

$$m_{\text{aq}}^{\text{des}}(\text{eq}) = m_{\text{m}}^{\text{des}}(\text{eq}) \cdot \frac{V_0}{V_r} - m_{\text{aq}}^{\text{A}} \quad (\mu\text{g}) \quad (14)$$

where:

- $C_s^{\text{des}}(\text{eq})$  = content of the test substance remaining adsorbed on the soil at desorption equilibrium ( $\mu\text{g g}^{-1}$ );  
 $m_{\text{m}}^{\text{des}}(\text{eq})$  = mass of substance determined analytically in the aqueous phase at desorption equilibrium ( $\mu\text{g}$ );  
 $m_{\text{aq}}^{\text{A}}$  = mass of the test substance left over from the adsorption equilibrium due to incomplete volume replacement ( $\mu\text{g}$ );  
 $m_{\text{aq}}^{\text{ads}}(\text{eq})$  = mass of the substance in the solution at adsorption equilibrium ( $\mu\text{g}$ );

$$m_{\text{aq}}^{\text{A}} = m_{\text{aq}}^{\text{ads}}(\text{eq}) \cdot \left( \frac{V_0 - V_{\text{R}}}{V_0} \right) \quad (15)$$

- $V_r^{\text{F}}$  = volume of the solution taken from the tube for the measurement of the test substance, at desorption equilibrium ( $\text{cm}^3$ );  
 $V_{\text{R}}$  = volume of the supernatant removed from the tube after the attainment of adsorption equilibrium and replaced by the same volume of a 0.01 M  $\text{CaCl}_2$  solution ( $\text{cm}^3$ );

The Freundlich desorption equation is shown in (16):

$$C_s^{\text{des}}(\text{eq}) = K_{\text{F}}^{\text{des}} \cdot C_{\text{aq}}^{\text{des}}(\text{eq})^{1/n} \quad (\mu\text{g g}^{-1}) \quad (16)$$

or in the linear form:

$$\log C_s^{\text{des}}(\text{eq}) = \log K_{\text{F}}^{\text{des}} + 1/n \cdot \log C_{\text{aq}}^{\text{des}}(\text{eq}) \quad (17)$$

where:

- $K_{\text{F}}^{\text{des}}$  = Freundlich desorption coefficient;  
 $n$  = regression constant;  
 $C_{\text{aq}}^{\text{des}}(\text{eq})$  = mass concentration of the substance in the aqueous phase at desorption equilibrium ( $\mu\text{g cm}^{-3}$ ).

The equations (16) and (17) can be plotted and the value of  $K_{\text{F}}^{\text{des}}$  and  $1/n$  are calculated by regression analysis using the equation 17.

#### Remark:

If the Freundlich adsorption or desorption exponent  $1/n$  is equal to 1, the Freundlich adsorption or desorption binding constant ( $K_{\text{F}}^{\text{ads}}$  and  $K_{\text{F}}^{\text{des}}$ ) will be equal to the adsorption or desorption equilibrium constants ( $K_{\text{a}}$  and  $K_{\text{des}}$ ) respectively, and plots of  $C_s$  vs  $C_{\text{aq}}$  will be linear. If the exponents are not equal to 1, plots of  $C_s$  vs  $C_{\text{aq}}$  will be nonlinear and the adsorption and desorption constants will vary along the isotherms.

### TEST REPORT

95. The test report should include the following information:

- Complete identification of the soil samples used including:
  - geographical reference of the site (latitude, longitude);
  - date of sampling;
  - use pattern (e.g. agricultural soil, forest, etc.);
  - depth of sampling;

- sand/silt/clay content;
  - pH values (in 0.01 M CaCl<sub>2</sub>);
  - organic carbon content;
  - organic matter content;
  - nitrogen content;
  - C/N ratio;
  - Cation Exchange Capacity (mmol/kg);
  - all information relating to the collection and storage of soil samples;
  - where appropriate, all relevant information for the interpretation of the adsorption - desorption of the test substance;
  - reference of the methods used for the determination of each parameter.
- 
- information on the test substance as appropriate;
  - temperature of the experiments;
  - centrifugation conditions;
  - analytical procedure used to analyse the test substance;
  - justification for any use of solubilizing agent for the preparation of the stock solution of the test substance;
  - explanations of corrections made in the calculations, if relevant;
  - data according to the form sheet (Annex 7) and graphical presentations;
  - all information and observations helpful for the interpretation of the test results.

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## OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Adopted by the Council on 27<sup>th</sup> July 1995

### Partition Coefficient (n-octanol/water): Shake Flask Method

#### INTRODUCTION

1. This guideline is a revised version of the original Guideline 107 which was adopted in 1981. The only change is one of format. The substantive content of the original guideline was not changed.

#### INITIAL CONSIDERATIONS

2.  $P_{ow}$  values in the range  $\log P_{ow}$  between -2 and 4 (occasionally up to 5) can be experimentally determined by the method set out in this guideline (1)(2).  $P_{ow}$  values in the range  $\log P_{ow}$  between 0 and 6 can be estimated using high performance liquid chromatography as is set out in Guideline 117. Before deciding on what procedure to use, a preliminary estimate of the  $P_{ow}$  should be obtained from calculation (see the annex to Guideline 117), or where appropriate from the ratio of the solubilities of the test substance in the pure solvents (3).

3. Before determining  $P_{ow}$  it is useful to have preliminary information on the structural formula, dissociation constant, water solubility, hydrolysis, n-octanol solubility and surface tension of the substance.

4. The shake flask method is impossible to use with surface-active materials (for these, a  $P_{ow}$  can be calculated from individual solubilities in water and n-octanol).

#### DEFINITIONS AND UNITS

5. The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$P_{ow} = \frac{c_{n-octanol}}{c_{water}}$$

The partition coefficient, being the quotient of two concentrations, or the quotient of the fractions of the test substance in the two phases multiplied by a fixed volume ratio, is dimensionless and is usually given in the form of its logarithm to base ten.

### REFERENCE SUBSTANCES

6. Reference substances do not need to be employed. They serve primarily to check the performance of the method from time to time and to allow comparison with results from other methods.

### PRINCIPLE OF THE METHOD

7. The Nernst partition law applies at constant temperature, pressure and pH for dilute solutions. It strictly applies to a pure substance dispersed between two pure solvents and when the concentration of the solute in either phase is not more than 0.01 mol per litre. If several different solutes occur in one or both phases at the same time, this may affect the results. Dissociation or association of the dissolved molecules result in deviations from the partition law. Such deviations are indicated by the fact that the partition coefficient becomes dependent upon the concentration. Measurements should be made on ionizable substances only in their non-ionized form (free acid or free base) produced by the use of an appropriate buffer with a pH of at least one unit below (free acid) or above (free base) the pK.

### DESCRIPTION OF THE METHOD

#### Test substance and solvents

8. n-Octanol of analytical grade and distilled or double-distilled water should be used. Water taken directly from an ion exchanger should not be used. A stock solution of known concentration of the test substance in n-octanol, pre-saturated with water, is prepared. This solution should be stored under conditions which ensure its stability.

9. Before a partition coefficient is determined, the two solvents are mutually saturated at the temperature of the experiment. To do this, it is practical to shake two large stock bottles, one containing n-octanol and a sufficient quantity of water, and the other containing water and a sufficient quantity of n-octanol, for 24 hours on a mechanical shaker, and then to let them stand long enough to allow the phases to separate.

#### Test conditions

10. The test should be done at a temperature in the range 20 to 25°C, kept constant at  $\pm 1^\circ\text{C}$ .

11. For a first run, a volume ratio of n-octanol to water and a quantity of substance are chosen with regard given to:

- the preliminary estimation of the partition coefficient,
- the minimum concentration of test substance in each phase required for the analytical procedure,
- a maximum concentration of the test substance in each phase of 0,01 mol per litre.

For a second run, the originally chosen volume ratio is divided by two, and, for a third run, it is multiplied by two. In the second and third run the quantity of substance added may have to be different from that used in the first run in order to fulfill the above criteria.

12. Duplicate vessels containing accurately measured amounts of the two solvents and stock solution are used in all three runs.



**Establishment of the partition equilibrium**

13. The two-phase system should nearly fill the entire volume of the test vessels. This will help prevent loss of material due to volatilisation.

14. The test vessels are placed in a mechanical shaker or are shaken by hand. When using a centrifuge tube as the test vessel, it is recommended to rotate the tube through 180° about its transverse axis (approximately a hundred times during five minutes), allowing the trapped air to rise through the two phases.

**Phase separation**

15. The separation of the two phases, in general, is achieved by centrifugation. This should preferably be done at the test temperature. If a centrifuge without temperature control is used, the centrifuge tubes should be kept for equilibration at the test temperature for at least one hour before analysis.

**Analysis**

16. It is necessary to determine the concentrations of the test substance in both phases. For the measurement, substance-specific methods are preferred. Analytical methods which may be appropriate are: photometry, gas chromatography and high performance liquid chromatography. The total quantity of substance present in both phases should be calculated and compared with the quantity originally introduced.

17. The aqueous phase should be sampled by a procedure that minimizes the risk of including traces of n-octanol. This can be done using a syringe with a removable needle. The syringe should initially be partially filled with air. Air should be gently expelled while passing the n-octanol layer. The adequate volume of the aqueous solution is withdrawn. The syringe is quickly withdrawn and the needle is detached.

**DATA AND REPORTING****Data**

18. A  $P_{OW}$  value is calculated from the data of each run. Altogether six values are obtained since the test conditions are: three duplicate runs with different solvent ratios, and possibly also different quantities of test substance. The six  $\log P_{OW}$  values should fall within a range of  $\pm 0.3$  units.

**Test Report**

19. The test report must include the following information:

- chemical identity and impurities;
- the results of the preliminary estimation (when the shake flask method is not applicable, e.g. surface active material, a calculated value or an estimate based on the individual n-octanol and water solubilities should be provided);
- all information relevant for the interpretation of the results, especially with regard to impurities and physical state of the substance;
- the test conditions: temperature, the amount of test substance introduced in the test vessels, the volume of each phase in each vessel and the calculated total amount of test substance based on the analytical data;
- pH of the water used and of the aqueous phase during the experiment;

- justification for the use of buffers; composition, concentration and pH of the buffers; pH of the aqueous phase before and after the experiment;
- duration and speed of centrifugation, if used;
- the analytical procedures;
- the concentrations measured in each run (a total of 12 concentrations);
- $P_{ow}$  values and their mean for each set of test conditions and the overall mean (if there is the suggestion of concentration dependence of the partition coefficient, this should be noted);
- the standard deviation of individual  $P_{ow}$  values about their mean;
- the overall mean expressed as its logarithm to base 10;
- the theoretical  $P_{ow}$  when it has been calculated or when the measured value is above  $10^4$ .

#### LITERATURE

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OECD GUIDELINE FOR TESTING OF CHEMICALS

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Adopted:  
12 May 1981

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**"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.

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*Users of this Test Guideline should consult the Preface,  
in particular paragraphs 3, 4, 7 and 8.*

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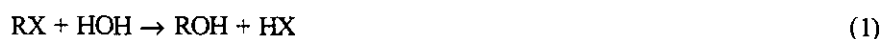
## "Hydrolysis as a Function of pH"

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:



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

$$\begin{array}{ll} \text{rate} & = k [\text{H}_2\text{O}] [\text{RX}] & \text{second order reaction} \\ \text{or} & = k [\text{RX}] & \text{first order reaction} \end{array}$$

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

$$k_{\text{obs}} = k \cdot [\text{H}_2\text{O}] \quad (2)$$

and can be determined from the expression

$$k_{\text{obs}} = \frac{2.303}{t} \log_{10} \frac{C_0}{C_t}$$

where  $t$  = time  
and  $C_0, C_t$  = 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

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

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## "Hydrolysis as a Function of pH"

- 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  $\text{H}_3\text{O}^+$  ( $\text{H}^+$ ) and  $\text{OH}^-$ , 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.

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

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*

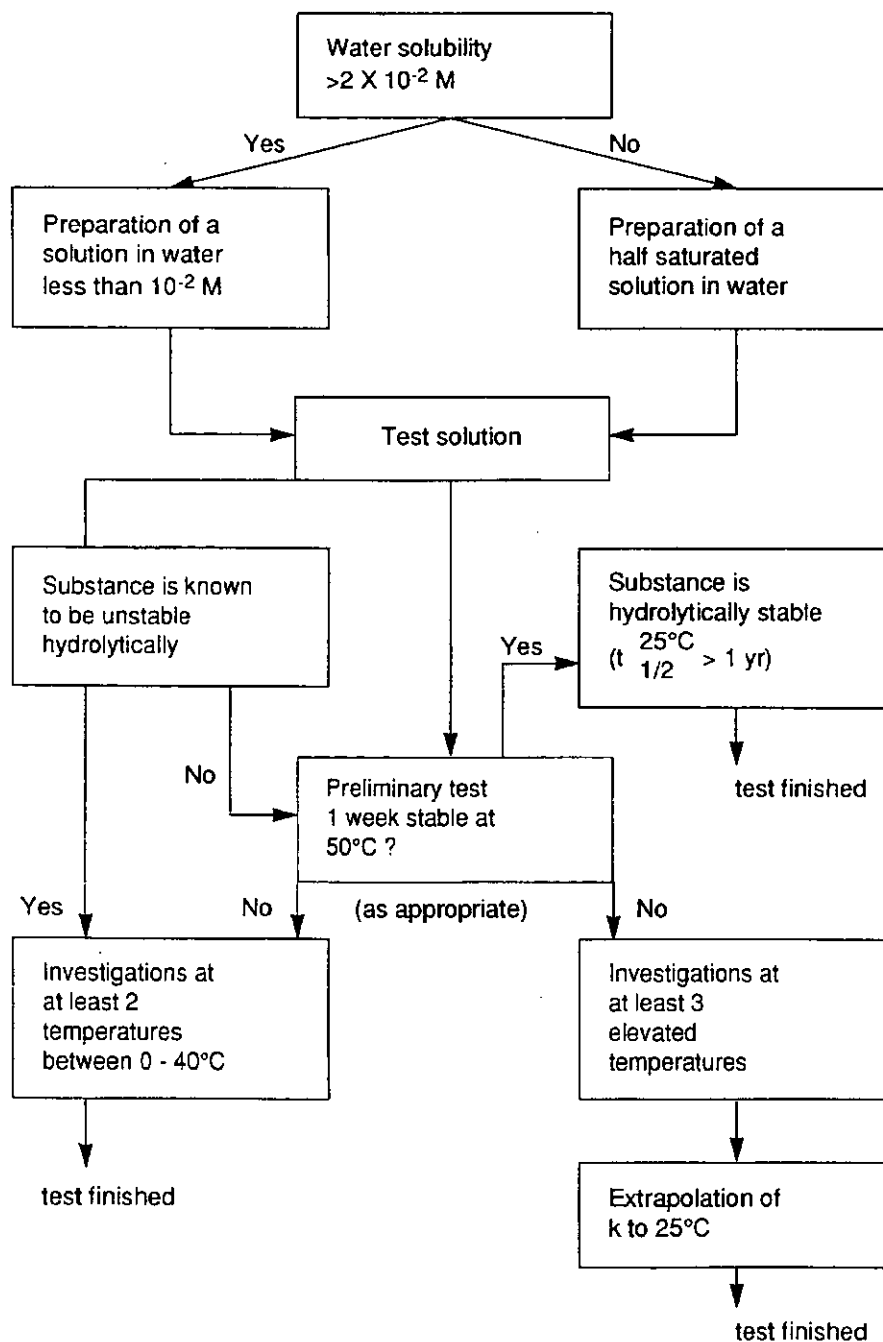
- Buffer solutions*

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

"Hydrolysis as a Function of pH"

Figure 1 HYDROLYSIS SCHEME



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## "Hydrolysis as a Function of pH"

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. Stopped 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



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## "Hydrolysis as a Function of pH"

- gas chromatography
- high pressure liquid chromatography
- extraction 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  $\pm 0.1^\circ\text{C}$ .

An appropriate constant temperature bath should be employed. If the hydrolytic behaviour of the substance is unknown, a preliminary test at  $50^\circ\text{C}$  is required. For tests beyond this preliminary stage data for temperatures in the range  $0 - 40^\circ\text{C}$  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  $10^\circ\text{C}$ .

### *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  $50 \pm 0.1^\circ\text{C}$  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:

## "Hydrolysis as a Function of pH"

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:

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

#### • 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

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**"Hydrolysis as a Function of pH"**

- 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. LITERATURE**

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 HCl and 0.2 N KCl at 20°**

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

**0.1 M potassium biphthalate + 0.1 N HCl at 20°**

46.70 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	2.2
39.60 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	2.4
32.95 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	2.6
26.42 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	2.8
20.32 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	3.0
14.70 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	3.2
9.90 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	3.4
5.97 ml. 0.1 N HCl + 50 ml. biphthalate to 100 ml . . . . .	3.6
2.63 ml. 0.1 N HCl + 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. biphthalate 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 Sørensen's standard equations (1909). The corresponding pH values are 0.04 unit higher than the tabulated values.