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SIST EN 14735:2005
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Karakterizacija odpadkov - Priprava vzorcev odpadka za ekotoksikološke preskuse

Characterization of waste - Preparation of waste samples for ecotoxicity tests

Charakterisierung von Abfällen - Herstellung von Abfallproben für ökotoxikologische Untersuchungen

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Caractérisation des déchets - Caractérisation des déchets - Préparation des échantillons de déchets en vue d'essais écotoxicologiques

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EUROPEAN STANDARD

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Characterization of waste - Preparation of waste samples for ecotoxicity tests

Caractérisation des déchets - Préparation des échantillons de déchets en vue d'essais écotoxicologiques

Charakterisierung von Abfällen - Herstellung von Abfallproben für ökotoxikologische Untersuchungen

This European Standard was approved by CEN on 3 October 2021.

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European foreword

This document (EN 14735:2021) has been prepared by Technical Committee CEN/TC 444 “Environmental characterization of solid matrices”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14735:2005.

The main changes with respect to the previous edition are:

— the modification of Clause 12 “Test report” to specify whether ecotoxicity tests have been repeated with pH adjustment of the test portion, allowing to clearly distinguish these results from those obtained without pH adjustment;

— the clarification of the notes of 10.5 and 11.2.3 and associated notes regarding the repetition of the tests with pH adjustment;

— the update of Annex B, describing the ecotoxicity tests considered to establish this document.

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Introduction

Ecotoxicity can be estimated using two approaches: a chemical-specific approach and a toxicity-based approach. Chemical analyses are compared, in the first case, to quality criteria or threshold values to estimate ecotoxicity. In the second one, ecotoxicity is measured directly using biological tests. These two approaches complement each other; indeed, determination of pollutants in complex mixtures of unknown composition, that is a characteristic of many wastes, does not allow a relevant estimation of ecotoxicity. For such samples, the toxicity-based approach is usually recognized to be appropriate to assess potential toxicity. Bioassays integrate, indeed, the effects of all contaminants including additive, synergistic and antagonistic effects. They are sensitive to the bioavailable fraction of the contaminants only. Finally, bioassays integrate the effects of all contaminants, including those, not considered or detected by chemical analyses.

Ecotoxicity tests can be applied to wastes to identify their potential hazardous properties with respect to the environment for classification purposes or to assess the risk related to a site-specific exposure scenario.

Identification of properties potentially hazardous to the environment for classification purposes

A classification system, based on the assessment of intrinsic properties, should be independent of an exposure scenario. The main requirement, in order to establish a relevant system for classifying wastes and for assessment of hazard properties, is to obtain comparable test results. This can only be obtained if the ecotoxicity tests on wastes are carried out according to a unique procedure describing more or less conventional test conditions (an exclusive dilution medium for terrestrial tests, a unique L/S ratio for preparation of water extracts, a unique liquid / solid separation step, etc.). This procedure should be applicable to a very wide range of waste materials whatever their physical properties are.

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Any strategy for the assessment of properties potentially hazardous to the environment used in a classification system should include test organisms representing the terrestrial and the aquatic compartment. Both types of tests should be considered because they expand the range of effect expression due to differences in species sensitivity and exposure.

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For this specific purpose, the water extracts preparations for toxicity testing do not simulate leaching from wastes under environmental conditions but measure the water available fraction of the toxic components of the wastes.

Site-specific exposure scenario

The second application of ecotoxicity tests to wastes refers to a risk assessment approach. In this particular case, the test strategy should model site specific exposure conditions and should take into account the transfer of contaminants via the food chain and to surface and ground water by run-off or leaching. This application concerns firstly the definition of generic scenarios frequently encountered (e.g. wastes deposit in stockpiles, re-use of wastes) and focus on the relevant way of exposure to terrestrial and aquatic organisms.

This document describes the necessary steps to be performed before carrying out ecotoxicity tests on wastes within the context of assessment of ecotoxic properties for classification purposes.

It should be kept in mind that the use of this standard is currently not mandatory for assessing the hazardous property HP14.

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1 Scope

This document describes the necessary steps to be performed before carrying out ecotoxicity tests on wastes. The purpose of this document is to provide guidance on the taking of the sample, transport, storage of wastes and to define preparation, for the determination of ecotoxicological properties of wastes under the conditions specified in this document by biological testing either as raw wastes or water extracts from wastes. Sample preparation for other applications (e.g. assessment of waste effects on aquatic and terrestrial organisms in a disposal scenario) is not considered.

Specifying a test battery to characterize ecotoxicological properties of wastes is not in the scope of this document.

This document is applicable to solid and liquid wastes.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12457-2:2002, *Characterisation of waste - Leaching - Compliance test for leaching of granular waste materials and sludges - Part 2: One stage batch test at a liquid to solid ratio of 10 l/kg for materials with particle size below 4 mm (without or with size reduction)*

EN 14899, *Characterization of waste - Sampling of waste materials - Framework for the preparation and application of a Sampling Plan)*

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EN 16720-1, *Characterization of sludges - Physical consistency - Part 1: Determination of flowability - Method by extrusion tube apparatus)*

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EN ISO 5667-3, *Water quality - Sampling - Part 3: Preservation and handling of water samples (ISO 5667-3)*

EN ISO 14238:2012, *Soil quality - Biological methods - Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes (ISO 14238: 2012)*

ISO 11465, *Soil quality - Determination of dry matter and water content on a mass basis - Gravimetric method*

EN ISO 10390, *Soil, sludge and treated biowaste - Determination of pH (ISO/FDIS 10390)*

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3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
dilution medium
liquid or solid used for the preparation of control vessels and the preparation of test mixtures

3.2
ecotoxicological properties
potential adverse effects to biological systems which a waste has an inherent capacity to cause

3.3
eluate
solution recovered from a leaching test

[SOURCE: EN 12457-2:2002, 3.3]

3.4
granular waste
waste not being monolithic, nor a liquid, a gas or a sludge

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[SOURCE: adapted from EN 12457-2:2002, 3.10]

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3.5
laboratory sample
sample or subsample(s) sent to or received by the laboratory (IUPAC definition)

Note 1 to entry: When the laboratory sample is further prepared (reduced) by subdividing, mixing, grinding or by combinations of these operations, the result is the test sample. When no preparation of the laboratory sample is required, the laboratory sample is the test sample. A test portion is removed from the test sample for the performance of the test or for analysis. The laboratory sample is the final sample from the point of view of sample collection, but it is the initial sample from the point of view of the laboratory.

Note 2 to entry: Several laboratory samples can be prepared and sent to different laboratories or to the same laboratory for different purposes. When sent to the same laboratory, the set is generally considered as a single laboratory sample and is documented as a single sample.

3.6
leachant
liquid used in a leaching test

Note 1 to entry: For the purpose of this document the leachant is water as specified in Clause 4.

3.7
leaching test
test during which a material is put into contact with a leachant and some constituents of the material are extracted

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3.8
liquid sludge
sludge in a liquid physical state, determined according to EN 16720-1

3.9
liquid waste
waste that completely flows out of a calibrated opening, down to the upper level of the opening within a limited period of time (see Annex B of EN 12457-2:2002)

3.10
monolithic waste
waste prepared with a certain minimum dimensions and physical and mechanical properties that ensure its integrity over a certain period of time in the considered scenario

[SOURCE: CEN/TS 16675:2018, 3.7]

3.11
paste-like material
material of soft plastic or wet cement consistency – usually smooth

3.12
sludge
mixture of liquid and solid separated from various types of liquid as a result of natural or artificial processes

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[SOURCE: adapted from EN 12457-2:2002, 3.8]

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3.13
test mixture
mixture of the test portion (waste or water extract) with the dilution medium (3.1)

3.14
test portion
amount or volume of the test sample taken for measurement of ecotoxicological properties by biological testing and/or other properties of interest, usually of known weight or volume (adapted from IUPAC definition)

Note 1 to entry: The test portion can be taken from the laboratory sample directly if no preparation of sample is required (e.g. with liquids), but usually it is taken from the prepared test sample.

Note 2 to entry: A unit or increment of proper homogeneity, size and fineness, needing no further preparation, can be a test portion.

3.15
test sample
sample, prepared from the laboratory sample, from which test portions are removed for biological testing or analysis (adapted from IUPAC definition)

Note 1 to entry: The preparation of the test sample can include particle size reduction, preparation of water extract, etc.

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3.16
water extract
solution obtained from a leaching test, a liquid/liquid extraction and a liquid/solid separation (centrifugation)

4 Equipment and reagents

Usual laboratory equipment and the following.

4.1 Sieving equipment, e.g. with sieves of 4 mm square mesh.

NOTE Due to sieving, contamination of the sample can occur to an extent that affects the leaching of some constituents of concern e.g. cobalt and tungsten from tungsten carbide equipment or chromium, nickel and molybdenum from stainless steel equipment.

4.2 Crushing equipment: jaw crusher or cutting device.

NOTE Due to crushing, contamination of the sample can occur to an extent that affects the leaching of some constituents of concern e.g. cobalt and tungsten from tungsten carbide equipment or chromium, nickel and molybdenum from stainless steel equipment.

4.3 Balance of accuracy of at least 0,1 g.

4.4 End-over-end tumbler (5 rpm to 10 rpm) **or rollertable** rotating at about 10 rpm.

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NOTE Other shaking or mixing devices can be used provided that they are proven to be equivalent.

4.5 Centrifuge that can attain a RCF between 2 000 g and 3 000 g.

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4.6 Filtering apparatus, either a vacuum filtration device (between 30 kPa and 70 kPa) (300 mbars to 700 mbars) or a high-pressure filtration apparatus (<0,5 MPa) (5 bars).

4.7 Large scale laboratory mixer.

4.8 pH meter.

4.9 Glass or high-density polyethylene (HDPE)/polypropylene (PP) bottles in accordance with EN ISO 5667-3, glass bottles having caps of inert material, for example polytetrafluoroethylene. Rinsing with sample material or water (4.10) is compulsory.

NOTE For inorganic constituents HDPE/PP bottles are preferred, except for samples containing mercury.

4.10 Water, distilled water, demineralized water or deionized water with a conductivity < 5 µS/cm.

5 Taking of laboratory sample

Obtain a representative laboratory sample of a quantity sufficient for the number of tests to be performed and in accordance with the requirements of biological standardized methods to be used.

Special precautions should be taken to avoid any contamination of laboratory samples by material of sampling devices and/or storage equipment, according to EN 14899.

NOTE Sampling devices are described in the technical report CEN/TR 15310-2.

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Some wastes are subject to chemical, physical and biological changes as soon as they are collected (e.g. wastes that are fermentable, subject to oxidation or carbonation and wastes that contain volatile substances). Possible changes shall be considered and sampling conditions shall be designed accordingly in order to limit the effects of such changes on the results of ecotoxicity tests.

However, addition of preservatives (e.g. acids, basic solutions, biocides) in order to reduce chemical and biological activity is prohibited.

6 Transport

Transport of laboratory samples shall be performed in the dark, in tight containers fully filled with the waste to be tested. However, special precautions should be taken for transport and storage of sludge or other microbial active wastes. Containers can become pressurized due to gas production and explosions may occur. For such laboratory samples, containers should not be completely filled. Nevertheless, headspace shall not exceed 10 % of the total capacity of the container. Manual release of pressure during and after transport may be necessary.

The container material shall be appropriate. The container, in which the waste laboratory sample is transported, and the stopper shall not react with the constituents of the sample and shall not be a cause of contamination. Wastes shall be stored in polyethylene, polypropylene, polytetrafluoroethene (PTFE) or glass containers. However, security aspects shall be considered, including the risk of explosion due to gas generation (for example, glass vessels are not suitable for sludge samples).

Transport of waste laboratory samples should be as short as possible. Possible changes shall be considered and transport conditions shall be designed accordingly in order to limit the effects of such changes on the results of ecotoxicity tests. Transport time shall be regarded as part of storage time.

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A transport time of less than 48 h and/or a low temperature conditions (4 ± 2) °C are appropriate to maintain the properties of laboratory samples.

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7 Storage

7.1 General

Storage should be carried out in the containers defined in Clause 6. Possible changes shall be considered and storage conditions shall be designed accordingly in order to limit the effects of such changes on the results of ecotoxicity tests.

7.2 Waste sample

Storage time starting from reception of laboratory sample and ending with the start of definitive tests should be as short as possible.

A storage time of less than two months and low temperature conditions (4 ± 2) °C are usually appropriate to maintain the properties of waste samples (i.e. granular wastes (3.4), monolithic wastes (3.10), paste-like materials (3.11)).

Liquid wastes should be stored under the conditions defined in 7.3.

NOTE Freezing can induce changes of characteristics of the waste sample.

7.3 Water extracts

Water extracts should be stored at (4 ± 2) °C in polyethylene, polypropylene, polytetrafluoroethene (PTFE) or glass containers. Before testing, the containers shall be filled with a headspace less than 5 %.

NOTE 1 Freezing can induce changes of characteristics of water extracts.

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It is recommended to minimize the time between the start of the different tests to be performed on the same laboratory sample in order to minimize its changes.

Ecotoxicological tests shall start immediately after production of water extract as specified in the applicable standard for the considered ecotoxicity test and in no case later than 72 h after production of water extract. If a range-finding test and a definitive test are performed, the definitive test shall be completed within 10 days after production of the water extract.

For longer tests (e.g. semi-static chronic tests), several water extracts shall be produced and used within 10 days after production.

If definitive test results are not in accordance with the range-finding test, water extraction shall be repeated and the test shall be performed on the new water extract.

NOTE 2 It could be possible to extend the duration of storage if it has been proved that no modification of toxicity occurred within the storage period (e.g. carry out the same ecotoxicity test immediately after extraction period and at the end of storage period).

8 Waste characterization

The following characteristics shall be determined prior to the performance of ecotoxicity tests:

— For granular waste (3.4), monolithic waste (3.10), paste-like waste (3.11) and sludge (3.12):

- pH, according to EN ISO 10390;
- dry matter content, according to ISO 11465 (see Clause 9);
- water holding capacity, according to Annex A of EN ISO 14238:2012 (see note).

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NOTE The method described in Annex A of EN ISO 14238:2012 has been found to be appropriate for most kinds of waste.

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— For liquid waste:

- pH;
- Dissolved oxygen concentration;
- Conductivity.

9 Waste pre-treatment: particle size reduction (granular waste, monolithic waste, paste-like waste and sludge)

Both ecotoxicological and leaching tests are performed on material which originally and after pretreatment has a particle size less than 4 mm.

The tests shall be made on material with a grain size of at least 95 % (mass) less than 4 mm. Therefore, the laboratory sample shall be sieved (4.1). If oversized material exceeds 5 % (mass) the entire oversized fraction shall be crushed with crushing equipment (4.2). On no account shall the material be finely ground. Non-crushable material (e.g. metallic parts such as nuts, bolts, or scrap) in the sample shall be separated and the weight and nature of the material shall be recorded. The method of size-reduction applied shall be documented and recorded in the test report. Irrespective of any necessary size reduction, the separate fractions with the exception of the non-crushable material shall be mixed to constitute the test sample. If the laboratory sample cannot be crushed or sieved because of its moisture content, it is allowed, only in this case, to dry the laboratory sample. The drying temperature shall not exceed 40 °C.

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NOTE Fibrous materials, paste-like waste and plastics can often be size-reduced after cryogenic treatment. The sample is usually plunged into liquid nitrogen (- 196 °C) just before crushing to make it fragile and brittle. It also limits the overheating during crushing. As a result, the sample obtained is fine and perfectly homogeneous.

In order to minimize the possible contamination during the sieving, fragmentation and splitting, it is recommended, before preparing the test sample, to process a portion of the laboratory sample through the devices for sieving, fragmentation and splitting and to discard such material thereafter. This recommendation does not cover the situation described in the notes under 4.1 and 4.2.

For this document, any other waste pre-treatment is excluded; especially, the test sample which shall not be further dried. The determination of the dry matter content ratio and the moisture content ratio shall be determined on a dedicated test portion. The moisture content of the test sample shall be determined at (105 ± 5) °C. It shall be taken into account when adjusting the L/S ratio in leaching test. The dry mass of the sample shall be determined at (105 ± 5) °C according to ISO 11465 and the dry matter content ratio is calculated as follows:

$$DR = 100 \times \frac{M_D}{M_W} \quad (1)$$

where

DR is the dry matter content ratio (%);

M_D is the mass of the dried test portion (kg);

M_W is the mass of undried test portion (kg).

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The moisture content ratio is calculated as follows:

$$MC = 100 \times \frac{\left(M_W - M_D\right)}{M_D} \quad (2)$$

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where

MC is the moisture content ratio (%).

The basis for the calculation of the moisture content is the mass of the moisture content of the dry residue in this document, as specified in ISO 11465 (for the determination of the water content of soil). It should be noted that in EN 12880 (for the determination of water content of sludge), the water content is calculated on the basis of the raw mass.

The above moisture content determination could be not accurate enough in some cases (e.g. large amount of volatile or unstable compounds). In such cases a direct determination of the true water content should be performed and the moisture content ratio calculated accordingly.

10 Tests performed on terrestrial organisms

10.1 General considerations

The determination of ecotoxicological properties of wastes under conventional conditions requires using a dilution medium as inert as possible. This dilution medium shall allow the survival and the good development of organisms during the test period. Both requirements may be difficult to reconcile particularly considering plant growth inhibition tests and microbial tests (tests that required indigenous population of microorganisms).

In order to fulfil these requirements, the dilution medium called “artificial soil” shall be used unless otherwise specified in the standardized terrestrial test methods. The same medium shall be used for both control and dilution.

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Moreover, preparation of a medium should be reproducible to allow comparison of ecotoxicity tests results.

Several standardized ecotoxicity tests were considered to establish the following conditions for testing wastes on terrestrial organisms. This compilation of tests is given in Annex B.

Preparation of test mixtures may differ according to the type of waste and according to the ecotoxicity tests to be performed. Preparation of the different test mixtures is summarized in Table A.1 of Annex A.

10.2 Dilution medium

The dilution medium shall have the following composition (such as defined in ISO 11268-1):

— sphagnum peat finely ground and with no visible plant remains: 10 % (percentage expressed on dry mass basis);

— kaolinite clay containing not less than 30 % kaolinite: 20 % (percentage expressed on dry mass basis);

— industrial quartz sand (more than 50 % of particle size from 0,05 mm to 0,20 mm): 70 % (percentage expressed on dry mass basis).

Calcium carbonate ($CaCO_3$), pulverised and of recognized analytical grade is added to bring the pH of the wetted substrate to 6,0 ± 0,5 (generally between 0,5 % and 1 % of the mass of the dry ingredients).

Water (4.10) or mixture of water extract with water is added to the dilution medium to reach the percentage of the total water holding capacity recommended for each test organism.

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10.3 Introduction of waste into the dilution medium

10.3.1 General

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Preparation of test mixtures differs according to the waste to be tested (see Annex A). The different methods of preparation are described below.

10.3.2 Monolithic waste, granular waste, paste-like waste and sludge

Different methods can be applied to introduce the test portion into the dilution medium. Several parameters influence the selection of introduction method such as physical properties of waste or amounts to be tested. The following methods are recommended:

— *for small amounts*, introduce the test portion in the water (or in part of it) necessary to wet the dilution medium, then mix this suspension thoroughly with the dilution medium;

— *for large amounts*, mix the test portion thoroughly with the already hydrated dilution medium;

— *for hydrophobic waste*, mix the test portion thoroughly with the dilution medium, then add the water necessary to wet this mixture.

Test mixtures are expressed in percentages (dry mass of waste per total dry mass of test mixture).

10.3.3 Liquid sludge

Introduce the test portion in the water (or in part of it) necessary to wet the dilution medium, then mix this suspension thoroughly with the dilution medium in order to obtain the test mixture to perform the considered ecotoxicological test. Test mixtures are expressed in percentages (mass of waste per total dry mass of test mixture).