

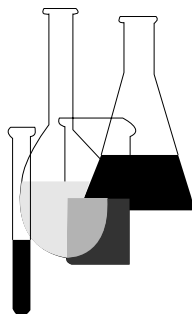


---

# Fate, Transport and Transformation Test Guidelines

## OPPTS 835.2130

### Hydrolysis as a Function of pH and Temperature



## INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

**Final Guideline Release:** This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

**OPPTS 835.2130 Hydrolysis as a function of pH and temperature.**

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are OPPT 796.3510, OPP 161–1 Hydrolysis Studies, and OECD 111 Hydrolysis as a Function of pH.

(b) **Introduction**—(1) **Background and purpose.** (i) The majority of the earth's surface is covered by water in the form of oceans, seas, rivers, lakes, streams, or ponds. As a result, chemical substances or mixtures released to the environment are likely to enter aqueous media and could undergo transformation via hydrolysis. Hydrolysis represents the transformation of a chemical substance by reaction with water into new chemicals different from their precursors. Certain classes of these substances, upon entering aquatic media, can undergo hydrolysis, which is one of the most common chemical reactions controlling stability and is, therefore, one of the principal chemical transformation pathways of these substances in the environment.

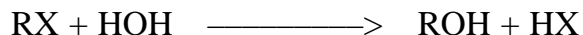
(ii) Since hydrolysis can be such an important chemical transformation pathway for certain classes of chemical substances, it is necessary, in assessing the fate of these chemicals in the environment, to know whether, at what rate, and under what conditions a substance will hydrolyze. Some of these reactions can occur so rapidly that there may be greater concern for the transformation products than for the parent substance. In other cases, a chemical substance will be resistant to hydrolysis under typical environmental conditions, while in other instances, the substance may have an intermediate stability that can result in the need for an assessment of both the parent substance and the transformation products. The importance of abiotic hydrolysis in aqueous media in the environment can be determined quantitatively from data on hydrolysis rate constants and half-lives.

(iii) This test guideline was developed to determine hydrolysis rate constants and half-lives of substances at any environmentally relevant pH and temperature anywhere in the United States.

(2) **Definitions and units.** The following definitions apply to this guideline:

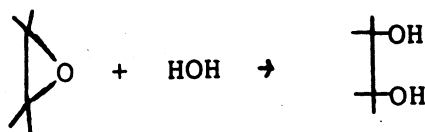
*Hydrolysis* is defined as a chemical reaction of an organic chemical with water such that one or more bonds are broken and the reaction products incorporate the elements of water (H<sub>2</sub>O). This type of transformation often results in the net exchange of the group X, in an organic chemical substance RX, for the OH group from water. This reaction can be written as:

*Equation 1*



Another result of hydrolysis can be the incorporation of both H and OH in a single product. An example of this reaction is the hydrolysis of an epoxide which can be represented by the reaction:

*Equation 2*



*Elimination reaction* means the reaction of an organic chemical substance RX in water in which the X group (as HX) is lost. These reactions generally follow the same type of rate laws that hydrolysis reactions follow, and, therefore, are also covered in this test guideline.

*First-order reaction* means a reaction in which the rate of disappearance of the test substance is directly proportional to the concentration of the test substance and is not a function of the concentration of any other substance present in the reaction mixture.

*Second-order reaction* means a reaction in which the rate of disappearance of a test substance is directly proportional to the product of the first power of the concentration of the test substance and the first power of the concentration of another species in the reaction mixture.

*Half-life* ( $t_{1/2}$ ) of a test substance means the time required for the concentration of the test chemical to be reduced to one-half its initial concentration.

*pH* of an aqueous solution means the negative decadic logarithm of the activity of the hydronium ion in solution. For practical purposes, the activity of the hydronium ion is taken as the molar concentration of the hydronium ion  $[\text{H}_3\text{O}^+]$ . Thus, pH is defined mathematically as:

Equation 3

$$\text{pH} = -\log [\text{H}_3\text{O}^+]$$

*Ion product of water* ( $K_w$ ) means the product of the activities of the hydronium and hydroxide ions in solution. For practical purposes, the activity of the hydronium ion is taken as the molar concentration of the hydronium ion  $[\text{H}_3\text{O}^+]$ , while the activity of the hydroxide ion is taken as the molar concentration of the hydroxide ion  $[\text{OH}^-]$ . Thus:

Equation 4

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

$\text{p}K_w$  means the negative decadic logarithm of  $K_w$ .

Equation 5

$$\text{p}K_w = -\log K_w$$

*Specific acid catalyzed rate constant* ( $k_H$  or  $k_A$ ) means the second-order rate constant for the hydrolysis of a chemical catalyzed by the hydronium ion ( $\text{H}_3\text{O}^+$ ). The units of  $k_H$  are in  $\text{molar}^{-1} \text{time}^{-1}$ .

*Specific base catalyzed rate constant* ( $k_{\text{OH}}$  or  $k_B$ ) means the second-order rate constant for the hydrolysis of a chemical substance catalyzed by the hydroxide ion ( $\text{OH}^-$ ). The units of  $k_{\text{OH}}$  are in  $\text{molar}^{-1} \text{time}^{-1}$ .

*Neutral water rate constant* ( $k_N$ ) means the pseudo first-order rate constant for the reaction of a chemical substance with water. The units of  $k_N$  are in  $\text{time}^{-1}$ .

(3) **Principle of the test method**—(i) **Rate of hydrolysis as a function of pH at a fixed temperature.** (A) At a fixed temperature, the rate law for the hydrolysis of a substrate RX can be put in the form:

Equation 6

$$-\frac{d[\text{RX}]}{dt} = k_h[\text{RX}] = k_H[\text{H}_3\text{O}^+][\text{RX}] + k_{\text{OH}}[\text{OH}^-][\text{RX}] + k'_N[\text{H}_2\text{O}][\text{RX}]$$

where  $k_H$ ,  $k_{\text{OH}}$ , and  $k'_N$  are the second-order rate constants for acid and base catalyzed and neutral water processes, respectively. In dilute solutions, such as are encountered in this guideline, water is present in great excess, and its concentration is, therefore, essentially constant during the course of the hydrolysis reaction. At a fixed pH, the reaction becomes pseudo first-order and the net hydrolysis rate constant  $k_h$  is given by the expression:

Equation 7

$$k_h = k_H[H_3O^+] + k_{OH}[OH^-] + k_N$$

where  $k_N$  is now the pseudo first-order neutral water rate constant. Since this is a first-order process, the half-life ( $t_{1/2}$ ) is independent of the concentration of a test substance and is given by the expression:

Equation 8

$$t_{1/2} = 0.693/k_h$$

At a fixed pH and temperature, equation 6 in this paragraph can be integrated to yield the first-order rate expression:

Equation 9

$$\ln [C_t] = - (k_h)t + \ln [C_0]$$

where  $[C_0]$  and  $[C_t]$  represent the molar concentration of RX, the test substance, at time zero and  $t$ ,  $\ln$  is the Napierian logarithm, and  $k_h$  is the net hydrolysis rate constant.

(B) In order to determine  $k_h$  as a function of pH, at a fixed temperature  $T_j$ , it is necessary to obtain the values of  $k_H$ ,  $k_{OH}$ , and  $k_N$  in equation 7 under paragraph (b)(3)(i)(A) of this guideline. This can be accomplished by measuring  $k_h$  at three different pH's at a fixed temperature  $T_j$  and solving the three equations for  $k_H$ ,  $k_{OH}$ , and  $k_N$ . This has been carried out mathematically and the results are summarized below. Equation 7, under paragraph (b)(3)(i)(A) of this guideline can be written in the following form:

Equation 10

$$k_{h(i)} = k_H[H_3^+]_i + k_{OH}[OH^-]_i + k_N$$

where  $i$  corresponds to the pH at which the hydrolysis rate constant  $k_h$  is measured. For the boundary conditions:

Equation 11

$$i = 1, \text{ pH} = x$$

$$i = 2, \text{ pH} = x + y$$

$$i = 3, \text{ pH} = x + y + z.$$

When the lowest pH is 3 and the increments are at least 2— (i.e., if  $x > 2$ ,  $y \ll 2$ ,  $z \ll 2$ ) then  $k_H$ ,  $k_{OH}$ , and  $k_N$  are given by the following mathematical expressions:

Equation 12

$$k_H = 10^x k_{h(1)} - 10^x k_{h(2)} + 10^{(x-z)} k_{h(3)}$$

Equation 13

$$k_{OH} = 10^{(pK_W - x - 2y - z)} k_{h(1)} - 10^{(pK_W - x - y - z)} k_{h(2)} + 10^{(pK_W - x - y - z)} k_{h(3)}$$

Equation 14

$$k_N = -10^y k_{h(1)} + k_{h(2)} - 10^{-z} k_{h(3)}$$

(C) The term  $pK_W$  that appears in equation 13 under paragraph (b)(3)(i)(B) of this guideline can be calculated from equation 5 under paragraph (b)(2)(viii) of this guideline and the following equation:

Equation 15

$$\log_{10} K_W = -(6014/T) - 23.65 \log_{10} T + 64.70$$

where  $T$  is the absolute temperature in K,  $T = t + 273.2$ , and  $t$  is the temperature in degrees centigrade.

(D) For 25 °C and  $x = 3$ ,  $y = z = 4$ , the pH values for  $i = 1, 2$ , and 3 correspond to 3, 7, and 11 (using equation 11 under paragraph (b)(3)(i)(B) of this guideline);  $pK_W = 14.00$  (using equation 5 under paragraph (b)(2)(viii) of this guideline and equation 15 under paragraph (b)(3)(i)(C) of this guideline).

(E) To determine  $k_H$ ,  $k_{OH}$ , and  $k_N$  at temperature  $T_j$  experimentally, the three pH's 3, 7, and 11 have been chosen. It should be noted that the pH's need not be precisely 3, 7, and 11, but must be close to these pH values (e.g.  $pH \pm 0.3$ ). However, the pH must be fixed and measured precisely to  $\pm 0.03$  pH units. Solutions of test substance are prepared at an initial molar concentration of  $10^{-3}$  M or less in buffered distilled water at pH's 3, 7, and 11 [ $C_0$ ]. The solutions are placed in a constant temperature bath controlled to  $\pm 0.1$  °C at temperature  $t_j$ (°C), or  $T_j$ (K), and the concentration of test substance is measured at regular time intervals [ $C_i$ ] to provide a minimum of 7 time points between 10 and 80 percent hydrolysis. Linear regression analysis of these data in equation 9 under paragraph (b)(3)(i)(A) of this guideline with  $t$  as the independent variable and  $\ln [C_i]$  as the dependent variable gives a slope equal to  $-k_h$ . From the exact values of the three pH's in equation 11 under paragraph (b)(3)(i)(B) of this guideline,  $x, y, z$  are calculated. Using the precise values of  $x, y,$  and  $z$  and the experimental values of  $k_{h(i)}$  at the three pH's ( $i = 1, 2, 3$ ) in equations 12, 13, and 14 under paragraph (b)(3)(i)(B) of this guideline, along with  $pK_W$  (calculated from equation 5 under paragraph

(b)(2)(viii) of this guideline and equation 15 under paragraph (b)(3)(i)(C) of this guideline at temperature  $T_j$ ,  $k_H$ ,  $k_{OH}$ , and  $k_N$  can be calculated.

(F) The hydrolysis rate constant at any pH of environmental concern at temperature  $T_j$  can be calculated using the values of  $k_H$ ,  $k_{OH}$ ,  $k_N$ ,  $[H_3O^+]$ , and  $[OH^-]$  in equation 7 under paragraph (b)(3)(i)(A) of this guideline. Finally, the half-life of the test substance can be calculated by substituting the value of  $k_h$  in equation 8 under paragraph (b)(3)(i)(A) of this guideline.

**(ii) The rate of hydrolysis as a function of pH and temperature.**

(A) In order to calculate  $k_h$  at any temperature of environmental concern, it is necessary to determine  $k_H$ ,  $k_{OH}$ , and  $k_N$  as a function of the temperature  $T$  [K]. This can be accomplished by using the Arrhenius equation for the three different rate processes:

*Equation 16*

$$k_H = A_H \exp(-E_H/RT)$$

*Equation 17*

$$k_{OH} = A_{OH} \exp(-E_{OH}/RT)$$

*Equation 18*

$$k_N = A_N \exp(-E_N/RT)$$

where  $A_H$ ,  $A_{OH}$ , and  $A_N$  are constants and  $E_H$ ,  $E_{OH}$ , and  $E_N$  correspond to the energy of activation for the acid, base, and neutral water processes;  $T$  is the absolute temperature in K; and  $R$  is the gas constant, which equals  $1.99 \times 10^{-3}$  kcal/mole or  $8.31 \times 10^{-3}$  kJ/mole. Equations 16, 17, and 18 in this paragraph are conveniently transformed to the following expressions:

*Equation 19*

$$\ln k_H = \ln A_H - (E_H/R)(1/T)$$

*Equation 20*

$$\ln k_{OH} = \ln A_{OH} - (E_{OH}/R)(1/T)$$

*Equation 21*

$$\ln k_N = \ln A_N - (E_N/R)(1/T)$$

(B) Paragraph (b)(3)(i) of this guideline describes a procedure for determining  $k_H$ ,  $k_{OH}$ , and  $k_N$  by measuring  $k_h$  at pH's 3, 7, and 11 in



buffered distilled water at temperature  $T_j$ . These experiments are repeated at two other elevated temperatures  $T_k$  and  $T_l$ , each temperature being separated by at least  $15^\circ\text{C}$  (15 K). These experiments yield  $k_H$ ,  $k_{OH}$ , and  $k_N$  as a function of the temperatures  $T_j$ ,  $T_k$ , and  $T_l$ . Using these data in equations 19, 20, and 21 under paragraph (b)(3)(ii)(A) of this guideline and linear regression analysis,  $A_H$ ,  $A_{OH}$ ,  $A_N$ ,  $E_H$ ,  $E_{OH}$ , and  $E_N$  can be determined. For example, for Equation 19, linear regression analysis with  $\ln k_h$  as the dependent variable and  $(1/T)$  as the independent variable yields a slope which is equal to  $-(E_H/R)$  and a y-intercept equal to  $\ln A_H$ .

(C) With the appropriate Arrhenius constants  $A$  and  $E$  in equations 16, 17, and 18 under paragraph (b)(3)(ii)(A) of this guideline,  $k_H$ ,  $k_{OH}$ , and  $k_N$ , can be calculated at any environmentally relevant temperature  $T_m$  of concern. At a fixed  $(\text{pH})_n$  of environmental concern,  $[\text{H}_3\text{O}^+]_n$  and  $[\text{OH}^-]_n$  can be calculated; and using these results in equation 7 under paragraph (b)(3)(i)(A) of this guideline along with  $k_H$ ,  $k_{OH}$ , and  $k_N$ ,  $k_h$  can be calculated for temperatures  $T_m$  and  $\text{pH}_n$ . Using the same procedure for other combinations of temperature and  $\text{pH}$ ,  $k_h$  can be computed as a continuous function of both  $\text{pH}$  and temperature within the experimental range. The corresponding half-life can be calculated by using  $k_h$  in equation 8 under paragraph (b)(3)(i)(A) of this guideline.

(D) The technical support document under paragraph (e)(2) of this test guideline gives a complete discussion of the scientific aspects of the kinetics of hydrolysis. This document should be carefully reviewed before carrying out these hydrolysis experiments.

**(4) Applicability and specificity.** (i) There are several different common classes of organic chemical substances that are subject to transformation by hydrolysis. These classes of substances include alkyl halides, epoxides, ethers, esters, amides, carbamates, phosphoric and phosphonic esters, lactones, and anhydrides. Processes other than nucleophilic displacement by water can also take place. Among these are elimination reactions that exhibit kinetic behavior similar to hydrolysis and are, therefore, also covered in this test guideline. This test guideline is not applicable to the above classes of chemicals which contain functional groups which ionize or protonate and are located close to the hydrolytic reaction center.

(ii) For most test substances, the hydrolysis experiments should be carried out at  $\text{pH}$ 's 3, 7, and 11. However, for a few chemicals, the rate of hydrolysis could be too rapid at  $\text{pH}$ 's 3 and 11 so that measuring the loss of test substance would be too difficult. For these substances, the hydrolysis experiments should be carried out at  $\text{pH}$ 's 5 and/or 9 because the rates are reduced by approximately a factor of 100 relative to  $\text{pH}$ 's 3 and/or 11. At  $\text{pH}$ 's 5, 7, and 9, equations 12, 13, and 14 under paragraph (b)(3)(i)(B) of this guideline are still applicable. If the rates are still too fast at  $\text{pH}$ 's 5 and/or 9, then the temperature should be adjusted to give rates that are easily measured in the laboratory.

(c) **Test procedures**—(1) **Test conditions**—(i) **Special laboratory equipment.** Special laboratory equipment shall include:

(A) A thermostatic bath that can be controlled to 0.1 °C in the temperature range 10–90 °C.

(B) A pH meter with an accuracy of  $\pm 0.03$  pH units or better.

(C) Stopped volumetric flasks (no grease), Mininert valves, or glass ampoules that can be sealed.

(ii) **Purity of water.** Reagent-grade water shall be used (e.g. water meeting ASTM Type IIA standards or an equivalent grade). ASTM Type IIA water is described in ASTM D—1193–77 “Standard Specification for Reagent Water.” Copies may be obtained from the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103.

(iii) **Sterilization.** It is required that all glassware be sterilized. Aseptic conditions shall be used in the preparation of all solutions and in carrying out all hydrolysis experiments to minimize or eliminate biodegradation. Glassware can be sterilized in an autoclave or by any other suitable non-chemical methods.

(iv) **Temperature controls.** All hydrolysis experiments shall be carried out at a temperature controlled to  $\pm 0.1$  °C.

(v) **Volatile chemical substances.** If a test substance is volatile, it is extremely important to take special precautions when carrying out hydrolysis experiments, especially at very high temperatures. Thus, the reaction vessels must be effectively sealed. Sealed tubes or tubes with gas-tight Mininert valves are recommended for elevated temperature studies. At lower temperatures, volumetric flasks or tubes with Teflon-lined screw caps can often be used successfully. Volumetric or other glass-stoppered flasks should be used without grease. In addition, the reaction vessel should be almost completely filled, and when conducting the hydrolysis experiments, especially at elevated temperatures, it is extremely important to submerge the reaction vessel completely below the fluid in the constant-temperature bath.

(vi) **pH.** (A) It is recommended that all hydrolysis experiments be performed at pH's of approximately 3, 7, and 11 (or 10, if necessary) but fixed precisely to  $\pm 0.03$  pH units. Buffers listed in paragraph (c)(2)(i)(A) of this guideline are strongly recommended. Alternatively, a pH-stat may be used to avoid the use of buffers and thus potential problems with buffer catalysis.

(B) In order to measure the pH accurately, the pH meter must be calibrated with NBS primary and secondary standards. In addition, some hydrophobic test substance could adsorb to the surface of the glass electrode in the pH meter and cause anomalous kinetic results. Hence, for

these hydrophobic substances, the glass electrode should be checked for contamination.

(vii) **Concentration of solutions of test substances.** It is required that the concentration of the test substance be less than one-half its solubility in water and not greater than  $10^{-3}$  M.

(viii) **Buffers.** For certain test substances, buffers may catalyze the hydrolysis reaction. If this is suspected, then hydrolysis rate determinations shall be carried out with the appropriate buffers and the same experiments shall be repeated at buffer concentrations lowered by at least a factor of 5. If the hydrolysis reaction produces a change of greater than 0.03 pH units in the lower concentration buffers at the end of the measurement time, then the test substance concentration shall be lowered by at least a factor of 5. Alternatively, test substance concentration and buffer concentration may be both lowered simultaneously by a factor of 5. A sufficient criterion for minimization of buffer catalysis is an observed equality in the hydrolysis rate constant of two different solutions differing in buffer or test substance concentration by a factor of 5.

(ix) **Light sensitive test chemicals.** The solution absorption spectrum should be employed to determine whether a particular test chemical is potentially subject to photolytic transformation upon exposure to light. The absorption spectrum can be obtained by using test guideline OPPTS 830.7050, entitled "Absorption in aqueous solution: Ultraviolet/visible spectra." under paragraph (e)(3) of this guideline. For substances that absorb light of wavelengths greater than 290 nm, it is recommended that the experiments be carried out by wrapping the reaction vessels with aluminum foil, by the use of amber or red colored glassware, by the use of amber or red safelights, or any other suitable technique which will eliminate the possibility of photolytic transformation.

(x) **Substances susceptible to oxidation.** If a test substance is suspected of being susceptible to oxidation with air, the following experiments should be performed. At a fixed pH and temperature, the rate of hydrolysis should be determined with and without purging the reaction solutions with purified argon or nitrogen gas. If the rate constant  $k_h$  measured in the unpurged solution is faster than the rate constant in the purged solution, then air oxidation may be occurring and all reaction solutions should be purged before all hydrolysis rate measurements are performed. For volatile test substances, the buffer solution must be purged before adding the test substance.

(xi) **Chemical analysis of solutions of test substance.** In determining the concentration of the test substance in solution, the most applicable analytical method may be employed. Chromatographic methods are recommended because of their compound specificity in analyzing the parent chemical without interferences from impurities. Whenever practicable, the

chosen method should have a precision within  $\pm 5$  percent. The test guideline requires that the specific analytical technique utilized be completely described.

(2) **Preparations**—(i) **Reagents and solutions**—(A) **Buffer solutions.** (1) Prepare buffer solutions using reagent-grade chemicals and reagent-grade water as follows:

(i) pH 3: Use 500 mL of 0.100 M potassium hydrogen phthalate  $\text{KH}(\text{C}_8\text{H}_4\text{O}_4)$  and 223 mL of 0.100 M hydrochloric acid, diluted to 1.000 L with water.

(ii) pH 5: Use 250 mL of 0.100 M potassium hydrogen phthalate and 113 mL of 0.100 M sodium hydroxide, diluted to 500 mL with water.

(iii) pH 7: Use 500 mL of 0.100 M potassium dihydrogen phosphate  $(\text{KH}_2\text{PO}_4)$  and 291 mL of 0.100 M sodium hydroxide, diluted to 1.000 L with water.

(iv) pH 9: Use 250 mL of 0.0250 M borax  $(\text{Na}_2\text{B}_4\text{O}_7)$  and 23 mL of 0.100 M hydrochloric acid, diluted to 500 mL with water.

(v) pH 11: Use 500 mL of 0.0500 M sodium bicarbonate  $(\text{NaHCO}_3)$  and 227 mL of 0.100 M  $\text{NaOH}$ , diluted to 1.000 L with water.

(2) Recommended buffers at other pH's are listed in test guideline OPPTS 835.2110, under paragraph (e)(4) of this guideline.

(B) **Adjustment of buffer concentrations.** (1) The concentrations of all the buffer solutions are the maximum concentrations to be employed in carrying out hydrolysis experiments. If the initial concentration of the test substance is less than  $10^{-3}$  M, it is extremely important that the buffer concentrations be lowered by a corresponding amount, e.g. if the initial concentration of the test substance is  $10^{-4}$  M, then reduce the concentration of the buffers by a factor of 10. In addition, for those reactions in which an acid or base is not a reaction product, then use the minimum buffer concentration necessary for maintaining the pH within  $\pm 0.03$  units.

(2) Check the pH of all buffer solutions with a pH meter at temperature  $T_j$ , and adjust the pH to the proper value with acid or base, if necessary.

(C) **Preparation of test substance solution.** (1) If the test substance is readily soluble in water, prepare an aqueous solution of the test substance in the appropriate buffer and determine the concentration of the test substance ( $C_0$ ). Alternatively, a solution of the substance in pure water may be prepared and added to an appropriate buffer solution, and the concentration of the test substance may then be determined. In the latter case, it is important that the aliquot be small enough so that the concentration of the buffer in the final solution and the pH of the solution remain essen-

tially unchanged. Do not employ heat in dissolving the test substance. The final concentration of the test substance shall be no greater than one-half its solubility in water and not greater than  $10^{-3}$  M.

(2) If the test substance is too insoluble in pure water to permit reasonable handling and reliable analysis, it is recommended that the substance be dissolved in reagent-grade acetonitrile (or reagent-grade ethanol if the hydrolysis experiments are carried out at temperatures above  $80\text{ }^{\circ}\text{C}$ ), and buffer solution is then added to an aliquot of the acetonitrile (or ethanol) solution. Do not employ heat to dissolve the substance in acetonitrile (or ethanol). The final concentration of the test substance shall not be greater than one-half its solubility in water and not greater than  $10^{-3}$  M. In addition, it is extremely important that the final concentration of acetonitrile (or ethanol) be 1 volume percent or less.

(3) **Performance of the test.** (i) Prepare the test substance solution as described in paragraph (c)(2)(i)(C) of this guideline at pH's of approximately 3, 7, and 11. Measure the pH of each solution to  $\pm 0.03$  pH units at temperature  $T_j$ , where  $T_j$  corresponds to the temperature of the constant temperature bath where the hydrolysis experiments will be carried out. Record the exact values of the three pH's and the temperature,  $T_j$ , of the constant temperature bath to  $0.1\text{ }^{\circ}\text{C}$  ( $0.1\text{ K}$ ) or less. For each kinetic experiment at each pH: Measure the initial concentration of the test substance ( $C_0$ ) in triplicate; measure the concentration of test substance in triplicate at regular time intervals ( $C_t$ ) to provide a minimum of 7 time points with the extent of hydrolysis between 10 and 80 percent; and repeat each rate measurement once. Five of the seven time points should be between 20 and 70 percent hydrolysis. Rates should be rapid enough so that 70 to 80 percent of the test substance hydrolyzes within 1 week.

(ii) If the pH at the end of the concentration measurements has changed by more than 0.03 units from the initial pH, repeat the experiments using a solution having a test substance concentration lowered sufficiently to keep the pH variation within 0.03 pH units.

(iii) Repeat the above experiments at two elevated temperatures,  $T_k$  and  $T_l$  each temperature separated by at least  $15\text{ K}$  ( $15\text{ }^{\circ}\text{C}$ ) and separated from  $T_j$  by at least  $15\text{ K}$  ( $15\text{ }^{\circ}\text{C}$ ).

(4) **Analytical methodology.** Select an analytical method that is most applicable to the analysis of the test substance (paragraph (c)(1)(xi) of this guideline).

(d) **Data and reporting**—(1) **Treatment of results**—(i) **Rate of hydrolysis as a function of pH at a fixed temperature.** The objective of this set of experiments is to determine  $k_H$ ,  $k_{OH}$ , and  $k_N$  of the test substance at a fixed temperature  $T_j$ , from the rates of hydrolysis at the three pH's (3, 7, and 11). This can be accomplished by the following steps.

(A) Hydrolysis experiments shall be carried out at three pH's at a fixed temperature  $T_j$ , and the molar concentration of test substance is measured in triplicate as a function of the time. These data are used to determine  $k_{h(i)}$  at the three pH's (i.e., pH's 3, 7, and 11 corresponding to  $i = 1, 2, 3$ ), and using these data in equation 9 under paragraph (b)(3)(i)(A) of this guideline and linear regression analysis with  $\ln [C_t]$  as the dependent variable and  $t$  as the independent variable, the slope of the best straight line is obtained. The slope of this line is  $-k_{h(i)}$ .

(B) From equation 11 under paragraph (b)(3)(i)(B) of this guideline and the exact values of the pH's (measured precisely to 2 decimal places), calculate the values  $x$ ,  $y$ , and  $z$ .

(C) Calculate the value of  $pK_w$  at  $T_j$  from the value of  $\log K_w$  from equation 15 under paragraph (b)(3)(i)(C) of this guideline and  $pK_w = -\log K_w$ .

(D) Using 12, 13, and 14 under paragraph (b)(3)(i)(B) of this guideline and the values of  $k_{h(1)}$ ,  $k_{h(2)}$ ,  $k_{h(3)}$ ,  $x$ ,  $y$ ,  $z$ , and  $pK_w$ , calculate  $k_H$ ,  $k_{OH}$ , and  $k_N$ .

(ii) **Rate of hydrolysis as a function of pH and temperature.** The objective of this set of experiments is to determine the rate of hydrolysis and half-life of the test substance at any pH and temperature of environmental concern.

(A) **The rate constants  $k_H$ ,  $k_{OH}$ , and  $k_N$  as a function of temperature.** Hydrolysis experiments shall be carried out at three pH's at three different temperatures  $T_j$ ,  $T_k$ , and  $T_l$  and  $k_H$ ,  $k_{OH}$ , and  $k_N$  are calculated as described in paragraph (d)(1)(i) of this guideline. Using these data in equations 19, 20, and 21 under paragraph (b)(3)(ii)(A) of this guideline and linear regression analysis,  $A_H$ ,  $A_{OH}$ ,  $A_N$ ,  $E_H$ ,  $E_{OH}$ , and  $E_N$  can be calculated. For example, consider the experimental data for  $k_H$  at  $T_j$ ,  $T_k$ , and  $T_l$ . Using linear regression analysis of these data in equation 19 with  $\ln k_H$  as the dependent variable and  $(1/T)$  as the independent variable (with  $T$  in K), the slope and  $y$ -intercept can be calculated. From equation 19, the slope is equal to  $-(E_H/R)$ ;  $E_H$  can be calculated using the value of  $R = 8.3 \times 10^{-3}$  kJ/mole. From equation 19, the  $y$ -intercept is equal to  $\ln A_H$ ; hence,  $A_H$  can be calculated.

(B) **Rate of hydrolysis and the half-life in the environment.** The calculation of the rate of hydrolysis and the half-life of the test substance at any temperature  $T_m$  and  $(pH)_n$  of the environmental concern can be accomplished by the following steps.

(1) Using equations 16, 17, and 18 under paragraph (b)(3)(ii)(A) of this guideline and the values of  $A_H$ ,  $A_{OH}$ ,  $A_N$ ,  $E_H$ ,  $E_{OH}$ , and  $E_N$  determined above, calculate  $k_H$ ,  $k_{OH}$ , and  $k_N$  at any temperature  $T_m$  of environmental concern.

(2) Calculate the ion product of water,  $K_w$  at  $T_m$  using equation 15 under paragraph (b)(3)(i)(C) of this guideline.

(3) At a given  $(pH)_n$  of environmental concern,  $(pH)_n$  is equal to  $-\log [H_3O^+]_n$  and  $[OH^-]_n$  is equal to  $K_w/[H_3O^+]_n$ . From the value of  $(pH)_n$ , calculate  $[H_3O^+]_n$ , and from the value of  $K_w$  calculated from step (2) and  $[H_3O^+]_n$ , calculate  $[OH^-]_n$ .

(4) Calculate  $k_h$  using the values of  $[H_3O^+]_n$ ,  $[OH^-]_n$ ,  $k_H$ ,  $k_{OH}$ , and  $k_N$  in equation 7 under paragraph (b)(3)(i)(A) of this guideline.

(5) Calculate the half-life,  $t_{1/2}$ , by substituting the value of  $k_h$  in equation 8 under paragraph (b)(3)(i)(A) of this guideline.

(2) **Test data report**—(i) **Test conditions.** Report the following:

(A) The name, structure, purity of the test substance, and whether the substance absorbs light at wavelengths  $\ll 290$  nm.

(B) If the test chemical absorbs light at wavelengths  $\ll 290$  nm, indicate the method used to eliminate photolysis.

(C) The type of reaction vessels used.

(D) The exact pH's and temperature used for each experiment.

(E) The type of solvent, if used, to solubilize the test substance and the percent by volume.

(F) the initial and final pH for each experiment.

(G) A description of the buffers used if they are not listed in this test guideline.

(H) If buffer effects were observed, described how they were minimized or eliminated.

(ii) **Specific analytical and recovery procedures.** (A) Provide a detailed description or reference for the analytical procedures, including the calibration data and precision; and

(B) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

(iii) **Test data report.** Report the following:

(A) The initial molar concentration  $[C_0]$  of test substance for each replicate and the mean value for each hydrolysis experiment (i.e., at pH's corresponding to  $i = 1, 2, 3$ , and at temperatures  $T_j, T_k, T_1$ ).

(B) The three separate values of the molar concentration ( $C_t$ ) and the mean value for each time point for each hydrolysis experiment (i.e., at pH's corresponding to  $i = 1, 2, 3$ , and temperatures  $T_j, T_k, T_l$ ).

(C) The value of  $k_{h(i)}$  and the correlation coefficient for each set of experiments at temperatures  $T_j, T_k, T_l$ .

(D) The calculated values of  $k_H, k_{OH},$  and  $k_N$  at  $T_j, T_k, T_l$  (in K) in tabular form.

(E) Using the appropriate data from paragraph (d)(2)(iii)(E) of this guideline, report the values of  $A$  and  $E$  and the correlation coefficient for each process (e.g.  $A_H, E_H,$  and the correlation coefficient, etc.).

(F) If the compound was susceptible to oxidation, report the data under paragraph (d)(2)(iii)(A) through (F) of this guideline for the purged solutions.

(e) **References.** The following references should be consulted for additional background information on this test guideline:

(1) American Society for Testing and Materials, 1973, 1983, Annual Book of Standards, Part 31. Standard specification for water, Philadelphia, PA.

(2) U.S. Environmental Protection Agency, 1993, OPPTS 835.2130. Technical Support Document, "Hydrolysis as a function of pH and temperature."

(3) U.S. Environmental Protection Agency. (1993a). OPPTS 830.7050—Absorption in aqueous solution: Ultraviolet/visible spectra.