"Hydrolysis as a Function of pH"

1. INTRODUCTORY INFORMATION

- Prerequisites

– Water solubility
– Suitable analytical method

- Guidance information

– Vapour pressure curve

- 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.

- Standard documents

This Test Guideline is based on methods given in the references listed in Section 4 and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

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.

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 behaviour 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.

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.

- Definitions and units

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

$$RX + HOH \rightarrow ROH + HX \qquad (1)$$

The rate at which the concentration of RX decreases in this simplified process is given by

rate	$= k\,[H_20]\,[RX]$	second order reaction
or	$= k\,[RX]$	first order reaction

depending on the rate determining step. Because the 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

$$k_{obs} = k \,.\, [H_2O] \qquad (2)$$

and can be determined from the expression

$$K_{obs} = \frac{2.303}{t} \log_{10} \frac{C_o}{C_t}$$

where t = time

and C_o, 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 per cent of RX to react) is given by

$$T_{1/2} = 0.693/k_{obs} \qquad (3)$$

- Reference substances

– Aspirin
– Diazinon

These substances need not be employed in all cases when investigating a new substance. They 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 Annex 2.

- Principle of the test method

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.

The hydrolysis reaction may be influenced by acidic or basic species H_30^+ (H^+) and OH^-, in which case it is referred to as specific acid or specific base catalysis.

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 behaviour) gives the rate constant from the formula.

k_{obs} = - slope . 2.303 (if $\log_{10}$ is used).

When it is not practicable to directly determine a rate constant for a particular temperature, 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).

- Quality criteria

Repeatability

Mabey and Mill (2) report that measurements of hydrolysis rate constants on 13 classes of organic structures can be of high precision, often with less than 2 per cent standard deviation. The rate constants for one pH and one temperature should be determined in duplicate with a deviation of less than 2.5 per cent unless unusual circumstances (e.g. analytical difficulties) prevent achieving this and then the details of these circumstances should be reported. The repeatability can be improved by an improved control of the sensitive parameters, in particular pH and oxygen.

Sensitivity

Most hydrolysis reactions follow apparent first order reaction rates and, therefore, half-lives are independent of concentration (equation 3). This usually permits the application of laboratory results determined at 10^{-2} - 10^{-3} M to environmental conditions ($\leq 10^{-6}$ M) (2).

Specificity

Mabey and Mill (2) report 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 had been measured.

B. DESCRIPTION OF THE TEST PROCEDURE

The overall scheme is summarised in Figure 1, below.

- Preparations

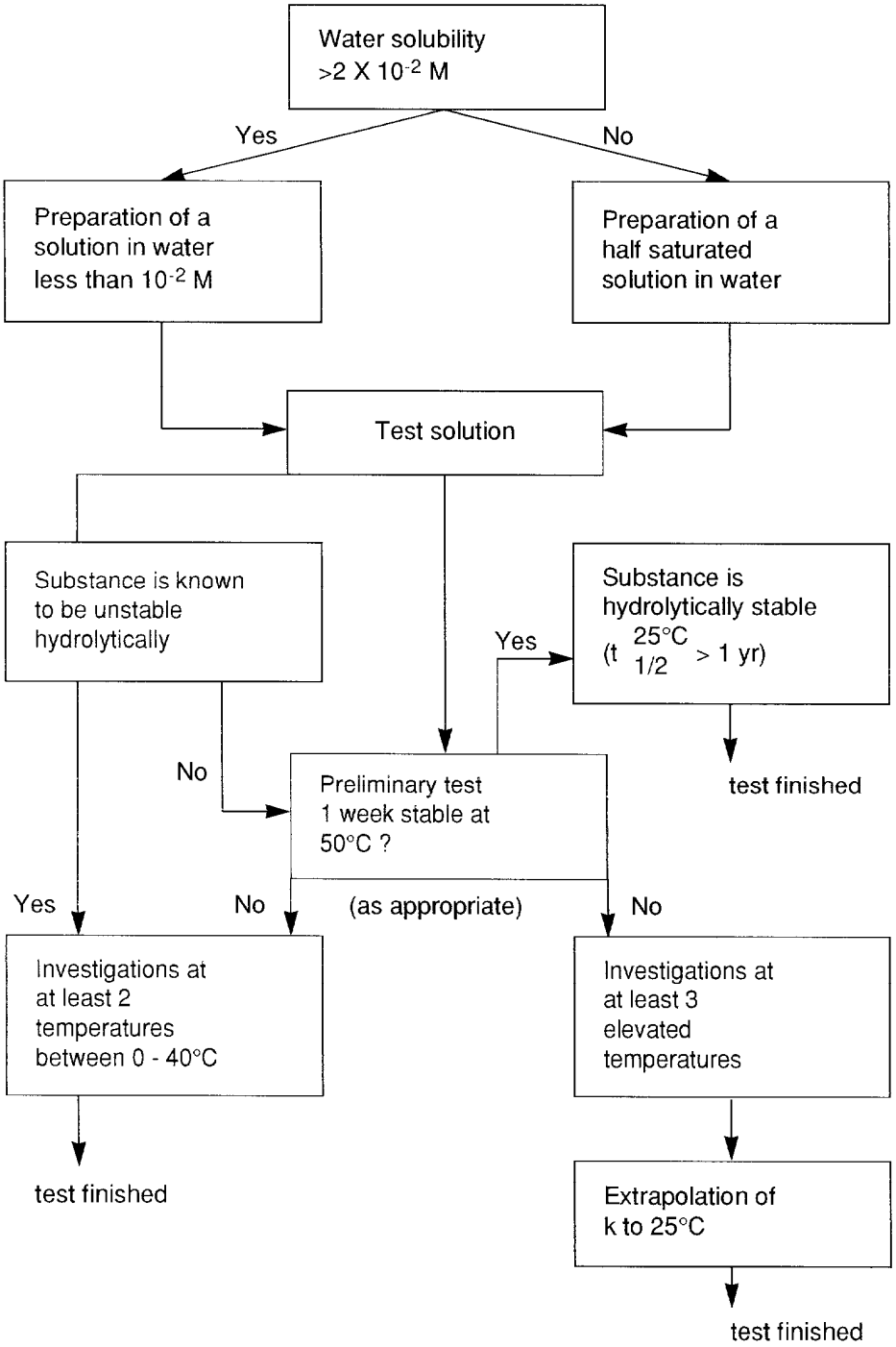
Materials

Buffer solutions

The hydrolysis test should be performed at four different values:

(1) at pH 1.2 (if physiologically important)
(2) at pH 4.0
(3) at pH 7.0
(4) at pH 9.0

Figure 1 HYDROLYSIS SCHEME



For this purpose, 0.05 M sterile buffer solutions should be prepared using reagent grade chemicals and distilled, sterile water. Some useful buffer systems are presented in Annex 1, 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. Mabey and Mill recommend the use of borate or acetate buffers instead of phosphate (2).

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.

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*, 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 of low water soluble substances the amount of solvent should be less than 1 per cent, and the solvent should not interfere with the hydrolysis process.

Glassware

All glassware, which must be inert in the pH range studied, should be sterilised. 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.

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

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* Test Guideline Water Solubility 105

– gas chromatography
– high pressure liquid chromatography
– extration and formation of derivative(s) and determination by a suitable analytical method.

• Test conditions

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 behaviour 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.

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 for 5 minutes before preparation of the solution).

• Performance of the test

(1) 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 per cent 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 per cent in the initial concentration.

(2) Hydrolysis of unstable substances. If the substance is unstable as defined by the preliminary test (above), the test procedure is to be as follows:

The buffered test solutions of the substance should be thermostated at the selected temperatures. To test for first-order behaviour, each reaction solution should be analysed in time intervals which provide a minimum of six spaced data points normally between 20 per cent and 70 per cent of hydrolysis of that test chemical. The reaction should be examined at three (4, 7, 9) pHs at each of the selected temperatures with replication at one of them (the middle temperature in the case of elevated temperature determinations).

(3) 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).

3. DATA AND REPORTING

- Treatment of results

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:

$$k_{obs} = 2.303 \cdot \text{slope} \qquad (5)$$

- Interpretation of results

If the data do not fall on a straight line, the reaction is not first order, and the data must be analysed by methods beyond the scope of this test principle.

- Test report

The test report should include information on

– sample purity

– any results appropriate to the procedure employing reference substances

– detailed test procedure including the temperature, pH and buffer for each set of experiments

– 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

– all concentration-time data points for reactions which were observed to originate a non-linear log concentration-time plot

– possibility of acid or base catalysis

A sample reporting form is found in Annex 3.

4. L I T E R A T U R E

1. I.M. Kolthoff and H.A. Laitinen, *pH and Electro-Titrations*, Second Ed, John Wiley & Sons, N.Y., pp. 34-36 (1941).

2. W. Mabey and T. Mill, "Critical Review of Hydrolysis of Organic Compounds in Water Under Environmental Conditions," J. Phys. Chem. Ref. Data *7* (2), 383-415 (1978).

3. H.M. Gomaa, I.H. Suffet, S.D. Faust, "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.

5. A N N E X

1. BUFFER MIXTURES (1)

A. CLARK AND LUBS

BUFFER MIXTURES OF CLARK AND LUBS*
0.2 N HC1 and 0.2 N KC1 at 20°

Composition	PH
47.5 ml. HC1 + 25 ml. KC1 dil. to 100 ml	1.0
32.25 ml. HC1 + 25 ml. KC1 dil. to 100 ml	1.2
20.75 ml. HC1 + 25 ml. KC1 dil. to 100 ml	1.4
13.15 ml. HC1 + 25 ml. KC1 dil. to 100 ml	1.6
8.3 ml. HC1 + 25 ml. KC1 dil. to 100 ml	1.8
5.3 ml. HC1 + 25 ml. KC1 dil. to 100 ml	2.0
3.35 ml. HC1 + 25 ml. KC1 dil. to 100 ml	2.2

0.1 M potassium biphthalate + 0.1 N HC1 at 20°

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 at 20°

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

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

"Hydrolysis as a Function of pH"

Composition	pH
0.1 M potassium biphthalate + 0.1 NaOH at 20°	
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 at 20°	
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 H_2B0_2 in 0.1 M KC1 + 0.1 N NaOH at 20°	
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

B. KOLTHOFF AND VLEESCHHOUWER

CITRATE BUFFERS OF KOLTHOFF AND VLEESCHHOUWER

0.1 M monopotassium citrate and 0.1 N HC1 at 18°
(Add tiny crystal of thymol or a few milligrams of mercury to prevent growth of molds)

Composition	pH
49.7 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	2.2
43.4 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	2.4
36.8 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	2.6
30.2 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	2.8
23.6 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	3.0
17.2 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	3.2
10.7 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	3.4
4.2 ml. 0.1 N HC1 + 50 ml. citrate to 100 ml	3.6

0.1 M monopotassium citrate and 0.1 N NaOH at 18°
(Add tiny crystal of thymol or a few milligrams of mercuric iodide to prevent growth of molds)

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

"Hydrolysis as a Function of pH"

C. SÖRENSEN

BORATE MIXTURES OF SÖRENSEN

0.05 M borax + 0.1 N HC1

Composition		Sörensen 18°	Walbum, pH at		
ml. Borax	ml. HCl		10°	40°	70°
5.25	4.75	7.62	7.64	7.55	7.47
5.50	4.50	7.94	7.98	7.86	7.76
5.75	4.25	8.14	8.17	8.06	7.95
6.00	4.00	8.29	8.32	8.19	8.08
6.50	3.50	8.51	8.54	8.40	8.28
7.00	3.00	8.08	8.72	8.56	8.40
7.50	2.50	8.80	8.84	8.67	8.50
8.00	2.00	8.91	8.96	8.77	8.59
8.50	1.50	9.01	9.06	8.86	8.67
9.00	1.00	9.09	9.14	8.94	8.74
9.50	0.50	9.17	9.22	9.01	8.80
10.0	0.00	9.24	9.30	9.08	8.86
0.05 M borax + 0.1 N NaOH					
10.0	0.0	9.24	9.30	9.08	8.86
9.0	1.0	9.36	9.42	9.18	8.94
8.0	2.0	9.50	9.57	9.30	9.02
7.0	3.0	9.68	9.76	9.44	9.12
6.0	4.0	9.97	10.06	9.67	9.28

2. RATE CONSTANTS AND HALF-LIVES OF SOME SELECTED CHEMICALS

A. REPORTED LITERATURE VALUES ON ASPIRIN AND DIAZINON

The following literature values have been reported:

Diazinon (0, 0-ondiethyl-0- (6-methyl-2-isopropyl-4-pyrimidinyl phosphrothioate) from H.M. Gomma, I.H. Suffet, S.D. Faust, Residue Reviews 29, 171 (1969):

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

Aspirin (2-acetylsalicylic acid) from L.J. Edwards, Trans. Faraday Soc. 723-735 (1950)

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

at 17°C

B. RESULTS OF OECD-EEC LABORATORY INTERCOMPARISON TESTING PROGRAMME, PART 2 (4)

– Aspirin
– Diazinon

In some cases the results presented here 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.

The Expert Group presents these results however, as they have been obtained using the Test Guidelines in the OECD/EEC Intercomparison Testing Programme, Part II. The first Guideline as presented here has been modified in the light of these results.

Summary Results of "Hydrolysis as a function of pH"

Reaction Rate Constant in 10^5. 1/s

Substance: Aspirin

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	1.013	1.278	126.2	0.109 to 1.916	2
3.0	20	0.080	0.056	70.3	0.040 to 0.119	2
	40	0.556	0.112	20.1	0.477 to 0.635	2
	60	-	-	-	-	-
7.0	20	0.205	0.033	15.9	0.182 to 0.228	2
	40	1.339	0.004	0.3	1.336 to 1.341	2
	60	-	-	-	-	-
9.0	20	0.309	0.160	51.7	0.196 to 0.442	2
	40	0.953	0.006	0.6	0.949 to 0.957	2
	60	-	-	-	-	-

"Hydrolysis as a Function of pH"

Summary Results of "Hydrolysis as a Function of pH"

Half Life Time in Hours ($t_{1/2}$)

Substance: Aspirin

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	93.71	118.3	126	10.05 to 177.36	2
3.0	20 40 60	319.7 35.4 -	222.5 7.1 -	70 20 -	162.3 to 477.0 30.3 to 40.4 -	2 2 (1 lab) -
7.0	20 40 60	95.1 14.4 -	15.1 0.0 -	20 - -	84.4 to 105.8 14.4 to 14.4 -	2 2 (1 lab) -
9.0	20 40 60	72.1 20.2 -	37.3 0.1 -	50 0.0 -	45.7 to 98.5 20.1 to 20.3 -	2 2 (1 lab) -

"Hydrolysis as a Function of pH"

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 10^5.1/s
Substance: Diazinon

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

Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: Diazinon

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	0.672	0.187	27.9	0.501 to 0.900	4
3.0	20	7.27	2.40	33	5.0 to 11.5	7
	40	2.25	0.57	25	1.4 to 3.3	11 (10 labs)
	50	9.00	11.53	128	2.24 to 22.31	3 (2 labs)
	60	0.57	0.17	29	0.37 to 0.75	6
7.0	20	1707.75	1875.18	110	5.3 to 3660.9	4
	40	215.16	166.12	77	21.5 to 460.7	8
	50	97.07	10.97	11	89.31 to 104.83	2
	60	38.63	20.98	54	2.0 to 63.5	8 (7 labs)
9.0	20	1150.75	1335.58	116	4.5 to 2840.5	4
	40	124.38	102.45	82	0.9 to 298.7	9
	50	66.40	7.74	12	60.92 to 71.87	2
	60	25.13	25.67	102	1.5 to 79.8	8 (7 labs)

C. FURTHER RESULTS OF OECD-EEC LABORATORY INTERCOMPARISON TESTING PROGRAMME (4)

Three other compounds were similarly tested in this programme and the results are presented below

- Atrazine
- Diethyl-hexylphthalate
- Ethylacetate

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 10^5. 1/s
Substance: Atrazine

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	0.546	0.416	76.3	0.076 to 0.948	4
3.0	20	0.014	0.008	56.8	0.005 to 0.020	3
	40	0.140	0.082	58.4	0.028 to 0.222	6
	60	0.808	0.397	49.1	0.282 to 1.283	5
7.0	20	-	-	-	-	2
	40	0.009	0.011	124.8	0.001 to 0.016	2
	60	-	-	-	-	-
9.0	20	0.013	0.016	130.1	0.001 to 0.024	-
	40	0.008	0.010	123.7	0.001 to 0.015	2
	60	-	-	-	-	-

Reaction Rate Constant in 10^5. 1/s
Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: Atrazine

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	54.76	44.36	81.0	20.3 to 115.35	4
3.0	20	1867.88	1446.58	77.0	980.7 to 3537.14	3
	40	240.50	239.61	99.6	111.71 to 696.79	6
	60	31.42	21.51	69.0	15.0 to 68.3	5
7.0	20	-	-	-	-	2
	40	9000.15	11033.77	123.0	1198.1 to 16802.2	2
	60	-	-	-	-	-
9.0	20	17921.05	24188.21	135.0	817.4 to 35024.7	2
	40	8005.35	9539.51	119.0	1259.9 to 14750.8	2
	60	-	-	-	-	-

"Hydrolysis as a Function of pH"

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 10^5. 1/s
Substance: DOP

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	-
3.0	20	0.048	0.051	107.8	0.009 to 0.106	3
	40	0.166	0.202	121.8	0.023 to 0.309	2
	60	0.084	0.022	26.3	0.068 to 0.099	2
7.0	20	0.073	0.064	88.8	0.027 to 0.118	2
	40	(1.627)	-	-	-	1
	60	(0.092)	-	-	-	1
9.0	20	0.040	0.047	116.7	0.007 to 0.073	2
	40	(0.126)	-	-	-	1
	60	-	-	-	-	-

Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: DOP

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	-
3.0	20	990.20	996.32	101	182.4 to 2103.5	3
	40	452.56	551.98	122	62.25 to 842.86	2
	60	239.33	63.75	27	194.25 to 284.41	2
7.0	20	440.30	391.35	89	163.57 to 717.02	2
	40	(11.83)	-	-	-	1
	60	(208.19)	-	-	-	1
9.0	20	1606.74	1898.79	118	265.51 to 2947.97	2
	40	(152.53)	-	-	-	1
	60	-	-	-	-	-

"Hydrolysis as a Function of pH"

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 105.1/s
Substance: Ethylacetate

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	1
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

Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: Ethylacetate

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	-
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

3. SUGGESTED FORM TO REPORT DATA

Laboratory:
Test substance:
Date:

Test Protocol

A. PRELIMINARY TEST

☐ yes ☐ no

buffer systems used:

pH 4.0 . . . . . .
pH 7.0 . . . . . .
pH 9.0 . . . . . .

pH approx. saturation concentration [mole/l]	4.0	7.0	9.0
C_o [mole/1] (start conc.)			
C_t [mole/1] (final conc. after t days; t_{max} = 5			
t			
$\frac{C_o - C_t}{C_o} \cdot 100\%$ at 50 ± 0.1°C			

B. DETERMINATION

- Test substance

Separate runs at pH 4.0, pH 7.0, and pH 9.0 at the chosen temperature(s) with replication at one of these (the middle temperature in the case of determination at elevated temperatures).

pH 4.0

buffer solution used: . . . . . .
temperature: . . . . . .
approximate saturation concentration: . . . . . .

t [ ]	0							
C_t [mole/1]								
log C_t								

pH 7.0

buffer solution used: . . . . . .
temperature: . . . . . .
approximate saturation concentration: . . . . . .

t [ ]	0							
C_t [mole/1]								
log C_t								

"Hydrolysis as a Function of pH"

pH 9.0

buffer solution used: . . . . . .
temperature: . . . . . .
approximate saturation concentration: . . . . . .

t [ ]	0							
C_t [mole/1]								
log C_t								

- Hydrolysis at pH 1-2

buffer solution used: . . . . . .
pH: . . . . . .
temperature: . . . . . .
approximate saturation concentration: . . . . . .

t [ ]	0							
C_t [mole/1]								
log C_t								

- Final data

pH	temperature in °C	start concentration C_o in mole/l	reaction rate constant k_{obs} in l/s x 10^5	half life $t_{1/2}$ in h	coefficient of correlation r^2

C. REPORT ON TEST METHOD

- Detailed description of the experimental conditions, e.g.

– for maintaining sterility
– to avoid photolytic effects
– to exclude oxygen
– to prepare the test solution

(Please use another sheet of paper.)

- Analytical combinations used

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: . . . . . .

- Do you ascertain the accuracy of this result in any additional way ?

Particular incidents:

Comments: