error variance. Then, for the regression analysis of the average of $\ln(C_o/C_t)$ versus t,

$$\text{var}(k) = \frac{\sigma_a^2/m}{\sum_i (t_i - \bar{t})^2} + \frac{\sigma_r^2}{\sum_i (t_i - \bar{t})^2} \qquad (6.28)$$

The first term is the imprecision due to the analytical method, while the second term is the imprecision due to the experimental reaction.

A repeatability statement for a kinetic test method can be made in the same manner as for a nonkinetic test method. The 95% repeatability interval for estimating a rate coefficient is

$$I_r = 1.96\sqrt{2}\left(\frac{\sigma_e^2}{\sum_i (t_i - \bar{t})^2}\right)^{1/2} \qquad (6.29)$$

The difference between estimates of the rate coefficient for two replicate experiments conducted within the same laboratory has a 95% probability of being less than the size of this interval. As before, σ_e^2 is estimated by S_ℓ^2 or S_p^2. The repeatability interval depends on the sampling times $\{t_i\}$ and the number of chemical analyses per time point, which will be reflected in the estimate of the residual variance.

The systematic error and accuracy of a kinetic test method can only be implied by comparing results among laboratories, since the true value of a rate coefficient is unknown. This was the purpose for the preceding presentation of statistical methods for comparing estimates of rate coefficients among laboratories. Rejecting the hypothesis that the rate coefficients for different laboratories are equal implies that laboratories have different systematic errors that are large compared

to within-laboratory imprecision. In this case, the reproducibility of the test method is poor. Conversely, accepting the hypothesis that the rate coefficients are equal would lead one to believe that the systematic error is small relative to the within-laboratory imprecision. However, the accuracy could still be poor if all the laboratories have the same systematic error, even though the reproducibility from laboratory to laboratory is good.

The between-laboratory variability of a kinetic test method can be studied by regarding the participating laboratories as a random selection each of which has a random effect on the rate coefficient. Employing the ln transformation for a first-order reaction and allowing a non-zero intercept, the random-effects model is

$$\ln(C_o/C_t) = a + a_\ell + (k + k_\ell)t_{\ell i} \quad . \tag{6.30}$$

In this model, a and k are the overall intercept and rate coefficient, where a_ℓ and k_ℓ are the random effects of the ℓth laboratory.

In terms of statistical theory and methodology, this random-effects model is fundamentally different than the fixed-effects model that is the basis of the methods for comparing a fixed selection of laboratories and that is implicit in the preceding discussion of within-laboratory precision. Unfortunately, random-effects models are more difficult to deal with than fixed-effects models, and no well-established statistical methods are available for treating the current problem. However, one statistical formulation is derived here to estimate the between-laboratory variability.

For the random-effects model, the between-laboratory variability of the kinetic test method corresponds to the variance of the random effect k_ℓ. The within-laboratory variability for the ℓth laboratory corresponds to the variance of the estimate of k_ℓ, conditional on the random effect k_ℓ. The within-laboratory regression estimate is denoted by $\hat{k}_\ell$,

to distinguish it from the random effect k_ℓ. Let $var_B(k_\ell)$ and $var_W(\hat{k}_\ell)$, denote the between- and within-laboratory variances. It can be shown that the total variance of $\hat{k}$ across all laboratories, $var_T(\hat{k}_\ell)$, is the sum of the between- and within-laboratory variance components,*

$$var_T(\hat{k}_\ell) = var_B(k_\ell) + var_W(\hat{k}_\ell) \tag{6.31}$$

Provided there are enough randomly selected laboratories, preferably ten or more, then the total variance across all laboratories of the within-laboratory regression estimates of the rate coefficients can be estimated by the standard formula for calculating a sample variance,

$$var_T(\hat{k}_\ell) = \frac{1}{N-1} \sum_\ell (\hat{k}_\ell - \bar{\hat{k}})^2 \tag{6.32}$$

The within-laboratory variance component is again estimated by the pooled estimate of the residual variance about the regression line for each laboratory,

$$var_W(\hat{k}_\ell) = S_p^2 = \frac{\sum_\ell (n_\ell - 2)S_\ell^2}{\sum_\ell n_\ell - 2N} \tag{6.33}$$

---

*Proof: For two random vairables x and z,

$$\sigma_x^2 = E\left[\sigma_{x|z}^2\right] + \sigma_{E(x|z)}^2 \qquad \text{(Rao, 1973)}$$

Let $x = \hat{k}_\ell$ and $z = k_\ell$.

Then $\sigma_{\hat{k}_\ell|k_\ell}^2 = E\left[(\hat{k}_\ell - k_\ell)^2 | k_\ell\right] = \frac{\sigma_e^2}{\sum_i (t_{\ell i} - \bar{t})^2}$

and $\sigma_{E(\hat{k}_\ell|k_\ell)}^2 = E\left\{\left[E(\hat{k}_\ell|k_\ell) - E(E(\hat{k}_\ell|k_\ell))\right]^2\right\}$

$= E\left\{[k_\ell - k]^2\right\} = \sigma_{k_\ell}^2$

The between-laboratory variance component can then be estimated by subtraction,

$$\begin{aligned} \mathrm{var}_B(k_\ell) &= \mathrm{var}_T(\hat{k}_\ell) - \mathrm{var}_w(\hat{k}_\ell) \\ &= \frac{1}{N-1}\sum_\ell (\hat{k}_\ell - \bar{\hat{k}})^2 - \frac{\sum_\ell (n_\ell - 2)s_\ell^2}{\sum_\ell n_\ell - 2N} \end{aligned} \tag{6.34}$$

Operational Steps of the Collaborative Test Program

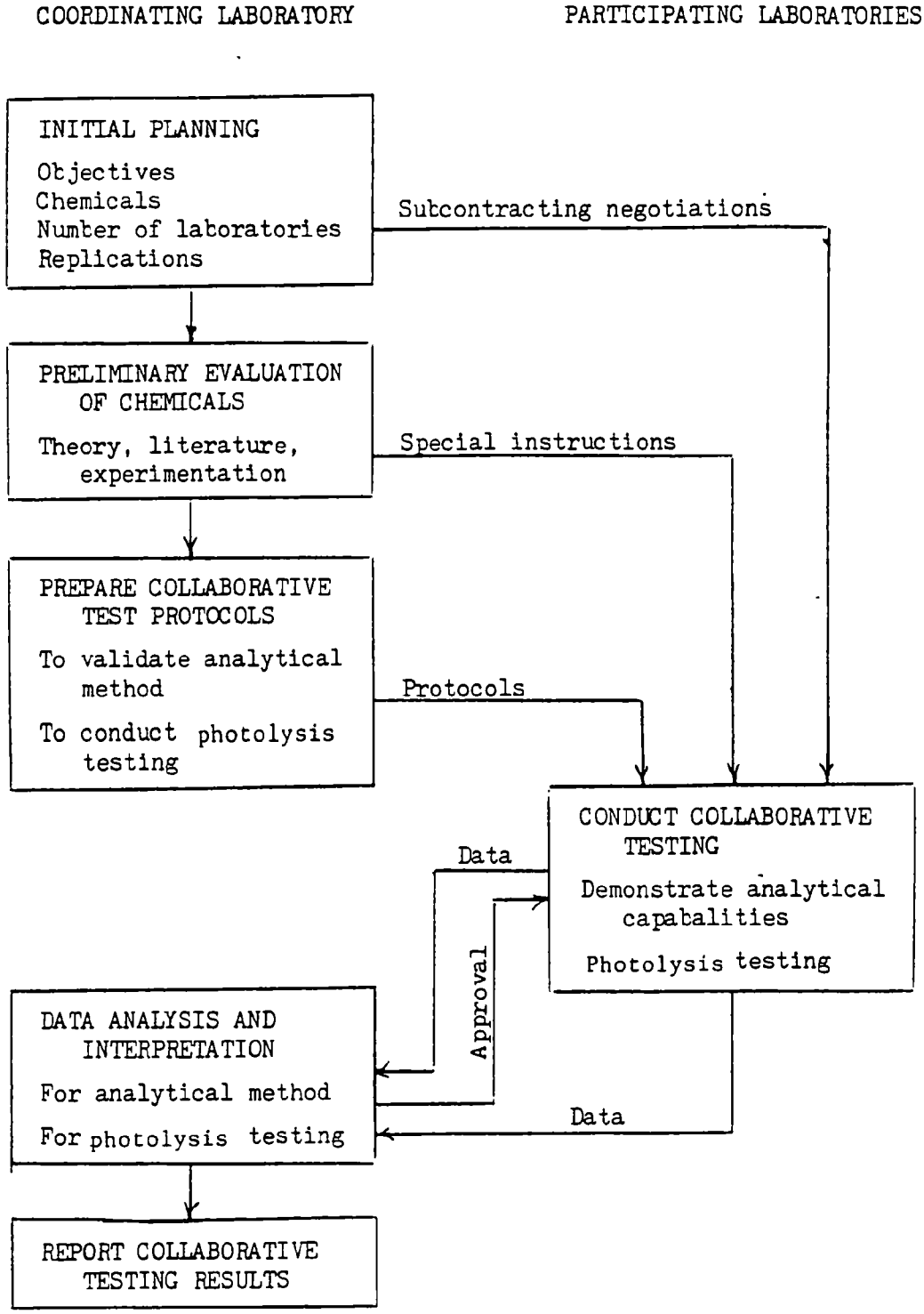
A collaborative test program consists of six steps, as shown in Figure 2; each step is described below.

Initial Planning

The initial planning serves to define the scope of a collaborative test program. The objectives of a particular collaborative study should be stated at the outset to establish a basis for planning decisions. Testing may be based on screening or detailed methods, or some combination of the two. The chemicals selected for testing may be familiar and well-behaved, may have special properties of interest, or may be of environmental concern. The number of laboratories participating in a study may be limited to a "fixed" selection of three to six laboratories or may involve a larger number of "randomly" selected laboratories that represent the population of all laboratories that might use the test method. There may or may not be a plan to replicate experiments within each laboratory. These and other planning decisions should be based on scientific principles as well as practical considerations.

Preliminary Evaluation

A preliminary evaluation of the chemicals is made by the coordinating laboratory to obtian information and data that will be used to plan and conduct the collaborative testing. A "zero-level" evaluation is based on theoretical and empirical literature and possibly ancillary laboratory work. Physical and chemical properties are studied in this way to aid in selecting an analytical measurement technique and to anticipate difficulties that might occur in conducting the screening or detailed test. It may be desirable to run the screening or detailed test on the selected chemicals at the coordinating laboratory. The advantage of such a preliminary evaluation is that problems can be identified and resolved in a more cost-effective and consistent manner than if left up to each participating laboratory. Alternatively, the selected chemicals may be submitted for collaborative testing with a minimum preliminary evaluation to determine how well the independent laboratories handle difficulties on their own.



OPERATIONAL ELEMENTS OF A COLLABORATIVE TESTING PROGRAM

Figure 2

Collaborative Testing Protocol

The collaborative test protocol consists of various instructions and forms for analytical measurements and photolysis testing. General instructions will relate various portions of the program protocol, which will be assembled to meet the needs of a particular collaborative study. The collaborative test protocol will usually provide advice or requirements for analytical procedures, including forms to record calibration data. However, on some occasions the choice of an analytical technique may be left to the discretion of each participating laboratory. The screening or detailed test method for photolysis will be summarized in the collaborative test protocol, with reference to the original document (Mill and Mabey, 1980) and other reports for elaboration regarding the test apparatus and procedure. Supplementary information about the chemicals being tested will be provided e.g., physical properties, toxicity, and handling precautions , including results of the preliminary evaluation. Data collection forms will be provided to (1) record quality control information that will ensure satisfactory control of experimental conditions and that will be used for monitoring test problems and (2) record test data that will be the raw data for the statistical analysis of single and multiple laboratory test results. Data analysis procedures will be provided for each laboratory to analyze its own results. The coordinating laboratory will also develop a plan to apply statistical methods to analyze collective results.

Collaborative Testing

Collaborative testing will be conducted under the guidance and monitoring of the coordinating laboratory. The collaborative testing protocol will be distributed to participating laboratories and meetings will be arranged according to the travel budget. Each participating laboratory will be required to demonstrate its analytical capability for the selected chemicals by providing test results for a set of unknown concentrations. On the basis of these chemicals analyses, the coordinating laboratory will decide whether the participating laboratory's procedures are acceptable. After this approval, each participating laboratory will conduct photolysis testing as specified in the collaborative

testing protocol. Each participating laboratory will be asked to follow the protocol as closely as possible and report any deviations. Laboratory data will be recorded on data collection forms, and each laboratory will analyze its own data as instructed by the collaborative testing protocol. All data will be submitted to the coordinating laboratory for further analysis.

Data Analyses and Interpretation

Test data received from participating laboratories will be analyzed and interpreted by the coordinating laboratory. Statistical analyses will be performed to (1) evaluate the precision and accuracy of each laboratory's analytical method, (2) compare photolysis test results between laboratories and evaluate error components, and (3) calculate the best overall estimates of rate coefficients, half-lives, within-laboratory precision, and between-laboratory reproducibility. Analytical methods will be evaluated by standard statistical techniques for collaborative testing based on replicate measurements or differences between paired samples. Photolysis testing methods will be evaluated by the statistical methods discussed earlier in this report. Rate coefficients will be estimated for each laboratory and for all laboratories to determine whether differences between laboratories are statistically significant. Within-laboratory precision will be evaluated by analyzing departures from a first-order rate law. If the number of laboratories in the study is adequate, the between-laboratory error variance of the rate coefficient will be estimated to evaluate the reproducibility of the hydrolysis test method. Graphs of rate coefficients or half-lives for pairs of chemicals plotted for each laboratory will show any tendency for systematic bias between laboratories. Additional statistical methods will be developed and applied if each experiment is replicated within each laboratory at the same or different initial concentrations. Such replication would provide another perspective on within-laboratory precision.

Reports Results

The coordinating laboratory will prepare and issue a final report of collaborative test results. This report will include a description of the study, test results, conclusions regarding the precision and accuracy of the test method, and recommendations for changes in the test method and for further collaborative testing.

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Appendix A

A GENERAL SOLUTION FOR k_A, k_B, AND k_N FROM THE OVERALL RATE CONSTANT

Three measurements of the rate constant, k_{h_i}, at a fixed temperature and at any three pHs can be used to solve for the values of the acid, base, and neutral rate constants, k_A, k_B, and k_N, respectively,

$$k_{h_i} = k_A[H^+]_i + k_N + k_B[OH^-]_i$$

where $i = 1, 2, 3$

$$k_A = \{k_{h_1}([OH^-]_2 - [OH^-]_3) - k_{h_2}([OH^-]_1 - [OH^-]_3) + k_{h_3}([OH^-]_1 - [OH^-]_2)\}/J$$

$$k_B = \{-k_{h_1}([H^+]_2 - [H^+]_3) + k_{h_2}([H^+]_1 - [H^+]_3) - k_{h_3}([H^+]_1 - [H^+]_2)\}/J$$

$$k_N = \{k_{h_1}([H^+]_2 \cdot [OH^-]_3 - [H^+]_3[OH^-]_2) - k_{h_2}([H^+]_1[OH^-]_3 - [H^+]_3[OH^-]_1) + k_{h_3}([H^+]_1[OH^-]_2 - [H^+]_2[OH^-]_1)\}/J$$

$$J = ([H^+]_2[OH^-]_3 - [H^+]_3[OH^-]_2) - ([H^+]_1[OH^-]_3 - [H^+]_3[OH^-]_1) + ([H^+]_1[OH^-]_2 - [H^+]_2[OH^-]_1)$$

The standard deviations of k_A, k_B, and k_N may be calculated from the above equations. Let $y = k_A$, k_B, or k_N; the above equations can then be written in the general form:

$$y = ak_{h_1} + bk_{h_2} + ck_{h_3}$$

where a, b, and c are the coefficients associated with k_{h_1}, k_{h_2}, and k_{h_3}, respectively. Since k_{h_1}, k_{h_2}, and k_{h_3} are independent, the variance of y, $\sigma^2 y$, and hence the standard deviation of y, σ_y, can be readily obtained from

$$\sigma^2 y = a^2\sigma^2 k_{h_1} + b^2\sigma_{k_{h_2}} + c^2\sigma^2 k_{h_3}$$

Appendix B

DATA COLLECTION FORMS

This appendix consists of sample data collection forms for the hydrolysis screening and detailed tests.

Appendix B

Data Collection Form

Hydrolysis Protocol (25°C)

Chemical _____

Experiment I. D. _____

pH _____

Time/Concentration Data:

Data Pt. No.	Date/Time	Elapsed Time (minutes)	Concentration Analysis			
			No. 1	No. 2	No. 3	Average
0						
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

Data Analysis Form

Chemical _____ Experiment I. D. _____

pH _____

Is there any reason why you believe the experiment at this pH is invalid? _____

If yes, explain: _____

_____

Are any particular data points invalid? _____

Data Pt. No.	Reason invalid
_____	_____
_____	_____
_____	_____

Calculate the average concentration from the chemical analyses for each time and enter the results on the data collection form. For each data point (i), record below the time (t_i) and the ratio of the average concentration (C_i) to the initial concentration (C_o):

i	t_i	C_i/C_o	i	t_i	C_i/C_o	i	t_i	C_i/C_o
0	0	1.000						
1								
2								
3								
4								
5								
6								

Plot C_t/C_o versus t on the attached semilog graph paper. Fit a straight line through these points (by eye). Can you think of any reasons that might explain noticeable departures from linearity (if any)? _____

If yes, explain: _____

_____

APPENDIX C

KINETICS OF HYDROLYSIS IN SOLUTIONS OF INADEQUATE BUFFERING CAPACITY

Hydrolysis reactions in solutions which are inadequately buffered against generation of acidic or basic products can lead to small changes in pH. These systematic changes introduce errors in the estimates of k_h based on simple first order kinetics because the usual calculation assumes a constant pH value. We have evaluated the error introduced into rate constants by such changes, and applied the error evaluation to the hydrolysis experiment at pH 11 for ethyl acetate.

From the data in Table 2 it can be shown that for ethyl acetate the rate of the base-catalyzed process is equal to the neutral process at pH values of 7.7 or 7.5, depending on whether the data from the SRI or EPA protocols, respectively, are used.* The hydrolysis rate constant k_h for ethyl acetate at pH 11 is then dominated by the base-catalyzed process. The acetic acid produced from hydrolysis of ethyl acetate neutralizes some hydroxide ion, and thereby lowers the pH of the solution. Under the experimental conditions described on pages 37 and 38 for the pH 11 experiment, the hydroxide ion concentration would drop approximately 32% if all the initial 10^{-3} M ethyl acetate were hydrolyzed to acetic acid and ethanol. The error analysis presented below has been applied to the estimation of k_B, which is the dominant term in the value of k_h at pH 11. Since neutral hydrolyses are not pH dependent, the error in k_B is the maximum error that will occur in k_h at pH values where acid-catalyzed processes do not occur.

The loss of ethyl acetate (C) due to hydrolysis at pH 11 is given by the equation

---

*This condition occurs when $[OH^-]k_B = k_N$; after solving for $[OH^-]$, the $[H^+]$ value used to calculate the pH is $[H^+] = K_w/[OH^-]$.

$$\frac{-dC}{dt} = k_B[C][OH^-] \qquad (C\text{-}1)$$

If the pH is constant throughout the experiment, the hydrolysis rate expression reduces to a simple first order kinetic relation

$$\frac{-dC}{dt} = k_B[OH_o][C_o\text{-}x]. \qquad (C\text{-}2)$$

Integrating equation (C-2) gives

$$tk_B = \frac{1}{[OH_o]} \ln \frac{C_o}{[C_o\text{-}x]} \qquad (C\text{-}3)$$

where $[OH_o^-]$ and $[C_o]$ are the initial concentrations of [OH] and ethyl acetate, respectively, and x is the amount of C reacted at time t. In the inadequately buffered solution the (OH^-) term is not constant; however some buffering does still occur and a simple second order kinetic expression does not apply (i.e. $[C_o\text{-}x]$ does not have a simple corresponding $[OH_o\text{-}x]$ relationship). From the buffering expression, however, it can be shown that

$$[OH^-] = \frac{K_W}{K_A}\left[\frac{A}{HA}\right] \qquad (C\text{-}4)$$

where A and HA are the $CO_3^=$ and HCO_3^- buffering components, respectively; K_w is the autoprotolysis constant and K_A is the acid dissociation constant for HA. The loss of ethyl acetate can then be expressed as

$$\frac{-dC}{dt} = k_B[C_o\text{-}x]\left[\frac{K_w}{K_a}\right]\left[\frac{A_o\text{-}x}{HA_o + x}\right] \qquad (C\text{-}5)$$

where A_o and HA_o are the initial concentrations of $CO_3^=$ and HCO_3^-. The solution to this differential equation is

$$tk_B = \frac{K_A}{K_W}\left[\frac{C_o + HA_o}{C_o - A_o}\right] \ln\frac{C_o - x}{C_o} + \frac{K_A}{K_W}\left[1 - \frac{C_o + HA_o}{C_o - A_o}\right] \ln\frac{A_o - x}{A_o} \qquad (C\text{-}6)$$

Rearranging and substituting appropriate terms with $C_o - x = C_t$, $A_o - x = A_t$

$$k_B t = \underbrace{\frac{1}{[OH]_o} \ln\frac{C_o}{C_t}}_{\text{"first order term"}} + \underbrace{\frac{K_A}{K_W}\left[\frac{HA_o + A_o}{A_o - C_o}\right]\left[\ln\frac{A_t}{A_o} - \frac{C_o}{A_o}\ln\frac{C_t}{C_o}\right]}_{\text{correction term}} \qquad (C\text{-}7)$$

The "correction term" above is then the part of the equation which is neglected when the [OH] concentration is assumed constant; the "first order term" is the part which is used in the regression analysis to calculate k_B assuming constant pH.

Table C-1 shows a comparison of the rate constants calculated from equations (C-3) and (3-7) using the pH 11 experimental data. The first three columns show the time t, the concentration of ethyl acetate at time t (C_t), and the calculated pH of the solution at time t (the change in pH was verified in experiments where appropriate amounts of acetic acid were added to the pH 11 buffer solutions). Columns 4 and 5 list rate constants calculated from equations (C-3) (k_B) and (C-7) (k_B'), respectively. The rate constants k_B or k_B' listed in the rows for each time point were calculated using C_o, C_t and t values directly, and represent a rate constant averaged over the time period (i.e., ΔC vs. Δt for two points.) The rate constants k_B or k_B' calculated regressing the right hand terms in equations (C-3) or (C-7) vs. t are given below the double lines in columns 4 and 5, respectively. The sixth column shows the increasing error in the "averaged" k_B values resulting from the use of equation (C-3) rather than the "true value" k_B' calculated using equation (C-7). The acetic acid produced in the pH 3 hydrolysis experiment has an insignificant effect on the pH, and therefore the k_h value for pH 3 is correct as shown in Table 1. the effect of acetic acid production on the pH 7 data is also minor. If the values of $k_{h(3)}$ and $k_{h(7)}$ are taken as shown in Table 1, and $k_{h(11)}$ is taken as 1.26×10^{-2} M^{-1} s^{-1} ($= k_B(10^{-3})$),

Values of k_N and k_A are 5.72×10^{-8} s^{-1} and 1.16×10^{-4} M^{-1} s^{-1}, respectively, are calculated according to regression analyses used for Table 2 data.

From the data in Table C-1 and comparison of equations (C-3) and (C-7), two relationships need be emphasized. The first is that the error in k_B resulting from a slight change in pH is not directly assessable from the pH change itself but rather is dependent on the initial concentrations of ethyl acetate and buffer salts. The second relationship evident from column six is that the error in k_B increases with the extent of the reaction, making it time dependent. Therefore a decision to use equation (C-3) or (C-7) will depend on the extent of conversion in the experiment: for the data in Table C-1, the error incurred up to 35% conversion is comparable to the error of analyses, and therefore the use of equation (C-3) may be acceptable.

Table C-1

COMPARISON OF RATE CONSTANTS k_B CALCULATED FROM pH 11 ETHYL ACETATE EXPERIMENT USING EQUATIONS C-3 AND C-7[a]

10^{-3} t (sec)	10^3 C_t [$EtOA_c$] (M)	pH	10^2 k_B (M^{-1} sec^{-1})	10^2 k_B'[d] (M^{-1} sec^{-1})	%Error in k_B[e]
0	1.04	11.05	--	--	--
310.8	1.01	--	9.42	9.46	0.45
2273.4	0.81	11.024	10.99	11.39	3 5
4571 4	0.645	10 999	10.79	11 51	6 3
7030.2	0.475	10.978	11.15	12.24	8.9
9433.0	0.365	10.964	11.04	12.39	10.9
11,722.2	0.290	10.955	10.90	12.42	12.3
14,255.4	0.225	10.948[b]	10.74	12.43	13.6
Rate constants calculated from regression analyses			10.8 ± 0.1	12.6 ± 0.1	14%

[a] See text for discussion and equations.
[b] pH change during experiment is equivalent to a 22% change in [OH] at 77% reaction of ethyl acetate.
[c] Calculated using equation (C-3).
[d] Calculated using equation (C-7).
[e] $[(k_B' - k_B)/k_B'] \times 100\%$.

APPENDIX D

ADDITIONAL ERROR ANALYSES FOR ETHYL ACETATE HYDROLYSIS EXPERIMENTS

Additional error analysis of the ethyl acetate experiments has been performed to evaluate the contribution of analytical error to overall error in the measured rate constants. The standard errors for k_h in Table 1 range from 0.9% at pH 11 to 10.7% at pH 9. For pHs 3, 9, and 11, the precision of the chemical analysis has been calculated from multiple determinations at each time point. The proportions of variance (the square of the standard error) of estimates of k_h due to analytical error are 10.5% at pH 3, 18.5% at pH 9, and 68.9% at pH 11. There was not sufficient data to perform an error analysis for k_h at pHs 5 and 7. These results suggest that for experiments of short duration analytical error will be dominant, whereas in the longer experiments systems errors will become more important (i.e. temperature variations, volatilization, other adventitious processes).

These estimation errors in the k_h contribute to the errors for k_A, k_B, and k_N through the equations given in Appendix A. The standard error given in Table 2 for EA are based on the standard errors for k_h at pHs 3, 9, and 11. For the SRI experiment, the standard error for k_A is dominated by the standard error for k_h at pH 3; the standard errors of k_B and k_N are dominated by the standard errors for k_h at pH 11. For the EPA experiment, the standard errors for k_A, k_B, and k_N only reflect the standard error for k_h at pH 9. In general, standard errors for k_A, k_B depend on both the standard error for k_h at the three pH levels and the pH-hydrolysis profile for a particular chemical.