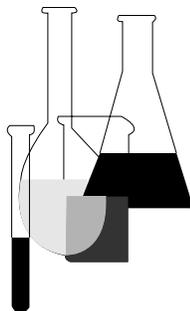




Fate, Transport and Transformation Test Guidelines

OPPTS 835.2110

Hydrolysis as a Function of pH



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 835.2110 Hydrolysis as a function of pH.

(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 40 CFR 796.3500 Hydrolysis as a Function of pH at 25 °C, and OECD guideline 111 Hydrolysis as a Function of pH.

(b) **Guidance information**—(1) **Prerequisites.** Water solubility data; suitable analytical method; vapor pressure curve.

(2) **Qualifying statements.** Pure and commercial grade substances can be tested with the method described here, but the potential effect of impurities on the results should be considered. This test guideline applies only to water soluble compounds. There is uncertainty in extrapolating high temperature results to environmentally relevant temperatures as a change in reaction mechanism could occur.

(3) **Standard documents.** This test guideline is based on methods given in paragraph (f) of this guideline and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

(c) **Method**—(1) **Purpose, relevance, application, and limits of test.**

(i) The testing of substances for hydrolysis is relevant to their persistence. Hydrolysis is one of the most common reactions controlling abiotic degradation and is therefore one of the main degradation paths of substances in the environment.

(ii) A procedure to determine hydrolysis rates is important also in indicating whether other testing should be carried out on a parent compound or on its hydrolysis products. It is the degradation products that are crucial. Hydrolysis behavior needs to be examined at pH values normally found in the environment (pH 4–9) and under more acidic conditions (pH 1–2) for physiological purposes.

(iii) Surface-controlled reactions can sometimes predominate over bulk solution hydrolysis, especially in the soil environment. This may result in different degradation rates than would be predicted from this guideline based upon rates in homogeneous solutions.

(2) **Definitions and units.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Hydrolysis refers to a reaction of a chemical RX with water, with the net exchange of the group X with OH at the reaction center:



Hydrolysis rate, the rate at which the concentration of RX decreases in this simplified process is given by:

$$\text{second order reaction rate} = k [\text{H}_2\text{O}] [\text{RX}]$$

or

$$\text{first order reaction rate} = k [\text{RX}]$$

depending on the rate determining step. Because water is present in great excess compared to the chemical, this type of reaction is usually described as a pseudo-first order reaction in which the observed rate constant is given by the relationship:

$$k_{\text{obs}} = k [\text{H}_2\text{O}]$$

and can be determined from the expression

$$K_{\text{obs}} = 2.303/t \log_{10}(C_0/C_t)$$

where t = time, and C_0 and C_t = concentrations of RX at times 0 and t .

The units of this constant have the dimension of $(\text{time})^{-1}$ and the half-life of the reaction (time for 50 percent of RX to react) is given by

$$t_{1/2} = 0.693/k_{\text{obs}}$$

(3) **Reference substances.** (i) Aspirin and Diazinon are used as references. These substances need not be employed in all cases when investigating a new substance. These data are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied. The results of the OECD/EEC-Laboratory Intercomparison Testing are included in the following Tables 1. and 2.:

Table 1.—Values of Rate Constants and Half-Lives for Aspirin^{1,2}

pH	K [10^5 sec^{-1}]	$t_{1/2}$ (h)
3.5	0.065	300
5.0	0.15	130
7.4	0.13	150
9.5	0.37	52
11.3	16	1.2

¹ Data taken at 17 °C

² Aspirin (CAS no. 50-78-2) (2-acetylsalicylic acid) data from L.J. Edwards, *Transactions of the Faraday Society* 723-735 (1950).

Table 2.—Values of Rate Constants and Half-Lives for Diazinon¹

pH	10 °C		20 °C		40 °C		60 °C	
	K [10 ⁵ sec ⁻¹]	t _{1/2} (h)	K [10 ⁵ sec ⁻¹]	t _{1/2} (h)	K [10 ⁵ sec ⁻¹]	t _{1/2} (h)	K [10 ⁵ sec ⁻¹]	t _{1/2} (h)
10.43	0.061	310	0.13	150	0.41	48	15	12
9.0	--	--	0.0059	3300	--	--	--	--
7.4	--	--	0.00043	4400	--	--	--	--
5.0	--	--	0.026	740	--	--	--	--
3.1	0.75	260	1.6	120	6.6	29	25	7.8

¹ Diazinon (CAS no. 333-41-5) (*O,O*-diethyl-*O*-(2-isopropyl-4-methyl)-6-pyrimidinyl phosphorothioate) data from H.M. Gomma et al. *Residue Reviews* 29:171 (1969).

(ii) Further results of OECD–EEC Laboratory intercomparison testing follow in Tables 3. through 12. In some cases the results presented have a variability which exceeds what is called for in the test guideline. This may particularly be the result of the use of different buffers in the test systems and the influence of oxygen. These results are presented, however, as they have been obtained using the Test Guidelines in the OECD/EEC Intercomparison Testing Programme, Part II. The guideline has been modified in the light of these results.

(A) Substance: Aspirin

Table 3.—Reaction Rate Constant in 10⁵sec⁻¹

pH	tem-perature, °C	mean value	stand-ard deviation	coeffi-cient of vari-ation, %	range	n* of results
1.2	35–40	1.013	1.278	126.2	0.109–1.916	2
3.0	20	0.080	0.056	70.3	0.040–0.119	2
	40	0.556	0.112	20.1	0.477–0.635	2
7.0	20	0.205	0.033	15.9	0.182–0.228	2
	40	1.339	0.004	0.3	1.336–1.341	2
9.0	20	0.309	0.160	51.7	0.196–0.442	2
	40	0.953	0.006	0.6	0.949–0.957	2

Table 4.—Half-life in hours

pH	tem- pera- ture, °C	mean value	stand- ard devi- ation	coeffi- cient of variation, %	range	n* of results
1.2	35–40	93.71	118.3	126	10.05– 177.36	2
3.0	20	319.7	222.5	70	162.3–477.0	2
	40	35.4	7.1	20	30.3–40.4	2 (1 lab)
7.0	20	95.1	15.1	20	84.4–105.8	2
	40	14.4	0.0	–	14.4–14.4	2 (1 lab)
9.0	20	72.1	37.3	50	45.7–98.5	2
	40	20.2	0.1	0.0	20.1–20.3	2 (1 lab)

(B) Substance: Diazinon

Table 5.—Reaction Rate Constant in 10^5sec^{-1}

pH	tem- pera- ture, °C	mean value	stand- ard de- viation	coeffi- cient of vari- ation, %	range	n* of results
1.2	35–40	30.36	8.10	27.0	21.40–38.46	4
3.0	20	2.866	0.825	28.8	1.675–3.841	7
	40	9.038	2.447	27.1	5.708– 14.165	11 (10 labs)
	50	5.77	4.27	74.0	0.86–8.58	3 (2 labs)
	60	36.085	11.088	30.7	25.535– 51.449	6
7.0	20	0.933	1.796	192.4	0.005–3.626	4
	40	0.231	0.294	127.3	0.042–0.895	8
	50	0.200	0.023	11.3	0.184–0.216	2
	60	1.638	3.154	192.6	0.303–9.413	8 (7 labs)
9.0	20	1.103	2.113	191.6	0.007–4.271	4
	40	2.568	6.900	268.7	0.064– 20.955	9
	50	0.292	0.034	11.6	0.268–0.316	2
	60	2.801	4.125	147.3	0.241– 12.604	8 (7 labs)

Table 6.—Half-life in hours

pH	tem- pera- ture, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	0.672	0.187	27.9	0.501–0.900	4
3.0	20	7.27	2.40	33	5.0–11.5	7
	40	2.25	0.57	25	1.4–3.3	11 (10 labs)
	50	9.00	11.53	128	2.24–22.31	3 (2 labs)
	60	0.57	0.17	29	0.37–0.75	6
7.0	20	1707.75	1875.18	110	5.3–3660.9	4
	40	215.16	166.12	77	21.5–460.7	8
	50	97.07	10.97	11	89.31– 104.83	2
	60	38.68	20.98	54	2.0–63.5	8 (7 labs)
9.0	20	1150.75	1335.58	116	4.5–2840.5	4
	40	124.38	102.45	82	0.9–298.7	9
	50	66.40	7.74	12	60.92–71.87	2
	60	25.13	25.67	102	1.5–79.8	8 (7 labs)

(C) Substance: Atrazine

Table 7.—Reaction Rate Constant in 10^5sec^{-1}

pH	tem- pera- ture, °C	mean value	stand- ard devi- ation	coefficient of variation, %	range	n* of results
1.2	35–40	0.546	0.416	76.3	0.76–0.948	4
3.0	20	0.014	0.008	56.8	0.005–0.020	3
	40	0.140	0.082	58.4	0.028–0.222	6
	60	0.808	0.397	49.1	0.282–1.283	5
7.0	40	0.009	0.011	124.8	0.001–0.016	2
9.0	20	0.013	0.016	130.1	0.001–0.024	–
	40	0.008	0.010	123.7	0.001–0.015	2

Table 8.—Half-life in hours

pH	tem- pera- ture, °C	mean value	standard deviation	coefficient of variation, %	range	n* of results
1.2	35–40	54.76	44.36	81.0	20.3–115.35	4
3.0	20	1867.88	1446.58	77.0	980.7– 3537.14	3
	40	240.50	239.61	99.6	111.71– 696.79	6
	60	31.42	21.51	69.0	15.0–68.3	5
7.0	40	9000.15	11033.77	123.0	1198.1– 16802.2	2
9.0	20	1792105	24188.21	135.0	817.4– 35024.7	2
	40	8005.35	9539.51	119.0	1259.9– 14750.8	2

(D) Substance: Di(2-ethylhexyl) phthalate (DOP)

Table 9.—Reaction Rate Constant in 10^5sec^{-1}

pH	tem- pera- ture, °C	mean value	stand- ard devi- ation	coefficient of variation, %	range	n* of results
3.0	20	0.048	0.051	107.8	0.009–0.106	3
9.0	20	0.040	0.047	116.7	0.007–0.073	2
	40	0.166	0.202	121.8	0.023–0.309	2
	60	0.084	0.022	26.3	0.068–0.099	2
7.0	20	0.073	0.064	88.8	0.027–0.118	2
	40	(1.627)	—	—	—	1
	60	(0.092)	—	—	—	1
	40	(0.126)	—	—	—	1

Table 10.—Half-life in hours

pH	tem- pera- ture, °C	mean value	standard devi- ation	coefficient of variation, %	range	n* of results
3.0	20	990.20	996.32	101	182.4–2103.5	3
	40	452.56	551.98	122	62.25–842.86	2
	60	239.33	63.75	27	194.25–284.41	2
7.0	20	440.30	391.35	89	163.57–717.02	2
	40	(11.83)	—	—	—	1
	60	(208.19)	—	—	—	1
	40	(152.53)	—	—	—	1

(E) Substance: Ethyl acetate

Table 11.—Reaction Rate Constant in 10^5sec^{-1}

pH	tem- pera- ture, °C	mean value	standard devi- ation	coefficient of variation, %	range	n* of results
3.0	20	(0.0012)	—	—	—	1
	40	(0.012)	—	—	—	1
	60	(0.355)	—	—	—	1
7.0	20	(0.003)	—	—	—	1
	40	(0.008)	—	—	—	1
	60	(0.137)	—	—	—	1
9.0	20	(0.063)	—	—	—	1
	40	(0.153)	—	—	—	1
	60	(1.547)	—	—	—	1

Table 12.—Half-life in hours

pH	tem- pera- ture, °C	mean value	standard devi- ation	coefficient of variation, %	range	n* of results
3.0	20	(1656)	—	—	—	1
	40	(1612.85)	—	—	—	1
	60	(54.30)	—	—	—	1
7.0	20	(7553.1)	—	—	—	1
	40	2511.81)	—	—	—	1
	60	(140.38)	—	—	—	1
9.0	20	(305.55)	—	—	—	1
	40	(125.71)	—	—	—	1
	60	(12.44)	—	—	—	1

(4) **Principle of the test method.** (i) In the environment, chemicals usually occur in dilute solution, which means that water is present in large excess, and, therefore, that the concentration of water remains essentially constant during hydrolysis. Hence, the kinetics of hydrolysis are generally pseudo-first order at fixed pH and temperature.

(ii) The hydrolysis reaction may be influenced by acidic or basic species H_3O^+ , (H^+) , and OH^- , in which case it is referred to as specific acid or specific base catalysis.

(iii) The concentration of the test substance is determined as a function of time. The logarithms of the concentrations are plotted against time and the slope of the resulting straight line (assuming first-order or pseudo-first order behavior) gives the rate constant from the formula (if \log_{10} is used):

$$k_{obs} = -\text{slope} \times 2.303$$

(iv) When it is not practicable to determine a rate constant for a particular temperature directly, it is usually possible to estimate the constant through the use of the Arrhenius relationship in which the logarithm of rate constants at other temperatures is plotted against the reciprocal of the absolute temperature (K).

(5) **Quality criteria**—(i) **Reproducibility.** Measurements of hydrolysis rate constants on 13 classes of organic structures can be of high precision, often with less than 2 percent standard deviation (see paragraph (f)(2) of this guideline). The rate constants for one pH and one temperature should be determined in duplicate with a deviation of less than 2.5 percent unless unusual circumstances (e.g. analytical difficulties) prevent achieving this and then the details of these circumstances should be reported. The reproducibility can be improved by an improved control of the sensitive parameters, in particular pH and oxygen.

(ii) **Sensitivity.** Most hydrolysis reactions follow apparent first order reaction rates and, therefore, half-lives are independent of concentration

$$t_{1/2} = 0.693/k_{\text{obs}}$$

This usually permits the application of laboratory results determined at 10^{-2} – 10^{-3} M to environmental conditions ($\leq 10^{-6}$ M) under paragraph (f)(2) of this guideline.

(iii) **Specificity.** Several examples of good agreement between rates of hydrolysis measured in both pure and natural waters for a variety of chemicals providing both pH and temperature have been measured (see paragraph (f)(2) of this guideline).

(d) **Description of the test procedure.** (1) If the water solubility of the substance is less than 2×10^{-2} M, a half-saturated solution in water is prepared. If the solubility is greater than 2×10^{-2} , the test solution is prepared at less than 10^{-2} M. Substances known to be hydrolytically unstable are tested at at least two temperatures between 0–40 °C. Other substances are put through preliminary testing. Substances found to be stable under these conditions are considered hydrolytically stable ($t_{1/2}$ at 25 °C is greater than 1 year). Those not stable at 50 °C for one week are submitted to testing at at least two temperatures between 0–40 °C, or, as appropriate, at at least three elevated temperatures, and the data extrapolated to produce k at 25°C.

(2) **Preparations—(i) Materials—(A) Buffer solutions.** (1) The hydrolysis test should be performed at four different pH's: at pH 1.2 (if physiologically important); pH 4.0; pH 7.0; and pH 9.0. Either buffers as described below or a pH-stat may be used. Use of a pH-stat avoids potential problems due to buffer catalysis.

(2) For this purpose, 0.05 M sterile buffer solutions should be prepared using reagent grade chemicals and distilled, sterile water. The buffer systems are based upon the analytical requirements for the chemical being tested. It should be noted that the buffer system used may influence the rate of hydrolysis and where this is observed an alternate buffer system should be employed. The use of borate or acetate buffers instead of phosphate has been recommended under paragraph (f)(2) of this guideline. The pH of each buffer solution must be checked with a calibrated pH meter at the required temperature to a precision of at least 0.1 pH units. Some useful buffer systems are presented in the following Tables 13., 14., and 15.:

Table 13.—Buffer Mixtures of Clark and Lubs^{1,2}

Composition	pH
0.2 N HCl and 0.2 N KCl	
47.5 ml HC1 + 25 ml KC1 diluted to 100 ml	1.0
32.25 ml HC1 + 25 ml KC1 diluted to 100 ml	1.2

Table 13.—Buffer Mixtures of Clark and Lubs^{1,2}—Continued

Composition	pH
20.75 ml HC1 + 25 ml KC1 diluted to 100 ml	1.4
13.15 ml HC1 + 25 ml KC1 diluted to 100 ml	1.6
8.3 ml HC1 + 25 ml KC1 diluted to 100 ml	1.8
5.3 ml HC1 + 25 ml KC1 diluted to 100 ml	2.0
3.35 ml HC1 + 25 ml KC1 diluted to 100 ml	2.2
0.1 M potassium biphthalate + 0.1 N HC1	
46.70 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.2
39.60 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.4
32.95 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.6
26.42 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	2.8
20.32 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.0
14.70 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.2
9.90 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.4
5.97 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.6
2.63 ml 0.1 N HC1 + 50 ml biphthalate to 100 ml	3.8
0.1 M potassium biphthalate + 0.1 N NaOH	
0.40 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.0
3.70 ml 0.1 N NaOH + 50 ml piphthalate to 100 ml	4.2
7.50 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.4
12.15 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.6
17.70 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	4.8
0.1 M potassium biphthalate + 0.1 NaOH	
23.85 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.0
29.95 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.2
35.45 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.4
39.85 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.6
43.00 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	5.8
45.45 ml 0.1 N NaOH + 50 ml biphthalate to 100 ml	6.0
0.1 M monopotassium phosphate + 0.1 N NaOH	
5.70 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.0
8.60 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.2
12.60 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.4
17.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.6
23.45 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	6.8
29.63 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.0
35.00 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.2
39.50 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.4
42.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.6
45.20 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	7.8
46.80 ml 0.1 N NaOH + 50 ml phosphate to 100 ml	8.0
0.1 M H₂B0₂ in 0.1 M KC1 + 0.1 N NaOH	
2.61 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	7.8
3.97 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.0
5.90 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.2
8.50 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.4
12.00 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.6
16.30 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	8.8
21.30 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.0
26.70 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.2
32.00 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.4
36.85 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.6
40.80 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	9.8
43.90 ml 0.1 N NaOH + 50 ml boric acid to 100 ml	10.0

¹ Data taken at 20 °C.

² The pH values reported in these tables have been calculated from the potential measurements using Sorensen's standard equations (1909). The corresponding pH values are 0.04 unit higher than the tabulated values.

Table 14.—Citrate Buffers of Kolthoff and Vleeschouwer¹²

Composition	pH
0.1 M monopotassium citrate and 0.1 N HCl	
49.7 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.2
43.4 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.4
36.8 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.6
30.2 ml 0.1 N HCl + 50 ml citrate to 100 ml	2.8
23.6 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.0
17.2 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.2
10.7 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.4
4.2 ml 0.1 N HCl + 50 ml citrate to 100 ml	3.6
0.1 M monopotassium citrate and 0.1 N NaOH	
2.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	3.8
9.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.0
16.3 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.2
23.7 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.4
31.5 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.6
39.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	4.8
46.7 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.0
54.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.2
61.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.4
68.0 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.6
74.4 ml 0.1 N NaOH + 50 ml citrate to 100 ml	5.8
81.2 ml 0.1 N NaOH + 50 ml citrate to 100 ml	6.0

¹ Data taken at 18 °C.

² Add tiny crystal of thymol or a few milligrams of mercury or mercuric iodide to prevent growth of molds.

Table 15.—Borate Buffer Mixtures of Sorensen

Composition			Sorensen (18 °C)	Walbum, pH at		
0.05 M borax (ml)	0.01 N HCl (ml)	0.01N NaOH (ml)		10 °C	40 °C	70 °C
5.25	4.75	0.00	7.62	7.64	7.55	7.47
5.50	4.50	0.00	7.94	7.98	7.86	7.76
5.75	4.25	0.00	8.14	8.17	8.06	7.95
6.00	4.00	0.00	8.29	8.32	8.19	8.08
6.50	3.50	0.00	8.51	8.54	8.40	8.28
7.00	3.00	0.00	8.08	8.72	8.56	8.40
7.50	2.50	0.00	8.80	8.84	8.67	8.50
8.00	2.00	0.00	8.91	8.96	8.77	8.59
8.50	1.50	0.00	9.01	9.06	8.86	8.67
9.00	1.00	0.00	9.09	9.14	8.94	8.74
9.50	0.50	0.00	9.17	9.22	9.01	8.80
10.00	0.00	0.00	9.24	9.30	9.08	8.86
10.0	0.00	0.0	9.24	9.30	9.08	8.86
9.0	0.00	1.0	9.36	9.42	9.18	8.94
8.0	0.00	2.0	9.50	9.57	9.30	9.02
7.0	0.00	3.0	9.68	9.76	9.44	9.12
6.0	0.00	4.0	9.97	10.06	9.67	9.28

(B) **Test solutions.** The chemical substance should be dissolved in distilled, sterile water with sterile buffer medium added to it. The concentrations should not exceed the lesser of 0.01 M or half the saturation concentration (see OPPTS 830.7840, Water solubility, shake flask method), and the purest available form of the substance should be employed

in making up the solutions. The use of mixed solvents is recommended only in case substances with low water solubility. The amount of solvent should be less than 1 percent, and the solvent should not interfere with the hydrolysis process.

(C) **Glassware.** All glassware, which must be inert in the pH range studied, should be sterilized. Stoppered volumetric flasks (no grease) should be used for carrying out the hydrolysis reactions. If the chemical or buffer system is volatile, or if the test is being conducted at elevated temperatures, sealed or septum-closed tubes are preferred and head space should be avoided.

(3) **Analytical method.** The analytical method will be determined by the nature of the substance being tested. It must be sufficiently sensitive and specific to allow determination of the different species at the test solution concentrations and may well consist of some combination of pH electrodes, UV-visible spectrophotometry, conductivity, gas chromatography, high pressure liquid chromatography, extraction and formation of derivative(s), and determination by a suitable analytical method.

(4) **Test conditions**—(i) **Temperature.** For extrapolation purposes, it is important to maintain the temperature of the determinations to at least ± 0.1 °C. An appropriate constant temperature bath should be employed. If the hydrolytic behavior of the substance is unknown, a preliminary test at 50 °C is required. For tests beyond this preliminary stage data for temperatures in the range 0–40 °C are sought. They may be obtained by measurement at two temperatures in this range or by extrapolation from three higher temperatures. In any event, the determinations should be done at temperatures differing from each other by at least 10 °C.

(ii) **Light and oxygen.** All of the hydrolysis reactions should be carried out using any suitable method to avoid photolytic effects. All suitable measures should be taken to exclude oxygen (e.g. by bubbling nitrogen or argon through the solvent for 5 minutes before preparation of the solution).

(5) **Performance of the test**—(i) **Preliminary test.** A preliminary test should be performed on the substance at 50 ± 0.1 °C at each of pH 4.0, 7.0, and 9.0. If less than 10 percent of the reaction is observed after 5 days ($t_{1/2} > 1$ year), the chemical is considered hydrolytically stable and no additional testing is required. If the substance is known to be unstable at environmentally relevant temperatures, the preliminary test is not required. The analytical method must be sufficiently precise and sensitive to detect a reduction of 10 percent in the initial concentration.

(ii) **Hydrolysis of unstable substances.** If the substance is unstable as defined by the preliminary test, the test procedure is to be as follows: The buffered test solutions of the substance should be thermostatted at the selected temperatures. To test for first-order behavior each reaction

solution should be analyzed in time intervals which provide a minimum of six spaced data points, normally between 20 percent and 70 percent of hydrolysis of that test chemical. The reaction should be examined at three (4, 7, 9) pH's at each of the selected temperatures with replication at one of them (the middle temperature in the case of elevated temperature determinations).

(iii) **Hydrolysis at pH 1.2.** The above test for a hydrolytically unstable compound should also be carried out at pH 1.2, employing a single, physiologically significant temperature (37 °C).

(e) **Data and reporting—(1) Treatment of results—(i) Confirmation of first order kinetics.** The data obtained should be plotted at $\log_{10} C_t$ versus t and the reaction rate constant k_{obs} calculated by regression analysis or from the slope:

$$k_{\text{obs}} = 2.303 \times \text{slope}$$

(ii) **Interpretation of results.** If the data do not fall on a straight line, the reaction is not first order, and the data must be analyzed by methods beyond the scope of this test principle.

(2) **Test report.** (i) The test report should include information on:

(A) Sample purity.

(B) Any results appropriate to the procedure employing reference substances.

(C) Detailed test procedure including the temperature, pH and, buffer for each set of experiments.

(D) Detailed analytical method used for the tested substance, including detailed method of extraction and recovery data if an extraction method is used to separate the chemical from the aqueous phase.

(E) All concentration-time data points for reactions which were observed to originate a nonlinear log concentration-time plot.

(F) Possibility of acid or base catalysis.

(ii) A suggested format for sample reporting form, which can be duplicated, is presented:

DATA SHEET FOR HYDROLYSIS STUDIES

Laboratory: _____

Date: _____

Test Substance: _____

Formula: _____

Name (IUPAC): _____

Test protocol

A. Preliminary test

yes _____

no _____

buffer systems used:

pH 4.0 _____

pH 7.0 _____

pH 9.0 _____

Approximate saturation concentration mole/L	pH		
	4.0	7.0	9.0
C_0 : Initial concentration
C_t : Final concentration after t days; $t_{\max} = 5$
t
$(C_0 - C_t)/C_0 \times 100$ at 50 ± 0.1 °C

B. Determination

Separate runs at pH 4.0, 7.0, and 9.0 at the chosen temperature(s) *with replication at one of these* (the middle temperature in the case of determination at elevated temperature). The same format would be used for each pH.

Buffer solution used _____

Temperature _____

Approximate saturation concentration _____

t [] 0

C _t [mole/L]
log C _t

Hydrolysis at pH 1–2

Buffer solution used _____

pH _____

Temperature _____

Approximate saturation concentration _____

t [] 0

C _t [mole/L]
log C _t

Final data

pH	temperature	initial concentration, C ₀ [mole/L]	reaction rate constant, k _{obs} [1/s × 10 ⁵]	half-life, t _{1/2}	coefficient of correlation, r ²
	°C			[h]	
.....
.....
.....

C. Report on test method

Provide detailed description of the experimental conditions, e.g. for maintaining sterility; to avoid photolytic effects; to exclude oxygen; to prepare the test solution, etc. (Please use separate sheet of paper.)

Analytical combinations used

h	h
pH electrodes
UV-visible spectrophotometry
Conductivity
Gas chromatography
High pressure liquid chromatography
Extraction and formation of derivative(s)

Details of the analytical performance:

Type of apparatus

Test conditions

Was the accuracy of this result determined in any additional way ?

Particular incidents:

Comments:

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Kolthoff, I.M. and Laitinen, H.A. *pH and Electro-Titrations*, 2nd Ed., Wiley, pp 34–36 (1941).

(2) Mabey, W. and Mill, T., Critical Review of Hydrolysis of Organic Compounds in Water Under Environmental Conditions. *Journal of Physical Chemistry Reference Data* 7:383–415 (1978).

(3) Gomaa, H.M. et al. Kinetics of Hydrolysis of Diazoxon. *Residue Reviews* 29:171 (1969).

(4) OECD Document A80.30, Summary of OECD-EEC Laboratory Intercomparison Testing Programme, Part 2, Umweltbundesamt, Berlin, May 1980.