Commission Regulation (EU) 2016/266 of 7 December 2015 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) 2016/266

of 7 December 2015

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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(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 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 contains twenty test methods: one new method for the determination of a physicochemical property, eleven new test methods and three updated test methods for the assessment of ecotoxicity, and five new test methods to assess the environmental fate and behaviour.

(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:

Changes to legislation: *There are currently no known outstanding effects for the Commission Regulation (EU) 2016/266. (See end of Document for details)*

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, 7 December 2015.

For the Commission

The President

Jean-Claude JUNCKER

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ANNEX

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

(1) A note is inserted at the beginning of the Annex, before Part A:
Note:

Before using any of the following test methods to test a multi-constituent substance (MCS), a substance of unknown or variable composition, complex reaction product or biological material (UVCB), or a mixture and where its applicability for the testing of MCS, UVCB, or mixtures is not indicated in the respective test method, it should be considered whether the method is adequate for the intended regulatory purpose.

If the test method is used for the testing of a MCS, UVCB or mixture, sufficient information on its composition should be made available, as far as possible, e.g. by the chemical identity of its constituents, their quantitative occurrence, and relevant properties of the constituents.

(2) Chapter A.24 is added:

A.24. **PARTITION COEFFICIENT (N-OCTANOL/WATER), HIGH PERFORMANCELIQUID CHROMATOGRAPHY (HPLC) METHOD**

INTRODUCTION

This test method is equivalent to OECD test guideline (TG) 117 (2004)

1. 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,

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

The partition coefficient being the quotient of two concentrations, is dimensionless and is usually given in the form of its logarithm to base ten.

2. P_{ow} is a key parameter in studies of the environmental fate of chemical substances. A highly-significant relationship between the P_{ow} of non-ionised form of substances and their bioaccumulation in fish has been shown. It has also been shown that P_{ow} is a useful parameter in the prediction of adsorption on soil and sediments and for establishing quantitative structure-activity relationships for a wide range of biological effects.

3. The original proposal for this test method was based on an article by C.V. Eadsforth and P. Moser (1). The development of the test method and an OECD inter-laboratory comparison test were coordinated by the Umweltbundesamt of the Federal Republic of Germany during 1986 (2).

INITIAL CONSIDERATIONS

4. log P_{ow} values in the range – 2 to 4 (occasionally up to 5 and more)[(4)] can be experimentally determined by the Shake-Flask method (Chapter A.8 of this Annex, OECD Test Guideline 107). The HPLC method covers log P_{ow} in the range of 0 to 6 (1)(2)(3)(4)(5). This method may require an estimation of P_{ow} to assign suitable reference substances and support any conclusions drawn from the data generated by the test. Calculation methods are briefly discussed in the Appendix to this test method. The HPLC operation mode is isocratic.

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5. The P_{ow} values depend on the environmental conditions such as temperature, pH, ionic strength etc, and these should be defined in the experiment for the correct interpretation of P_{ow} data. For ionisable substances, another method (e.g. draft OECD guideline on pH metric method for ionised substances (6)) may become available and could be used as an alternative method. Although this draft OECD guideline may appropriate be suitable to determine P_{ow} for those ionisable substances, in some cases it is more appropriate to use the HPLC method at an environmentally relevant pH (see paragraph 9).

PRINCIPLE OF THE METHOD

6. Reverse phase HPLC is performed on analytical columns packed with a commercially available solid phase containing long hydrocarbon chains (e.g. C_8, C_{18}) chemically bound onto silica.

7. A chemical injected on such a column partitions between the mobile solvent phase and the hydrocarbon stationary phase as it is transported along the column by the mobile phase. The substances are retained in proportion to their hydrocarbon-water partition coefficient, with hydrophilic substances eluted first and lipophilic substances last. The retention time is described by the capacity factor k given by the expression:

$$k = \frac{t_R - t_0}{t_0}$$

where t_R is the retention time of the test substance, and t_0 is the dead-time, i.e. the average time a solvent molecule needs to pass the column. Quantitative analytical methods are not required and only the determination of retention times is necessary.

8. The octanol/water partition coefficient of a test substance can be computed by experimentally determining its capacity factor k and then inputting k into the following equation:

$$\log P_{ow} = a + b \times \log k$$

where

a, b = linear regression coefficients.

The equation above can be obtained by linearly regressing the log of octanol/water partition coefficients of reference substances against the log of capacity factors of the reference substances.

9. Reverse phase HPLC method enables partition coefficients to be estimated in the log P_{ow} range between 0 and 6, but can be expanded to cover the log P_{ow} range between 6 and 10 in exceptional cases. This may require that the mobile phase is modified (3). The method is not applicable to strong acids and bases, metal complexes, substances which react with the eluent, or surface-active agents. Measurements can be performed on ionisable substances in their non-ionised form (free acid or free base) only by using an appropriate buffer with a pH below the pK_a for a free acid or above the pK_a for a free base. Alternatively, the pH-metric method for the testing of ionisable substances (6) may become available and could be used as an alternative method (6). If the log P_{ow} value is determined for the use in environmental hazard classification or in environmental risk assessment, the test should be performed in the pH range relevant for the natural environment, i.e. in the pH range of 5,0 - 9.

10. In some cases impurities can make the interpretation of the results difficult due to uncertainty in peak assignments. For mixtures which result in an unresolved band, upper and lower limits of log P_{ow}, and the area % of each log P_{ow} peak should

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be reported. For mixtures which are a group of homologues, the weighted average log P_{ow} should also be stated (7), calculated based on the single P_{ow} values and the corresponding area % values (8). All peaks that contribute an area of 5 % or more to the total peak area should be taken into consideration in the calculation (9):

$$weighted\ average\ \log P_{ow} = \frac{\sum_i (\log P_{owi})(area\ \%)}{total\ peak\ area\ \%} = \frac{\sum (\log P_{owi})(area\ \%_i)}{\sum_i area\ \%}$$

The weighed average log P_{ow} is valid only for substances or mixtures (e.g. tall oils) consisting of homologues (e.g. series of alkanes). Mixtures can be measured with meaningful results, provided that the analytical detector used has the same sensitivity towards all the substances in the mixture and that they can be adequately resolved.

INFORMATION ON THE TEST SUBSTANCE

11. The dissociation constant, structural formula, and solubility in the mobile phase should be known before the method is used. In addition, information on hydrolysis would be helpful.

QUALITY CRITERIA

12. In order to increase the confidence in the measurement, duplicate determinations must be made.

— Repeatability: The value of log P_{ow} derived from repeated measurements made under identical conditions and using the same set of reference substances should fall within a range of ± 0,1 log units.

— Reproducibility: If the measurements are repeated with a different set of reference substances, results may differ. Typically, the correlation coefficient R for the relationship between log k and log P_{ow} for a set of test substances is around 0,9, corresponding to an octanol/water partition coefficient of log P_{ow} ± 0,5 log units.

13. The inter-laboratory comparison test has shown that with the HPLC method log P_{ow} values can be obtained to within ± 0,5 units of the Shake-Flask values (2). Other comparisons can be found in the literature (4)(5)(10)(11)(12). Correlation graphs based on structurally related reference substances give the most accurate results (13).

REFERENCE SUBSTANCES

14. In order to correlate the measured capacity factor k of a substance with its P_{ow}, a calibration graph using at least 6 points has to be established (see paragraph 24). It is up to the user to select the appropriate reference substances. The reference substances should normally have log P_{ow} values which encompass the log P_{ow} of the test substance, i.e. at least one reference substance should have a P_{ow} above that of the test substance, and another a P_{ow} below that of the test substance. Extrapolation should only be used in exceptional cases. It is preferable that these reference substances should be structurally related to the test substance. log P_{ow} values of the reference substances used for the calibration should be based on reliable experimental data. However, for substances with high log P_{ow} (normally more than 4), calculated values may be used unless reliable experimental data are available. If extrapolated values are used a limit value should be quoted.

15. Extensive lists of log P_{ow} values for many groups of chemicals are available (14)(15). If data on the partition coefficients of structurally related substances are not available, a more general calibration, established with other reference substances, may be used. Recommended reference substances and their P_{ow} values are listed in Table