Commission Regulation (EU) No 260/2014 of 24 January 2014 amending, for the purpose of its adaptation to technical progress, 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) No 260/2014

of 24 January 2014

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(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(3) thereof,

Whereas:

(1) Commission Regulation (EC) No 440/2008[(2)] contains the test methods for the purposes of the determination of the physico-chemical properties, toxicity and eco-toxicity of substances 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 with priority new and updated alternative test methods recently adopted by the OECD, in order to obtain a reduction of 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 of 22 September 2010 on the protection of animals used for scientific purposes[(3)] and Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes[(4)].

(3) The adaptation contains two methods for the determination of physicochemical properties including an update of the water solubility test method and a new partition coefficient test method relevant for the persistent, bioaccumulative and toxic (PBT) assessment; four new and one updated method for the determination of ecotoxicity and environmental fate and behaviour; nine methods for the determination of toxicity and other health effects including four inhalation toxicity test methods, which include

an update of three methods and one new method to reduce the number of animals used and to improve assessment of effects, an update of the repeat dose 28-day oral toxicity test method to include parameters for assessment of endocrine activity, an update of the toxicokinetics test method relevant for the design and understanding of toxicological studies and an update of chronic, carcinogenicity and combined chronic and carcinogenicity test methods.

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

(5) 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 third 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, 24 January 2014.

For the Commission

The President

José Manuel BARROSO

***Changes to legislation:** There are currently no known outstanding effects for the Commission Regulation (EU) No 260/2014. (See end of Document for details)*

ANNEX

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

(1) Chapter A.6 is replaced by the following:

A.6. **WATER SOLUBILITY**

INTRODUCTION

1. This Test Method is equivalent to OECD Test Guideline (TG) 105 (1995). This Test Method is a revised version of the original TG 105 which was adopted in 1981. There is no difference of substance between the current version and that from 1981. Mainly the format has been changed. The revision was based on the EU Test Method "Water Solubility" ([1]).

INITIAL CONSIDERATIONS

2. The water solubility of a substance can be considerably affected by the presence of impurities. This Test Method addresses the determination of the solubility in water of essentially pure substances which are stable in water and not volatile. Before determining water solubility, it is useful to have some preliminary information on the test substance, like structural formula, vapour pressure, dissociation constant and hydrolysis as a function of pH.

3. Two methods, the column elution method and the flask method which cover respectively solubilities below and above 10^{-2} g/l are described in this Test Method. A simple preliminary test is also described. It allows the determination of approximately the appropriate amount of sample to be used in the final test, as well as the time necessary to achieve saturation.

DEFINITIONS AND UNITS

4. The water solubility of a substance is the saturation mass concentration of the substance in water at a given temperature.

5. Water solubility is expressed in mass of solute per volume of solution. The SI unit is kg/m^3 but g/l may also be used.

REFERENCE CHEMICALS

6. Reference chemicals do not need to be employed when investigating a test substance.

DESCRIPTION OF THE METHODS

Test conditions

7. The test is preferably run at 20 ± 0,5 °C. The chosen temperature should be kept constant in all relevant parts of the equipment.

Preliminary test

8. In a stepwise procedure, increasing volumes of water are added at room temperature to approximately 0,1 g of the sample (solid test substances must be pulverized) in a 10 ml glass-stoppered measuring cylinder. After each addition of an amount of water, the mixture is shaken for 10 minutes and is visually checked for any undissolved parts of the sample. If, after addition of 10 ml of water, the sample or parts of it remain undissolved, the experiment is continued in a 100 ml measuring cylinder. The approximate solubility is given in Table 1 below under that volume of water in which complete dissolution of the sample occurs. When the solubility is low, a long time may be required to dissolve a test substance and at least 24 hours should be allowed. If, after 24 hours, the test substance is still not dissolved, more time (up to 96

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hours) should be allowed or further dilution should be attempted to ascertain whether the column elution method or flask method should be used.

TABLE 1

ml of water for 0,1 g soluble	0,1	0,5	1	2	10	100	> 100
approximate solubility in g/l	≥ 1 000	1 000 to 200	200 to 100	100 to 50	50 to 10	10 to 1	< 1

Column elution method

Principle

9. This method is based on the elution of a test substance with water from a micro-column which is charged with an inert support material, previously coated with an excess of the test substance (2). The water solubility is given by the mass concentration of the eluate when this has reached a plateau as a function of time.

Apparatus

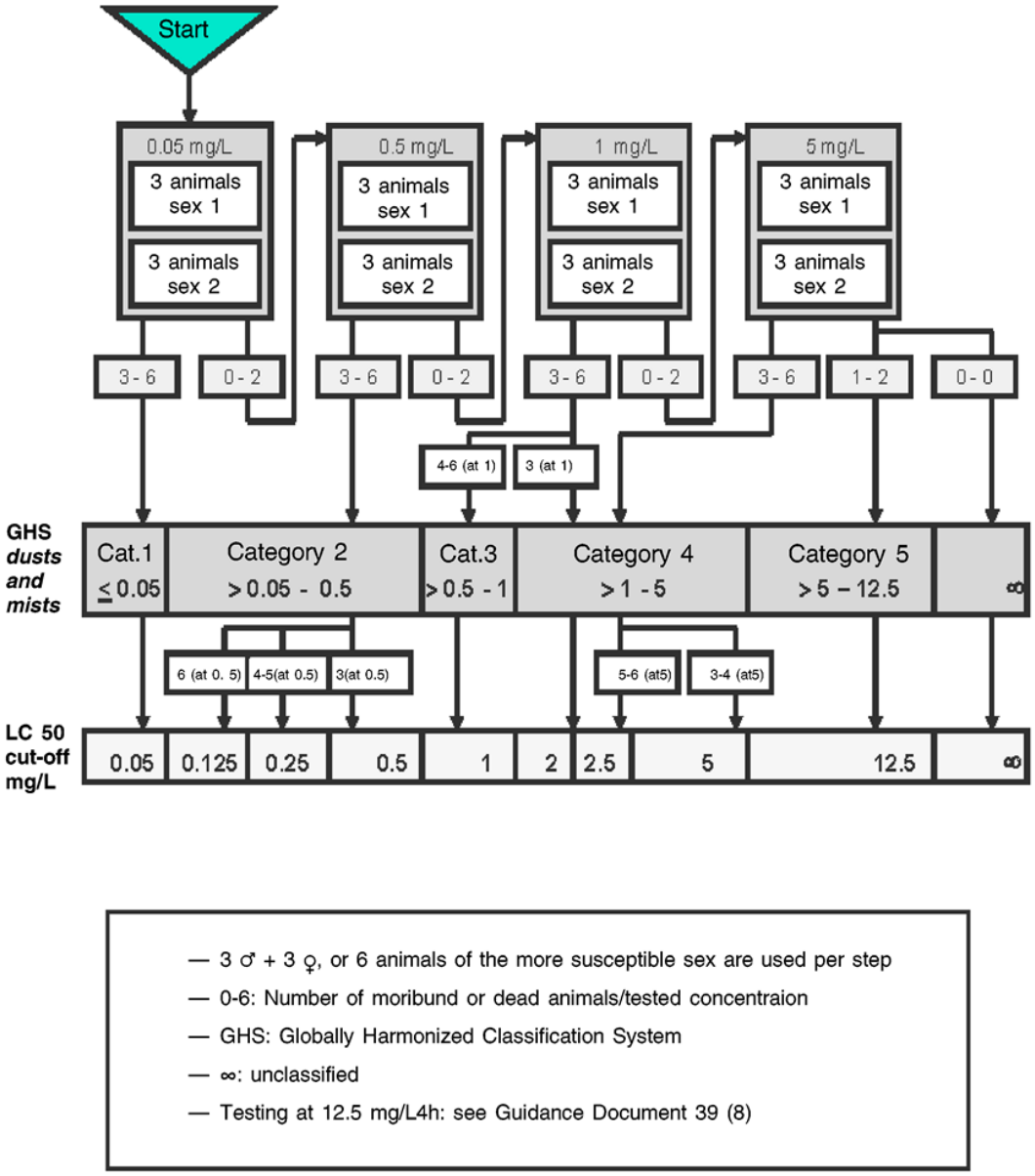
10. The apparatus consists of a microcolumn (Figure 1), maintained at constant temperature. It is connected either to a recirculating pump (Figure 2) or to a levelling vessel (Figure 3). The microcolumn contains an inert support held in place by a small plug of glasswool which also serves to filter out particles. Possible materials which can be employed for the support are glass beads, diatomaceous earth, or other inert materials.

11. The microcolumn shown in Figure 1 is suitable for the set-up with recirculating pump. It has a head space providing for five bed volumes (discarded at the start of the experiment) and the volume of five samples (withdrawn for analysis during the experiment). Alternatively, the size can be reduced if water can be added to the system during the experiment to replace the initial five bed volumes removed with impurities. The column is connected with tubing made of an inert material to the recirculating pump, capable of delivering approximately 25 ml/h. The recirculating pump can be, for example, a peristaltic or membrane pump. Care must be taken that no contamination and/or adsorption occur with the tube material.

12. A schematic arrangement using a levelling vessel is shown in Figure 3. In this arrangement the microcolumn is fitted with a one way stopcock. The connection to the levelling vessel consists of a ground glass joint and tubing made of an inert material. The flow rate from the levelling vessel should be approximately 25 ml/h.

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Figure



1