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Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium

Qualité de l'eau — Lignes directrices pour la préparation et le traitement des composés organiques peu solubles dans l'eau en vue de l'évaluation de leur biodégradabilité en milieu aqueux

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10634 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annex A of this International Standard is for information only.

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Introduction

The standardizing work carried out by ISO/TC 147/SC 5 has shown that the development of a single method for evaluating the biodegradability of organic compounds with a low solubility in water may not be envisaged in the immediate future. In fact, the selection of the most suitable working method to obtain a satisfactory emulsion or dispersion of these compounds in the test media depends particularly on their physico-chemical properties. Consequently, the selection of the most suitable method has to be left to the judgement of laboratories responsible for the tests based on their experience and the product information supplied by the applicant. It is for this reason that this International Standard describes various techniques for treating poorly soluble organic compounds before they are investigated in biodegradability tests, with the aim of reaching a stage where, for any given technique, the same working method is used by all laboratories, thus making it easier to compare results.

The techniques described in this International Standard do not necessarily produce the same results if they are used in parallel. The use of solvents and dispersing or emulsifying techniques may be additional sources of errors and may lead to test results which differ from those obtained without using these techniques. Furthermore, dispersions or emulsions may be produced which would not exist as such in nature and where the rate and degree of biodegradability is enhanced because very fine particles are present. These facts have to be considered for the evaluation and interpretation of the results of biodegradation tests. It is recommended to perform biodegradability tests first, with the direct addition of a test compound, or to perform this test in parallel to tests using dispersion techniques.

Normally, only pure or virtually pure compounds should be tested. If mixtures or multi-componental substances are tested, the use of solvents and dispersion techniques may lead to unrepresentative heterogeneous distributions and to misleading test results in the subsequent biodegradability tests.

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Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium

1 Scope

This International Standard describes four techniques for preparing poorly water-soluble organic compounds and introducing them into test vessels for a subsequent test on biodegradability in an aqueous medium using standard methods. The test compounds concerned are not sufficiently soluble in water to perform the biodegradability tests in the normal manner, as described in the respective test methods indicated in clause 2.

The preparation techniques are as follows:

— direct addition (clause 3): this technique is restricted to non-volatile test compounds if inert supports or solvents are used;

— ultrasonic dispersion (clause 4): this technique may be applied to non-volatile liquid and solid compounds;

— adsorption on an inert support (clause 5);

— dispersions or emulsions with an emulsifying agent (clause 6).

The subsequent tests on biodegradability are primarily methods using the analysis of the released carbon dioxide (see ISO 9439) and the determination of the oxygen consumption (see ISO 9408). This International Standard does not describe the test methods; it is restricted to describing the techniques for introducing the test substances into the test medium and to keep them in a dispersed state. These techniques are implemented while observing the experimental conditions described in the standardized methods for evaluating biodegradability. It should be noted that volatile chemicals may not be tested by the carbon dioxide method specified in ISO 9439.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 9408:1991, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by determining the oxygen demand in a closed respirometer.*

ISO 9439:1990, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide.*

3 Direct addition

Any of the following techniques can be used

— The test compound is weighed and directly introduced into the test vessels which are subjected to continuous agitation.

NOTE 1 Some organic compounds which are sparingly soluble in water dissolve more readily when alkali or acid is added. They may be introduced as an acid or

alkaline stock solution, provided that no substantial reaction of the test compound takes place. The test medium is adjusted to neutral before the inoculum is added.

— The test compound is weighed onto a suitable inert support and introduced into the test vessels which are subjected to continuous agitation.

— A solution of the test compound is prepared in a volatile organic solvent and is introduced into the test vessels which are subjected to continuous agitation.

The solvent, which shall be used in minimal amounts, is then removed, if possible completely, by agitation before the test medium is added.

3.1 Reagents

A volatile organic solvent is selected for its capacity to dissolve the test compound.

The chosen solvent shall not react with the test compound or with any component of the medium.

The solvent shall be non-biodegradable and non-toxic to bacteria under the conditions of the subsequent biodegradability tests, especially if it cannot be removed sufficiently.

Suitable solvents are acetone or dichloromethane.

3.2 Apparatus

3.2.1 Inert supports, which may be introduced into the test vessels, e.g. microscope slides.

3.2.2 Stirrers, in sufficient numbers to ensure that all the test vessels used in the respective biodegradability tests can be agitated.

Stirrer-rods shall be of such a material that no ingredients of a plastics coat will contaminate the test medium and no adsorption of test compounds will occur. Heating up the test vessels by stirring and raising the test temperature shall be avoided.

3.3 Procedure

3.3.1 Direct addition

Test compounds with crystalline structures may be weighed and directly added to the test vessels.

Non-viscous liquid compounds may be added with a high precision volumetric syringe.

Test compounds which are sufficiently soluble in water under acidic or alkaline conditions may be introduced as stock solution. Prepare a stock solution of such a test compound in deionized water adjusted with inorganic acid or alkali to a sufficiently high or low pH. Add an appropriate amount of the stock solution to the test medium to obtain the desired concentration of the test compound in the test vessels. Measure the pH of the medium and adjust it as necessary before the inoculum is added.

3.3.2 Using a support

Grind solid test compounds as finely as possible before weighing them. Weigh the liquids, including viscous substances, without treatment or, if possible, solidify in liquid nitrogen and grind finely before weighing.

Weigh onto the support (3.2.1) a quantity of the compound corresponding to the initial concentation or organic carbon required by the test method to be used.

Introduce a support into each of the test vessels, and also introduce a support without the test compound into each of the control vessels.

Maintain agitation using the stirrers (3.2.2) throughout the biodegradability test.

3.3.3 Using a solvent

Prepare a solution of the test compound in a minimum of the chosen organic solvent (3.1).

Introduce, into the test vessels, the quantity of solution needed to obtain the initial concentration of organic carbon required by the test method used.

Introduce the same quantity of the solvent, without any test compound, into each of the control vessels.

Evaporate the solvent, if possible completely, by appropriate methods.

NOTE 2 The test solution may be spread over the base of the test vessels and the system is then purged with gas and/or stirred. The last traces of solvent are difficult to remove. Interferences may occur if the solvent is biodegradable or inhibitory to bacteria.

Then carry out the biodegradability test.

4 Ultrasonic dispersion

An emulsion or dispersion of the compound to be tested is prepared using an ultrasonic probe and is

introduced into the test vessels, which are continuously agitated (see 3.2.2).

4.1 Apparatus

4.1.1 Ultrasonic transducer, capable of producing a frequency of approximately 20 kHz.

4.1.2 Stirrers, in sufficient numbers to ensure that all the test vessels can be agitated (see 3.2.2).

4.2 Procedure

Add, for example, 10 g or 10 ml of the test compound to a 500 ml beaker containing approximately 400 ml of deionized water.

The test compound shall be present in excess so that a saturated solution is obtained. Install the ultrasonic transducer (4.1.1) in such a way that its tip is as close as possible to the interface between the water and the test compound.

Use a stirrer (4.1.2) to agitate the beaker so that the compound is drawn down to the bottom.

Set the transducer to give a frequency of about 20 kHz and maintain this for about 30 min.

Switch off the transducer and leave the emulsion or dispersion to settle for 15 min to 30 min, then decant it off from the excess test compound into another container.

NOTES

3 The proportions and figures are given only as a guide. They depend on the characteristics of the test compound.

4 Some substances are subjected to thermal decomposition if heat generation occurs at the probe tip. This may also lead to an increase in the temperature of the bulk solution. This problem may be avoided by measuring and controlling the temperature, reduction of the power of the sonifier or intermittent sonification. In some cases, problems may be encountered because of the destruction of the chemical. If this is the case, a different method should be used.

Using an appropriate analytical method [specific analysis or total organic carbon (TOC) analysis], analyse an aliquot of the emulsion or dispersion obtained and determine the concentration of the test compound.

Introduce an appropriate volume of emulsion or dispersion into the test vessels to obtain the initial concentration of organic carbon required by the test method to be used.

Sustain the agitation throughout the biodegradability test.

NOTE 5 It may be difficult to obtain a stable emulsion or dispersion. Special care is therefore required when aliquots are distributed to the test vessels. If it proves impossible to obtain a sufficiently stable emulsion or a sufficiently high concentration to carry out the test, the test compound may be introduced directly into the test medium and be dispersed ultrasonically in the test vessels before the inoculum is added.

5 Adsorption on an inert support

The test compound is adsorbed onto an inert support and introduced into the test vessels. It is kept dispersed in the medium by continuous agitation.

5.1 Reagents

5.1.1 Inert support

Silica gel, glassfibre filters or other non-biodegradable inert supports which do not release organic or inorganic carbon into the aqueous medium may be used. It should be validated by preliminary work that the support is inert and carbon-free; to avoid or minimize surface area effects, the quantity of the support shall be minimal. The test compound should be adsorbed on the surface but not be adsorbed and fixed too intensively on the support.

NOTE 6 For example, if silica gel is used, the following supports are suitable:

— silica gel used for thin-layer chromatography (15 µm particle size);

— silica gel used for column chromatography (200 µm to 500 µm particle size).

5.1.2 Solvent

A volatile solvent is selected for its capacity to dissolve the test compound.

It shall be non-toxic to bacteria and, if possible, non-biodegradable under the conditions of the subsequent biodegradability tests. This shall be tested in advance or in the subsequent biodegradability test.

Depending on the test compound, acetone or dichloromethane may be suitable.

5.2 Apparatus

5.2.1 Stirrers, in sufficient numbers to ensure that all the vessels can be agitated (see 3.2.2).

5.3 Procedure

Prepare the quantity of test compound to be impregnated on the support required by the biodegradability test method to be used.

For example, mix together, by agitation in a 250 ml vessel for 2 h, 30 g of the support (5.1.1) and 150 ml of a solution of 1 g/l of the test compound in the chosen solvent (5.1.2). At the same time, carry out the same procedure using only the support and the solvent as a control.

In both cases, recover the support and dry by totally evaporating the solvent. This may be performed using, in succession, a rotary evaporator, a ventilated oven and a vacuum oven at about 45 °C.

NOTE 7 The last traces of solvent may be difficult to remove. Interferences may occur if the solvent is biodegradable or inhibitory to bacteria.

Determine the amount of the compound impregnated on the support in three samples of 1,5 g or more, using one of the following methods:

- elemental quantitative analysis of the amount of carbon originating from the compound, using a high-temperature total carbon analyser, and then deducting the values obtained for the support treated with solvent only;
- determination of the chemical oxygen demand of the compound impregnated on the inert support and then deducting the values obtained for the support treated with solvent only;
- extraction of the compound using an organic solvent and quantitative analysis using a specific analytical method.

From the amount of test compound on the support, determine the quantity of support to be introduced into the test vessels to obtain the initial concentration of organic carbon of the test compound required by the test method used.

Introduce the same quantity of support treated with the solvent alone into each of the control vessels.

Any biodegradability of the solvent shall be taken into account when calculating the test results.

Sustain continuous agitation throughout the biodegradability test.

6 Dispersion with an emulsifying agent

A dispersion or emulsion of the test compound is prepared using an emulsifying agent and is introduced into the test vessels which are continuously agitated.

6.1 Reagents

6.1.1 Solvent for the emulsifying stage

A solvent, volatile if necessary and sufficiently miscible in water, is chosen for its capacity to dissolve the test compound. It may be difficult to eliminate solvents which are highly miscible in water. In this case, they should not be used.

The solvent shall be non-toxic to bacteria and non-biodegradable under the conditions of the subsequent biodegradability test (e.g. pentane, hexane or 1,1-dichloro-1-fluoroethane).

6.1.2 Emulsifying agent

The emulsifying agent shall be non-biodegradable and non-toxic under the conditions of the subsequent biodegradability tests.

NOTE 8 If the biodegradability and the inhibition to bacteria of the emulsifying agent are unknown, they should be investigated in advance or in additional assays of the subsequent biodegradability test, e.g. using one vessel containing only emulsifying agent for measuring biodegradability and one vessel containing emulsifying agent and a reference substance such as sodium benzoate for measuring inhibition.

As an example, the following substances[1)] may be used:

a) a block of copolymer of ethylene oxide and propylene oxide, with a hydrophilic-lipophilic balance value of about 9;

b) a block of copolymer of ethylene oxide and propylene oxide, with a hydrophilic-lipophilic balance value of about 13,5;

c) polyethylene sorbitan trioleate.

1) Synperonic PE/P94, Synperonic PE/P103 or Tween 85 are examples of suitable products available commercially for substances a), b) and c), respectively. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of these products.

6.2 Apparatus

6.2.1 Stirrers, in sufficient numbers to ensure that all the test vessels can be agitated (see 3.2.2).

6.3 Preliminary tests for selecting the emulsifying agent

As an example, prepare three solutions of the test compound (x mg) in y ml of the solvent (6.1.1) (in the case of 1,1-dichloro-1-fluoroethane, approximately 10 ml may be used) with each of the following emulsifying agents:

a) substance [6.1.2 a)] alone at $\frac{x}{2}$ mg;

b) substance [6.1.2 b)] alone at $\frac{x}{2}$ mg;

c) a mixture of substance [6.1.2 a)] at $\frac{x}{4}$ mg plus substance [6.1.2 b)] at $\frac{x}{4}$ mg.

The amount of the compound dissolved in the solvent (x mg) is calculated to obtain the required concentration of organic carbon in the test compound in the test medium of the test method to be used.

Homogenize by steady agitation for 10 min, then add the solution obtained drop by drop to the volume of test medium required for each test vessel specified for the biodegradability test. Remove the solvent by persisting with the agitation (in the case of 1,1-dichloro-1-fluoroethane, e.g. for 1 h at 30 °C) or by any other appropriate method.

Select by visual assessment the preparation a), b) or c) which produces the most homogeneous emulsion.

6.4 Procedure

Prepare a sufficient amount of the emulsion or dispersion needed to carry out the biodegradability test to be used in accordance with the procedure selected as a result of the preliminary tests.

Prepare an emulsion or dispersion containing only the test medium and the emulsifying agent, to provide a control vessel to verify that the emulsifying agent does not have a degradation in excess of about 10 % in comparison with the test compound.

The results obtained for this vessel shall be taken into account in calculating the test results.

Sustain continuous agitation throughout the biodegradability test.

7 Test report

The test report on the biodegradability of compounds which are poorly soluble in water shall contain, first of all, the data required by the standard biodegradability method. In addition, it shall contain reference to this International Standard, specifying the method used and any modification to the procedure, and include the following information:

a) any pretreatment of the compound before the test;

b) method of introduction of the test compound;

c) duration and intensity of treatment;

d) nature and amount of the support, the solvent or the emulsifying agent;

e) concentration of the compound in the emulsion or dispersion;

f) degree of recovery of the adsorbed test compound;

g) rate of degradation of the emulsifying agent obtained during the test in the control vessel;

h) obvious behaviour of the test compound during the biodegradability test (e.g. formation of aggregates, appearance of phases).