OECD/OCDE 111

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# OECD GUIDELINES FOR THE TESTING OF CHEMICALS

## Hydrolysis as a Function of pH

### INTRODUCTION

1. Chemicals can enter surface waters by such routes as direct application, spray drift, run-off, drainage, waste disposal, industrial, domestic or agricultural effluent and atmospheric deposition and may be transformed in those waters by chemical (e.g. hydrolysis, oxidation), photochemical and/or microbial processes. This Guideline describes a laboratory test method to assess abiotic hydrolytic transformations of chemicals in aquatic systems at pH values normally found in the environment (pH 4 – 9) and is based on existing Guidelines (1)(2)(3)(4)(5)(6)(7).

2. The experiments are performed to determine (i) the rate of hydrolysis of the test substance as a function of pH and (ii) the identity or nature and rates of formation and decline of hydrolysis products to which organisms may be exposed. Such studies may be required for chemicals which are directly applied to water or that are likely to reach the environment by the other routes described above.

### PRINCIPLE OF THE TEST

3. Sterile aqueous buffer solutions of different pH values (pH 4, 7 and 9) are treated with the test substance and incubated in the dark under controlled laboratory conditions (at constant temperatures). After appropriate time intervals, buffer solutions are analysed for the test substance and for hydrolysis products. With labelled test substance (e.g. $^{14}C$), a mass balance can be more easily established.

4. This Guideline is designed as a tiered approach which is shown and explained in Annex 1. Each tier is triggered by the results of the previous tier.

### APPLICABILITY OF THE TEST

5. The method is generally applicable to chemical substances (unlabelled or labelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile and non-volatile compounds of sufficient solubility in water. The test should not be applied to chemicals that are highly volatile from water (e.g. fumigants, organic solvents) and thus cannot be kept in solution under the experimental conditions of this test. The test may be difficult to conduct with substances of minimal solubility in water (8).

### INFORMATION ON THE TEST SUBSTANCE

6. Non-labelled or labelled test substance can be used to measure the rate of hydrolysis. Labelled material is generally preferred for studying the pathway of hydrolysis and for establishing mass balance; however, in special cases, labelling may not be absolutely necessary. $^{14}C$-labelling is recommended but the use of other isotopes, such as $^{13}C$, $^{15}N$, $^{3}H$, may also be useful. As far as possible, the label should be positioned in the most stable part(s) of the molecule. For example, if the test substance contains one ring,

labelling on this ring is required; if the test substance contains two or more rings, separate studies may be needed to evaluate the fate of each labelled ring and to obtain suitable information on formation of hydrolysis products. The purity of the test substance should be at least 95%.

7. Before carrying out a hydrolysis test, the following information on the test substance should be available:

(a) solubility in water [OECD Guideline 105] (9);
(b) solubility in organic solvents;
(c) vapour pressure [OECD Guideline 104] (9) and/or Henry's Law constant;
(d) n-octanol/water partition coefficient [OECD Guidelines 107 and 117] (9);
(e) dissociation constant ($pK_a$) [OECD Guideline 112] (9);
(f) direct and indirect phototransformation rate in water where appropriate.

8. Analytical methods for quantification of the test substance and, if it is relevant, for identification and quantification of hydrolysis products in aqueous solutions should be available (see also paragraphs 12 and 13).

## REFERENCE SUBSTANCES

9. Where possible, reference substances should be used for the identification and quantification of hydrolysis products by spectroscopic and chromatographic methods or other suitably sensitive methods.

## DEFINITIONS AND UNITS

10. See Annex 2.

## QUALITY CRITERIA

### Recovery

11. Analysis of, at least, duplicate buffer solutions or of their extracts immediately after the addition of the test substance gives a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances (when labelled material is used). Recoveries should range from 90 % to 110 % for labelled and nonlabelled chemicals (7). In case it is technically difficult to reach this range, a recovery of 70 % for nonlabelled chemicals is acceptable, but justification should be given.

### Repeatability and sensitivity of analytical method

12. Repeatability of the analytical method(s) used to quantify the test substance and hydrolysis products at later times can be checked by duplicate analysis of the same buffer solutions (or of their extracts) after sufficient quantities of hydrolysis products have formed for quantification.

13. The analytical method should be sufficiently sensitive to quantify test substance concentrations down to 10 % or less of the initial concentration. If relevant, analytical methods should also be sufficiently

sensitive to quantify any hydrolysis product representing 10 % or more of applied (at any time during the study) down to 25 % or less of its peak concentration.

**Confidence intervals for hydrolysis kinetic data**

14. Confidence intervals should be computed and presented for all regression coefficients, rate constants, half-lives, and any other kinetic parameters (e.g. $DT_{50}$).

## DESCRIPTION OF THE TEST METHOD

**Equipment and apparatus**

15. The study should be performed in glass containers (e.g. test tubes, small flasks) under dark and sterile conditions, if necessary, unless preliminary information (such as the n-octanol-water partition coefficient) indicates that the test substance may adhere to glass. In such cases, alternative materials (such as Teflon) may have to be considered. It may also be possible to alleviate the problem of adherence to glass by using one or more of the following methods:

- determine the mass of test substance and hydrolysis products sorbed to the test vessel;
- use of an ultrasonic bath;
- ensure a solvent wash of all glassware at each sampling interval;
- use of formulated products;
- use an increased amount of co-solvent for addition of test substance to the system; if a co-solvent is used it should be a co-solvent that does not hydrolyse the test substance.

16. Temperature-controlled water bath shakers or thermostatically-controlled incubators for incubation of the various test solutions are normally required.

17. Standard laboratory equipment is required, including, in particular, the following:

- pH meter;
- analytical instruments such as GC, HPLC, TLC equipment, including the appropriate detection systems for analysing radiolabelled and non-labelled substances or inverse isotopes dilution method;
- instruments for identification purposes (e.g. MS, GC-MS, HPLC-MS, NMR, etc.);
- liquid scintillation counter;
- separating funnels for liquid-liquid extraction;
- instrumentation for concentrating solutions and extracts (e.g. rotating evaporator);
- temperature control devise (e.g. water bath).

18. Chemical reagents include, for example:

- organic solvents, analytical grade, such as hexane, dichloromethane, etc.;
- scintillation liquid;
- buffer solutions (for details see paragraph 23).

19. All glassware, reagent-grade water and buffer solutions used in the hydrolysis tests should be sterilised.

**Test substance application**

20. The test substance should be applied as an aqueous solution into the different buffer solutions (see Annex 3). If it is necessary for adequate dissolution, the use of low amounts of water miscible solvents (such as acetonitrile, acetone, ethanol) is permitted for application and distribution of the test substance but this should not normally exceed 1 % v/v. In case a higher concentration of solvents is considered (e.g. in the case of poorly soluble test substances), this could only be allowed when it can be shown that the solvent has no effect on the hydrolysis of the test substances.

21. The use of formulated product is not routinely recommended, as it cannot be excluded that the formulation ingredients may influence the hydrolysis process. However, for poorly water-soluble test substances or for substances that adhere to glass (see paragraph 15), the use of formulated material may be an appropriate alternative.

22. One concentration of the test substance should be used; it should not exceed 0.01 M or half of the saturation concentration (see Annex 1).

**Buffer solutions**

23. The hydrolysis test should be performed at pH values of 4, 7 and 9. For this purpose, buffer solutions should be prepared using reagent grade chemicals and water. Some useful buffer systems are presented in Annex 3. 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[1].

24. The pH of each buffer solution should be checked with a calibrated pH meter to a precision of at least 0.1 at the required temperature.

**Test conditions**

**Test temperature**

25. The hydrolysis experiments should be carried out at constant temperatures. For extrapolation purposes, it is important to maintain the temperature to at least ± 0.5 °C.

26. A preliminary test (Tier 1) should be conducted at a temperature of 50°C if the hydrolytic behaviour of the test substance is unknown. Higher Tier kinetic tests should be carried out with a minimum of three temperatures (including the test at 50 ºC) unless the test substance is stable to hydrolysis as determined by the Tier 1 testing. A suggested temperature range is 10-70°C (preferably with at least one temperature below 25°C utilised), which will encompass the reporting temperature of 25°C and most of the temperatures encountered in the field.

**Light and oxygen**

27. All of the hydrolysis tests should be carried out using any suitable method to avoid photolytic effects. All suitable measures should be taken to avoid oxygen (e.g. by bubbling helium, nitrogen or argon for 5 minutes before preparation of the solution).

[1] Mabey and Mill recommend the use of borate or acetate buffers instead of phosphate (11).

**Test duration**

28. The preliminary test should be carried out for 5 days whereas the higher Tier tests should be conducted until 90 % hydrolysis of the test substance is observed or for 30 days whichever comes first.

**Performance of the test**

**Preliminary test (Tier 1)**

29. The preliminary test is performed at 50 ± 0.5°C and pH 4.0, 7.0 and 9.0. If less than 10 per cent of hydrolysis is observed after 5 days ($t0.5_{25°C}$ > 1 year), the test substance is considered hydrolytically stable and, normally, no additional testing is required. If the substance is known to be unstable at environmentally relevant temperatures[2], 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.

**Hydrolysis of unstable substances (Tier 2)**

30. The higher Tier (advanced) test should be performed at the pH values at which the test substance was found unstable as defined by the preliminary test above. The buffered solutions of the test 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 10 % and 90 % hydrolysis of the test substance. Individual replicate test samples (a minimum of duplicate samples contained in separate reaction vessels) should be removed and the contents analysed at each of at least six sampling times (for a minimum of twelve replicate data points). The use of a single bulk sample from which individual aliquots of the test solution are removed at each sampling interval is considered to be inadequate, as it does not allow for the analysis of data variability and it may lead to problems with contamination of the test solution. Sterility confirmation tests should be conducted at the end of the higher Tier test (i.e. at 90% hydrolysis or 30 days). However, if no degradation (i.e. transformation) is observed, sterility tests are not considered necessary.

**Identification of hydrolysis products (Tier 3)**

31. Any major hydrolysis products at least those representing ≥ 10% of the applied dose should be identified by appropriate analytical methods.

**Optional tests**

32. Additional tests at pH values other than 4, 7 and 9 may be required for a hydrolytically unstable test substance. For example, for physiological purposes a test under more acidic conditions (e.g. pH 1.2) may be required employing a single physiologically relevant temperature (37 °C).

[2] Such information may come from other sources such as hydrolysis data of structurally similar compounds from the literature or from other preliminary, semi-quantitative hydrolysis tests with the test substance at an earlier development stage.

## DATA AND REPORTING

### Treatment of results

33. The amounts of test substance and of hydrolysis products, if relevant, should be given as % of applied initial concentration and, where appropriate, as mg/L for each sampling interval and for each pH and test temperature. In addition, a mass balance should be given in percentage of the applied initial concentration when labelled test substance has been used.

34. A graphical presentation of the log-transformed data of the test substance concentrations against time should be reported. Any major hydrolysis products at least those representing $\geq$ 10% of the applied dose should be identified and their log-transformed concentrations should also be plotted in the same manner as the parent substance to show their rates of formation and decline.

35. More accurate determinations of half-lives or $DT_{50}$ values should be obtained by applying appropriate kinetic model calculations. The half-life and/or $DT_{50}$ values (including confidence limits) should be reported for each pH and temperature together with a description of the model used the order of kinetics and the coefficient of determination ($r^2$). If appropriate, the calculations should also be applied to the hydrolysis products.

36. In the case of rate studies carried out at different temperatures, the pseudo first-order hydrolysis rate constants ($k_{obs}$) should be described as a function of temperature. The calculation should be based on both the separation of $k_{obs}$ into rate constants for acid catalysed, neutral, and base catalysed hydrolysis ($k_H$, $k_{neutral}$, and $k_{OH}$ respectively) and the Arrhenius equation:

$$k_{obs} = k_H[H^+] + k_{neutral} + k_{OH}[OH^-] = \sum_{i=H,neutral,OH} A_i e^{-B_i/T}$$

where $A_i$ and $B_i$ are regression constants from the intercept and slope, respectively, of the best fit lines generated from linearly regressing ln $k_i$ against the reciprocal of the absolute temperature in Kelvin (T). Through the use of the Arrhenius relationships for acid, neutral and base catalysed hydrolysis, pseudo first-order rate constants, and thus half-lives can be calculated for other temperatures for which the direct experimental determination of a rate constant is not practicable (10).

### Test report

37. The report must include the following information:

Test substance:

- common name, chemical name, CAS number, structural formula (indicating position of label when radiolabelled material is used) and relevant physical-chemical properties (see paragraph 7);
- purity (impurities) of test substance;
- label purity of labelled chemical and molar activity (where appropriate).

Buffer solutions:

- dates and details of preparation;
- buffers and waters used;

- molarity and pH of buffer solutions.

Test conditions:

- dates of the performance of the studies;
- amount of test substance applied;
- method and solvents (type and amount) used for application of the test substance;
- volume of buffered test substance solutions incubated;
- description of the incubation system used;
- pH and temperature during the study;
- sampling times;
- method(s) of extraction;
- methods for quantification and identification of the test substance and its hydrolysis products in the buffer solutions;
- number of replicates.

Results:

- repeatability and sensitivity of the analytical methods used;
- recoveries (% values for a valid study are given in paragraph 11);
- replicate data and means in a tabular forms;
- mass balance during and at the end of the studies (when labelled test substance is used);
- results of preliminary test;
- discussion and interpretation of results;
- all original data and figures.

The following information is only required when hydrolysis rate is determined:

- plots of concentrations versus time for the test substances and, where appropriate, for the hydrolysis products at each pH value and temperature;
- tables of results of Arrhenius equation for the temperature 20 °C/25 °C, with pH, rate constant [$h^{-1}$ or $day^{-1}$], half-life or $DT_{50}$, temperatures [°C] including confidence limits and the coefficients of correlation ($r^2$) or comparable information;
- proposed pathway of hydrolysis.

**Interpretation and evaluation of results**

38. Most hydrolysis reactions follow apparent first order reaction rates and, therefore, half-lives are independent of the concentration (see equation 4 in Annex 2). This usually permits the application of laboratory results determined at $10^{-2}$ to $10^{-3}$ M to environmental conditions ($\leq 10^{-6}$ M) (10). Several examples of good agreement between rates of hydrolysis measured in both pure and natural waters for a variety of chemicals were reported by Mabey and Mill (11), provided both pH and temperature had been measured.

**LITERATURE**

(1) OECD (1981). Hydrolysis as a Function of pH. OECD Guideline for Testing of Chemicals Nr. 111, adopted 12 May 1981.

(2) US-Environmental Protection Agency (1982). 40 CFR 796.3500, Hydrolysis as a Function of pH at 25 °C. Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate.

(3) Agriculture Canada (1987). Environmental Chemistry and Fate Guidelines for registration of pesticides in Canada.

(4) European Union (EU) (1995). Commission Directive 95/36/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Annex V: Fate and Behaviour in the Environment.

(5) Dutch Commission for Registration of Pesticides (1991). Application for registration of a pesticide. Section G: Behaviour of the product and its metabolites in soil, water and air.

(6) BBA (1980). Merkblatt Nr. 55, Teil I und II: Prüfung des Verhaltens von Pflanzenbehandlungsmitteln im Wasser (October 1980).

(7) SETAC (1995). Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides. Mark R. Lynch, Ed.

(8) OECD (2000). Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD Environmental Health and Safety Publications Series on Testing and Assessment Nr.23.

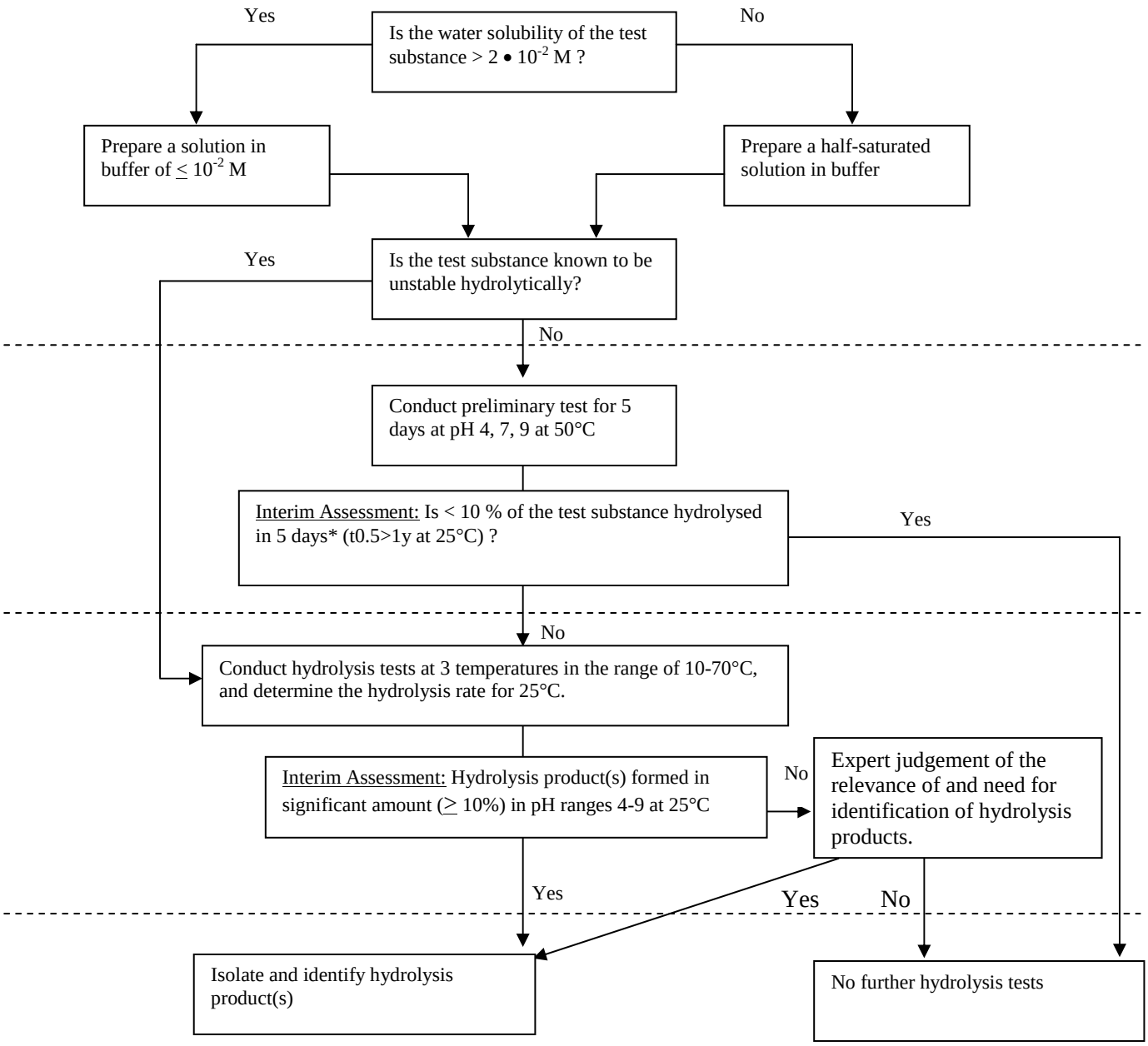
(9) OECD (1993). Guidelines for the Testing of Chemicals. Paris. OECD (1994 – 2000): Addenda 6-11 to Guidelines for the Testing of Chemicals.

(10) Nelson, H, Laskowski D, Thermes S, and Hendley P. (1997) Recommended changes in pesticide fate study guidelines for improving input to computer models. (Text version of oral presentation at the 14th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Dallas TX, November 1993).

(11) Mabey, W. and Mill, T. (1978). Critical review of hydrolysis of organic compounds in water under environmental conditions. J. Phys. Chem. Ref. Data 7, 383-415.

## ANNEX 1

## TIERED HYDROLYSIS TEST SCHEME




* 10 % hydrolysis of a test substance at 50 °C corresponds to a half-life of approx. 30 days which corresponds to a value of approx. 1 year at 25°C.

## ANNEX 2

## DEFINITION AND UNITS

Standard International (SI) units should be used in any case.

Test substance: any substance, whether the parent compound or relevant transformation products.

Transformation products: all substances resulting from biotic or abiotic transformation reactions of the test substance.

Hydrolysis products: all substances resulting from hydrolytic transformation reactions of the test substance.

Hydrolysis refers to a reaction of a test substance 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

$$\text{rate} = k\,[H_2O]\,[RX] \qquad \text{second order reaction}$$

or

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

depending on the rate determining step. Because the water is present in great excess compared to the test substance, 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*

$$k_{obs} = \frac{1}{t} \ln \frac{C_o}{C_t} \qquad [3]$$

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

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

$$t_{0.5} = \frac{\ln 2}{k_{obs}} \qquad [4]$$

* If the plot of the log-transformed data *vs.* time does not indicate a linear function (equated with a first-order reaction rate), then the use of equation [3] is not appropriate for determining the hydrolysis rate constant of the test compound.

Half-life ($t_{0.5}$) is the time taken for 50% hydrolysis of a test substance when the reaction can be described by first order kinetics: it is independent of the concentration.

$DT_{50}$ (Disappearance Time 50) is the time within which the concentration of the test substance is reduced by 50%; it is different from the half-life $t_{0.5}$ when the reaction does not follow first order kinetics.

Estimation of k at different temperature

When the rate constants are known for two temperatures, the rate constants at other temperatures can be calculated using the Arrhenius equation:

$$k = A \times e^{-\frac{E}{R \times T}} \quad \text{or} \quad \ln k = \frac{-E}{R \times T} + \ln A$$

A plot of ln k versus 1/T gives a straight line with a slope of –E/R

where:

k = rate constant, measured at different temperatures
E = activation energy [kJ/mol]
T = absolute temperature [K]
R = gas constant [8.314 J/mol*K]
The activation energy was calculated by regression analysis or the following equation:

$$E = R \times \frac{\ln k_2 - \ln k_1}{\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where: $T_2 > T_1$.

## ANNEX 3

## BUFFER SYSTEMS

**A. CLARK AND LUBS:** Buffer mixtures of CLARK and LUBS*

| Composition | pH |
|---|---|
| **0.2 N HC1 AND 0.2 N KC1 AT 20 °C** | |
| 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 °C** | |
| 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 °C** | |
| 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 |
| 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 |

* 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 units higher than the tabulated values.

**A. CLARK AND LUBS:** Buffer mixtures of CLARK and LUBS (Continued)

| **0.1 M monopotassium phosphate + 0.1 N NaOH at 20 °C** | |
|---|---|
| 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 $H_3B0_3$ in 0.1 M KC1 + 0.1 N NaOH at 20 °C** | |
| 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

| Composition | pH |
|---|---|
| **0.1 M monopotassium citrate and 0.1 N HC1 at 18 °C** * | |
| 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 |
| | |
| **0.1M monopotassium citrate and 0.1 N NaOH at 18 °C** * | |
| 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 |

* Add tiny crystal of thymol or a similar substance to prevent growth of molds.

**C. <u>SÖRENSEN:</u>** Borate mixtures of SÖRENSEN

| Composition | | Sörensen | Walbum, pH at | | |
|---|---|---|---|---|---|
| ml. Borax | ml. HC1/NaOH | 18 °C | 10 °C | 40 °C | 70 °C |
| **0.05 M borax + 0.1 N HC1** | | | | | |
| 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 |
| 10.00 | 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 |

**Phosphate mixtures of SÖRENSEN**

| Composition | pH |
|---|---|
| **0.0667 M Monopotassium phosphate + 0.0667 M Disodium phosphate at 20 °C** | |
| 99.2 ml. $KH_2PO_4$ + 0.8 ml $Na_2HPO_4$ | 5.0 |
| 98.4 ml. $KH_2PO_4$ + 1.6 ml $Na_2HPO_4$ | 5.2 |
| 97.3 ml. $KH_2PO_4$ + 2.7 ml $Na_2HPO_4$ | 5.4 |
| 95.5 ml. $KH_2PO_4$ + 4.5 ml $Na_2HPO_4$ | 5.6 |
| 92.8 ml. $KH_2PO_4$ + 7.2 ml $Na_2HPO_4$ | 5.8 |
| 88.9 ml. $KH_2PO_4$ + 11.1 ml $Na_2HPO_4$ | 6.0 |
| 83.0 ml. $KH_2PO_4$ + 17.0 ml $Na_2HPO_4$ | 6.2 |
| 75.4 ml. $KH_2PO_4$ + 24.6 ml $Na_2HPO_4$ | 6.4 |
| 65.3 ml. $KH_2PO_4$ + 34.7 ml $Na_2HPO_4$ | 6.6 |
| 53.4 ml. $KH_2PO_4$ + 46.6 ml $Na_2HPO_4$ | 6.8 |
| 41.3 ml. $KH_2PO_4$ + 58.7 ml $Na_2HPO_4$ | 7.0 |
| 29.6 ml. $KH_2PO_4$ + 70.4 ml $Na_2HPO_4$ | 7.2 |
| 19.7 ml. $KH_2PO_4$ + 80.3 ml $Na_2HPO_4$ | 7.4 |
| 12.8 ml. $KH_2PO_4$ + 87.2 ml $Na_2HPO_4$ | 7.6 |
| 7.4 ml. $KH_2PO_4$ + 92.6 ml $Na_2HPO_4$ | 7.8 |
| 3.7 ml. $KH_2PO_4$ + 96.3 ml $Na_2HPO_4$ | 8.0 |