Commission Regulation (EU) 2017/735 of 14 February 2017 amending, for the purpose of its adaptation to technical progress, the Annex to Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (Text with EEA relevance)

COMMISSION REGULATION (EU) 2017/735

of 14 February 2017

amending, for the purpose of its adaptation to technical progress, the Annex to Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC[(1)], and in particular Article 13(2) thereof,

Whereas:

(1) Commission Regulation (EC) No 440/2008[(2)] contains the test methods for the purposes of the determination of the physicochemical properties, toxicity and ecotoxicity of chemicals to be applied for the purposes of Regulation (EC) No 1907/2006.

(2) It is necessary to update Regulation (EC) No 440/2008 to include new and updated test methods recently adopted by the Organisation for Economic Cooperation and Development (OECD) in order to take into account technical progress, and to ensure the reduction in the number of animals to be used for experimental purposes, in accordance with Directive 2010/63/EU of the European Parliament and of the Council[(3)]. Stakeholders have been consulted on this draft.

(3) The adaptation to technical progress contains 20 test methods: one new method for the determination of a physicochemical property, five new and one updated test methods for the assessment of ecotoxicity, two updated test methods to assess the environmental fate and behaviour, and four new and seven updated test methods for the determination of effects on human health.

(4) The OECD regularly reviews its test guidelines in order to identify those which are scientifically obsolete. This adaptation to technical progress deletes six test methods for which the corresponding OECD test guidelines have been cancelled.

Changes to legislation: *There are currently no known outstanding effects for the Commission Regulation (EU) 2017/735. (See end of Document for details)*

(5) Regulation (EC) No 440/2008 should therefore be amended accordingly.

(6) The measures provided for in this Regulation are in accordance with the opinion of the Committee established under Article 133 of Regulation (EC) No 1907/2006,

HAS ADOPTED THIS REGULATION:

Article 1

The Annex to Regulation (EC) No 440/2008 is amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 14 February 2017.

For the Commission

The President

Jean-Claude JUNCKER

***Changes to legislation:** There are currently no known outstanding effects for the Commission Regulation (EU) 2017/735. (See end of Document for details)*

ANNEX

The Annex to Regulation (EC) No 440/2008 is amended as follows:

(1) In Part A, the following Chapter is added:

A.25 **DISSOCIATION CONSTANTS IN WATER (TITRATION METHOD — SPECTROPHOTOMETRIC METHOD — CONDUCTOMETRIC METHOD)**

INTRODUCTION

This test method is equivalent to OECD test guideline 112 (1981)

Prerequisites

— Suitable analytical method

— Water solubility

Guidance information

— Structural formula

— Electrical conductivity for conductometric method

Qualifying statements

— All test methods may be carried out on pure or commercial grade substances. The possible effects of impurities on results should be considered.

— The titration method is not suitable for low solubility substances (see Test solutions, below).

— The spectrophotometric method is only applicable to substances having appreciably different UV/VIS-absorption spectra for the dissociated and undissociated forms. This method may also be suitable for low solubility substances and for non-acid/base dissociations, e.g. complex formation.

— In cases where the Onsager equation holds, the conductometric method may be used, even at moderately low concentrations and even in cases for non-acid/base equilibria.

Standard documents

This test method is based on methods given in the references listed in the section "Literature" and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

METHOD — INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The dissociation of a substance in water is of importance in assessing its impact upon the environment. It governs the form of the substance which in turn determines its behaviour and transport. It may affect the adsorption of the chemical on soils and sediments and absorption into biological cells.

Definitions and units

Dissociation is the reversible splitting into two or more chemical species which may be ionic. The process is indicated generally by

$$RX \# R^{+} + X^{-}$$

and the concentration equilibrium constant governing the reaction is

$$K = \frac{[R^{+}][X^{-}]}{[RX]}$$

For example, in the particular case where R is hydrogen (the substance is an acid), the constant is

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$$K_a = [H^+] \times \frac{[X^-]}{[HX]}$$

or

$$pK_a = pH - \log \frac{[X^-]}{[HX]}$$

Reference substances

The following reference 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.

	pKa[a]	Temp. in °C
p-Nitrophenol	7,15	25[a]
Benzoic acid	4,12	20
p-Chloroaniline	3,93	20

a No value for 20 °C is available, but it can be assumed that the variability of measurement results is higher than the temperature dependence to be expected.

It would be useful to have a substance with several pKs as indicated in Principle of the method, below. Such a substance could be:

Citric acid	pK_a (8)	Temp. in °C
	(1) 3,14	20
	(2) 4,77	20
	(3) 6,39	20

Principle of the test method

The chemical process described is generally only slightly temperature dependent in the environmentally relevant temperature range. The determination of the dissociation constant requires a measure of the concentrations of the dissociated and undissociated forms of the chemical substance. From the knowledge of the stoichiometry of the dissociation reaction indicated in Definitions and units, above, the appropriate constant can be determined. In the particular case described in this test method the substance is behaving as an acid or a base, and the determination is most conveniently done by determining the relative concentrations of the ionised and unionised forms of the substance and the pH of the solution. The relationship between these terms is given in the equation for pK_a in Definitions and units, above. Some substances exhibit more than one dissociation constant and similar equations can be developed. Some of the methods described herein are also suitable for non-acid/base dissociation.

Quality criteria

Repeatability

The dissociation constant should be replicated (a minimum of three determinations) to within ± 0,1 log units.

DESCRIPTION OF THE TEST PROCEDURES

There are two basic approaches to the determination of pK_a. One involves titrating a known amount of substance with standard acid or base, as appropriate; the other

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involves determining the relative concentration of the ionised and unionised forms and its pH dependence.

Preparations

Methods based on those principles may be classified as titration, spectrophotometric and conductometric procedures.

Test solutions

For the titration method and conductometric method the chemical substance should be dissolved in distilled water. For spectrophotometric and other methods buffer solutions are used. The concentration of the test substance 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. If the substance is only sparingly soluble, it may be dissolved in a small amount of a water-miscible solvent prior to adding to the concentrations indicated above.

Solutions should be checked for the presence of emulsions using a Tyndall beam, especially if a co-solvent has been used to enhance solubility. Where buffer solutions are used, the buffer concentration should not exceed 0,05 M.

Test conditions

Temperature

The temperature should be controlled to at least ± 1 °C. The determination should preferably be carried out at 20 °C.

If a significant temperature dependence is suspected, the determination should be carried out at least at two other temperatures. The temperature intervals should be 10 °C in this case and the temperature control ± 0,1 °C.

Analyses

The method will be determined by the nature of the substance being tested. It must be sufficiently sensitive to allow the determination of the different species at each test solution concentration.

Performance of the test

Titration method

The test solution is determined by titration with the standard base or acid solution as appropriate, measuring the pH after each addition of titrant. At least 10 incremental additions should be made before the equivalence point. If equilibrium is reached sufficiently rapidly, a recording potentiometer may be used. For this method both the total quantity of substance and its concentration need to be accurately known. Precautions must be taken to exclude carbon dioxide. Details of procedure, precautions, and calculation are given in standard tests, e.g. references (1), (2), (3), (4).

Spectrophotometric method

A wavelength is found where the ionised and unionised forms of the substance have appreciably different extinction coefficients. The UV/VIS absorption spectrum is obtained from solutions of constant concentration under a pH condition where the substance is essentially unionised and fully ionised and at several intermediate pHs. This may be done, either by adding increments of concentrated acid (base) to a relatively large volume of a solution of the substance in a multicomponent buffer, initially at high (low) pH (ref. 5), or by adding equal volumes of a stock solution of the substance in e.g. water, methanol, to constant volumes of various buffer solutions covering the desired pH range. From the pH and absorbance values at the chosen wavelength, a sufficient number of values for the pK_a is calculated using data from at