Designation: E 895 – 89 (Reapproved 2001)

Standard Practice for Determination of Hydrolysis Rate Constants of Organic Chemicals in Aqueous Solutions[1]

This standard is issued under the fixed designation E 895; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice describes specific procedures for obtaining solution hydrolysis rate constants and half-lives of organic chemicals that may enter the aquatic environment.

1.2 Solution hydrolysis data are obtained in sterile, buffered water using laboratory studies in which the concentration of a chemical as a function of time is measured.

1.3 A four-tiered approach is described. The testing procedures are designed to provide basic and easily obtainable information in the first tier. More detailed and costly experiments are proposed in subsequent tiers. This approach is more cost effective than one which provides for no sequential assessment.

1.4 Since all details are not covered in this practice, successful execution of the described tests will require some training or experience in the area of hydrolysis. Familiarity with the material in the references is essential.

1.5 This practice describes laboratory studies. It is not designed to provide data directly applicable to the environment. Extrapolations to specific environmental situations may require additional data or tests not included in this practice.

1.6 This practice does not consider the possible hydrolytic influences of dissolved organic matter or of adsorption/catalysis by suspended material.

1.7 This practice is written to minimize competitive processes such as oxidation, reduction, substitution, and microbial reactions.

1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 *Definitions:*

2.1.1 *half-life*—the time required for the chemical concentration to decrease to half its initial value (see section 7.2.4).

2.1.2 *hydrolysis*—any reaction that takes place in water, in the absence of light and microorganisms, in which the compound is transformed to a different compound as the result of a reaction with water.

2.1.3 *rate constant*—see 7.2.3.

3. Summary of Practice

3.1 This practice consists of separate tests arranged in a tier or hierarchal system. The testing procedures are designed to provide hydrolysis information in a cost effective manner. Basic and easily obtainable information will result from the first tier. The higher tiers are more stringent and provide additional information. Progression guidelines are provided so that a testing program can proceed from one tier to the next when additional data are desirable.

3.2 *Tier 1*—A study is performed on the chemical at 50 ± 1°C in acidic (pH 5) and basic (pH 9) solutions. These conditions are designed to provide an accelerated test procedure. Since the rate of hydrolysis increases with temperature, the rate constant measured at 50°C will always be greater than that at 25°C. If less than 10 % hydrolysis is detected after seven days, at both acidic and basic pH levels, the chemical is considered hydrolytically stable and no additional testing is required. If hydrolysis is detected and additional information is desired, proceed to Tier 2.

3.3 *Tier 2*—The rate of hydrolysis is determined in acidic, neutral, and basic solutions. One incubation temperature and one chemical concentration is used to determine a pseudo first order rate constant.

3.4 *Tier 3*—The rate of hydrolysis is determined in acidic, neutral, and basic solutions. Three incubation temperatures and two concentrations are used to define kinetic rate expressions and corresponding rate constants. Progression to Tier 3 is dependent on an estimation of the importance of hydrolysis relative to other degradation processes in the environment; greater precision and additional kinetic data such as Arrhenius parameters (activation energies and frequency factors) may be of interest.

3.5 *Tier 4*—Hydrolysis products are characterized if hydrolysis is expected to be important under environmental conditions.

4. Significance and Use

4.1 Hydrolysis is one of several factors which may influence

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the degradation of organic chemicals in the environment. Hydrolysis may be the dominant pathway for the transformation of many chemicals. Hydrolysis kinetics are, therefore, a necessary component of any mathematical model to determine the fate of chemicals in the environment **(1, 2, 3, 4, 5, 6, 7, 8, 9, 10)**.[2]

5. Guidelines for Test Progression

5.1 As a guideline to obtain additional information, proceed to Tier 2 if greater than 10 % hydrolysis occurs during 7 days, at either pH 5 or pH 9.

5.2 *Guidelines to Proceed to Tier 3*—Proceed to Tier 3 under the following conditions:

5.2.1 Hydrolysis appears to be the most important degradative mechanism in the environment.

5.2.2 Greater precision or additional kinetic data such as Arrhenius parameters (activation energies and frequency factors) are desired.

5.3 *Guidelines to Proceed to Tier 4*:

5.3.1 Characterization of hydrolysis products should be done if data from related compounds indicate potential formation of a product which is toxic and persistent.

5.3.2 If no environmental data exist for the chemical in question or related compounds.

6. Tier 1

6.1 *Procedure*:

6.1.1 Prepare acidic (pH 5.0) and basic (pH 9.0) solutions using commercially available buffers. The buffers should be made up in sterile, distilled, or deionized water. Measure the pH value of each buffer solution to ± 0.1 unit. Borate or acetate buffers should be used instead of phosphate buffers to minimize possible catalysis. The buffer concentration should be as low as possible to avoid possible buffer catalysis. As a guide, the buffer concentration should not exceed 0.02 *M*.

6.1.2 Use borosilicate glass containers to minimize possible wall reactions. Clean all sample containers and autoclave using good laboratory practice. Sterilize the solutions using 0.22-µm filters. Wash the 0.22-µm filters before use to remove impurities.

6.1.3 Use the highest purity chemicals available. Report the purity. A mixture of compounds requires an analytical procedure that will assay for each of the components of concern.

6.1.4 For certain chemicals, it may be necessary to prepare a stock solution of the test chemical using acetonitrile or other solvent. Acetonitrile is preferable because it has a dielectric constant approximately the same as water. Restrict the organic solvent in concentration to 1 % or less in the test solution and use at the same concentration in all the tests.

6.1.5 Place the buffer solutions in test containers and add the stock chemical solution to obtain the desired chemical concentration.

6.1.6 The initial concentration of the chemical must be below its water solubility and should not exceed $1 \times 10^{-4} M$. This will help to ensure first order kinetics.

6.1.7 Maintain the hydrolysis solutions in closed containers in darkness at a temperature of 50 ± 1°C. Use sealed containers sufficient to prevent volatilization losses in all studies to avoid possible volatilization. The containers are to be completely filled, sealed, and incubated at a constant temperature. Sample the solutions after 7 days. Extract the containers to remove compounds adsorbed to the container walls (refer to 1.7).

6.1.8 Use appropriate analytical methodology for chemical assay.

6.1.9 Carry out replicated (two) experiments.

6.2 *Results Report*:

6.2.1 Describe all analytical procedures used.

6.2.2 Include all raw data.

6.2.3 Determine the percent hydrolysis in the 7-day study.

7. Tier 2

7.1 *Procedure*:

7.1.1 Prepare solutions in acidic (pH 3.0 to 5.5), neutral (pH 5.5 to 8.0), and basic (pH 8.0 to 10.0) ranges using commercially available buffers. Separate chosen pH values by at least two pH units. Make up the buffers in sterile, distilled, or deionized water. Determine the pH value of each buffer solution at the start of the kinetic experiment and at the end of the experiment. Use borate or acetate buffers instead of phosphate buffers to minimize possible catalysis. The concentration of buffers should be kept as low as possible to avoid possible buffer catalysis. As a guide, the buffer concentration should not exceed 0.02 *M*. (Refer to 6.1.2-6.1.4.)

7.1.2 Add the buffer solutions to the stock chemical solution to obtain the desired chemical concentration.

7.1.3 The initial concentrations of the chemical must be below its water solubility and should not exceed $1 \times 10^{-4} M$. This will help to ensure first order kinetics.

7.1.4 Maintain the hydrolysis solutions in stoppered containers in darkness at a temperature of 25 ± 1°. Use sealed tubes in all studies to avoid possible volatilization. Sample the hydrolysis solutions at 4 intervals, such as 0, 1, 5, and 14 days. Extract the containers to remove compound adsorbed to the container walls. (Refer to 1.7.)

7.1.5 Use appropriate analytical methodology to follow the chemical concentration as a function of time.

7.1.6 Carry out replicated experiments (two) each with duplicate analysis to provide a data base for error analysis.

7.2 *Calculation*:

7.2.1 Plot the logarithm of the concentration of the chemical versus time. A straight line indicates the hydrolysis is a pseudo first order reaction over the measured time period. Because natural systems are usually buffered, hydrolysis reactions in the environment are generally pseudo first order.

7.2.2 If data do not fall in a straight line, the reaction is not first order and the data must be analyzed by methods beyond the scope of this standard practice.

7.2.3 Assuming first order reaction kinetics, hydrolysis rate constants for each pH and each temperature may be described as follows:

$$k = -dc/dt \times 1/c \quad (1)$$

[2] The boldface numbers in parentheses refer to the list of references at the end of this practice.

where:
k = first order rate constant,
c = concentration of chemical, and
t = time, s.

Integration:

$$k\int_0^t dt = -\int_{c_0}^{c} dc/c \quad (2)$$

and:

$$kt = -\ln(c/c_0) = \ln(c_o/c) \quad (3)$$

$$k = (\ln c_0 - \ln c)/t, \quad (4)$$

where:
c_0 = initial concentration, and
c = concentration at time t.

A plot of the logarithm of the concentration of the chemical versus time is a straight line:

$$\ln c = -kt + \ln c_0 \quad (5)$$

Thus the rate constant may be obtained from Eq 4 or from the slope of the line of ln c versus time as described by Eq 5. The slope of this line can be calculated by linear regression analysis. The standard error estimate of k should be included.

7.2.4 Half-life values may be calculated as follows:

$$kt = \ln(c_0/c) \quad (6)$$

Since the half-life is defined as the time required for the chemical concentration to decrease to half its initial value,

$$c = \tfrac{1}{2}c_0$$

and,

$$kt_{1/2} = \ln(c_0/\tfrac{1}{2}c_0) = \ln 2 \quad (7)$$

$$t_{1/2} = \ln 2/k = 0.693/k$$

where:
k = the first order hydrolysis rate constant (at 25°C for each pH value).

7.3 *Results Report*:

NOTE 1—The method applies to nonionic organic chemicals. It is also applicable to ionic or ionizable chemicals where the ionic or ionizable portion of the molecule is sufficiently removed from the hydrolyzable portion.

7.3.1 Describe all analytical procedures used.

7.3.2 Include all raw data.

7.3.3 Express results as follows:

7.3.3.1 A table containing the hydrolysis rate constant k_{obs} of the chemical at 25°C at each pH.

7.3.3.2 A plot of the logarithm of the concentration as a function of time for each pH.

7.3.3.3 A table containing the calculated rate constants and half-lives for each pH.

7.3.3.4 An overall rate expression as follows:

$$k_{obs} = k_b k_w/[H^+] + k_a[H^+] + k_n \quad (8)$$

where:
k_{obs} = observed first-order rate constant, s^{-1},
k_a = rate constant for second-order acid-catalyzed hydrolysis, $M^{-1}\cdot s^{-1}$,
k_b = rate constant for second-order base-catalyzed hydrolysis, $M^{-1}\cdot s^{-1}$,
k_w = $[H^+][OH^-] = 10^{-14}$ at 25°C. k_w varies with temperature.
k_n = first-order rate constant for neutral reaction, that is, pH independent, $M^{-1}\cdot s^{-1}$.

Using determination of k_{obs} at three values of pH (pH = x, $x + y$, and $x + y + z$), the observed first order reaction rates are expressed as follows:

$$k_{obs}(pH = x) = 10^{-x}k_a + k_n + 10^{(-14+x)}k_b \quad (9)$$

$$k_{obs}(pH = x + y) = 10^{-(x+y)}k_a + k_n + 10^{-14+(x+y)}k_b \quad (10)$$

$$k_{obs}(pH = x + y + z) = 10^{-(x+y+z)}k_a + k_n + 10^{-14+(x+y+z)}k_b \quad (11)$$

x, y, and z must be positive values.
The solution of these equations is:

$$k_a = 1/\beta\{[10^x(1 - 10^{-z})k_n(x)] - [10^x(1 - 10^{-y-z})k_n(x + y)] + [10^{x-z}(1 - 10^{-y})k_n(x + y + z)]\} \quad (12)$$

$$k_n = 1/\beta\{-10^{-y}(1 - 10^{-2z})k_n(x) + [(1 - 10^{-2y-2z})k_n(x + y)] - [10^{-z}(1 - 10^{-2y})k_n(x + y + z)]\} \quad (13)$$

$$k_b = 1/\beta\{10^{14-x-2y-z}(1 - 10^{z})k_n(x) - 10^{14-x-y-z}(1 - 10^{-y-z})k_n(x + y) + 10^{14-x-y-z}(1 - 10^{-x})k_n(x + y + z)\} \quad (14)$$

where:
$\beta = 1 - 10^{-Y} - 10^{-z} - 10^{-2y-2z} + 10^{-2y-z} + 10^{-y-2z}$

second-order rate constants may be calculated using the following relationship:

where:
k_2 = second-order rate constant, and
k_1 = first-order rate constant.

8. Tier 3

8.1 *Procedure*:

8.1.1 Prepare five buffer solutions; two acidic pH solutions, two basic pH solutions, and one neutral solution to determine if hydrolysis is first-order in acid and base. Suggested pH values are 2, 5, 7, 9, and 12. Prepare buffers as outlined in Section 7. Refer to 6.1.2-6.1.4.

8.1.2 Add the buffer solution to the test container containing the appropriate stock chemical solution to obtain the two desired chemical concentrations. One chemical concentration should be restricted to levels expected in the environment. Results of Tier 2 may be used for this concentration. The second concentration is higher or lower from the first concentration by a factor of 10. As an alternative to a second concentration, hydrolysis of a single concentration may be followed through two half-lives to confirm that first order kinetics describes the reaction over a wide range of concentrations.

8.1.3 Equilibrate the hydrolysis solutions in darkness at three temperatures with 20°C intervals between each temperature. Remove aliquots at intervals ranging up to 28 days or two half-lives for each of the hydrolysis conditions. Each reaction solution should be analyzed at regular time intervals to provide a minimum of six time points between 20 % and 80 % hydrolysis of the chemical.

8.1.4 Use sealed tubes in all studies to avoid possible

volatilization. Fill and seal the tubes completely. To obtain the numerous data points necessary to determine hydrolysis kinetics at the five pHs and the three temperatures, the following schedule is suggested:

Sample	Hydrolysis Period
1	0 hours
2	1 hours
3	4 hours
4	8 hours
5	24 hours
6	3 days
7	7 days
8	14 days
9	21 days
10	28 days

8.1.5 Use appropriate analytical methodology to follow chemical concentration versus time. Extract the containers to remove compound adsorbed to container walls (Refer to 1.7).

8.1.6 Carry out replicated experiments each with duplicate analysis to provide a data base for error analysis.

8.2 *Calculation*:

8.2.1 Refer to 7.2.1-7.2.3.

8.2.2 The experimentally determined hydrolysis rate constants are used to obtain Arrhenius parameters so that rate constants may be calculated for other temperatures.

8.2.3 The Arrhenius parameters, E_a(activation energy) and A (frequency factor or pre-exponential factor) may be determined from the following:

$$k = Ae^{-E_a/RT} \quad (15)$$

where:

R = gas constant = 1.99 cal·mol^{-1}·K^{-1},
A = frequency factor or pre-exponential factor, s^{-1},
E_a = activation energy, cal·mol^{-1}, and
T = absolute temperature, K

and:

$$\ln k = \ln A - E_a/RT \quad (16)$$

or:

$$\ln k = -E_a/RT + \ln A \quad (17)$$

8.2.4 A plot of ln k versus 1/T (temperature in units of K) is used to determine the parameters E_a and A. Linear regression analysis may be used to calculate parameters E_a and A.

8.2.5 Once E_a and A are known, k may be calculated at specific temperatures encountered in the environment using Eq 10.

8.3 *Results Report* (see Note in 7.3):

8.3.1 Describe all analytical methods used.

8.3.2 Include all raw data.

8.3.3 Express results as follows:

8.3.3.1 A table containing the hydrolysis rate constant (k) of the chemical for each pH and temperature experiment.

8.3.3.2 A plot of the ln of the concentration as a function of time for each pH and temperature experiment.

8.3.3.3 A table containing the calculated rate constants and half-lives for each pH and temperature experiment.

8.3.3.4 Plots of ln k versus 1/T for the different temperatures for each pH.

8.3.3.5 A table of the calculated Arrhenius parameters E_a and A, for each pH.

8.3.3.6 A table of rate constants and half-lives for normal environmental temperatures.

8.3.3.7 An overall rate expression:

$$k_{obs} = k_b k_w/[H^+] + k_a[H^+] + k_n$$

where:

k_{obs} = observed first-order rate constant, s^{-1},
k_a = rate constant for second-order acid-catalyzed hydrolysis, M^{-1}·s^{-1},
k_b = rate constant for second-order base-catalyzed hydrolysis, M^{-1}·s^{-1},
k_w = $[H^+][OH^-] = 10^{-14}$ at 25°C; k_w varies with temperature, and
k_n = rate constant for first-order neutral reaction, that is, pH independent, M^{-1}·s^{-1}.

(*a*) Using determinations of k_{obs} at three values of pH (pH = x, $x + y$, and $x + y + z$), the observed first order reaction rates are expressed as shown in Eq 8-13.

(*b*) Second-order rate constants may be calculated using the following relationship:

$$k_2 = k_1/([H^+] \quad \text{or} \quad [OH^-])$$

where:

k_2 = second-order rate constant, and
k_1 = first-order rate constant.

8.3.3.8 A plot of rate constants or half-lives as a function of pH at various temperatures.

9. Tier 4

9.1 *Procedure*:

9.1.1 Use results of Tier 3 to select experimental conditions to most easily obtain 50 to 70 % hydrolysis of the chemical.

9.1.2 Hydrolysis products may be characterized if methodology is available.

10. Precision and Bias

10.1 The precision and bias of this practice have not yet been determined.

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