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Utgåva 1

Vattenundersökningar – Bestämning av akut toxicitet hos marina- eller brackvattensediment för märlkräftor (amfipoder) (ISO 16712:2005)

Water quality – Determination of acute toxicity of marine or estuarine sediment to amphipods (ISO 16712:2005)

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EUROPEAN STANDARD

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English Version

Water quality - Determination of acute toxicity of marine or estuarine sediment to amphipods (ISO 16712:2005)

Qualité de l'eau - Détermination de la toxicité aiguë des sédiments marins et estuariens vis-à-vis des amphipodes (ISO 16712:2005)

Wasserbeschaffenheit - Bestimmung der akuten Toxizität mariner Sedimente oder von Sedimenten aus Flussmündungsgebieten gegenüber Amphipoden (ISO 16712:2005)

This European Standard was approved by CEN on 11 September 2006.

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Foreword

The text of ISO 16712:2005 has been prepared by Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 16712:2006 by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by April 2007, and conflicting national standards shall be withdrawn at the latest by April 2007.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 16712:2005 has been approved by CEN as EN ISO 16712:2006 without any modifications.

Introduction

This International Standard outlines procedures for conducting acute tests for sediment toxicity, using one or more amphipod species that are found primarily below the sediment surface in coastal marine and estuarine waters. The biological endpoint for the test is percent mortality at day 10.

Water quality — Determination of acute toxicity of marine or estuarine sediment to amphipods

1 Scope

This International Standard specifies a method for the determination of acute toxicity to amphipods exposed over a period of 10 d to

a) samples of contaminated marine or estuarine sediment,

b) chemical, industrial or municipal sludge, or other solid wastes that may combine with marine or estuarine sediments, or

c) chemicals or preparations spiked into clean sediment.

2 Principle

Marine or estuarine amphipods which typically live below the sediment surface are exposed for 10 d to contaminated sediment or to sediment spiked with a test chemical. The endpoint for the test is percent mortality. The test is performed in 1-litre vessels with 175 ml of solid-phase sediment and overlying water. Salinity and temperature are dependent on the species of amphipod used in testing.

3 Test environment

3.1 Facilities

The test facility shall be well ventilated, isolated from physical disturbances and free from dust and fumes.

3.2 Lighting

All test vessels shall receive direct, overhead illumination that provides normal laboratory lighting (i.e. 500 lx to 1 000 lx) at the water surface. Illumination should be uniform and shall be continuous throughout the test period to inhibit the nocturnal migration of amphipods into the water column[39].

4 Reagents and materials

4.1 Test organism

4.1.1 General

One of the marine or estuarine sediment-dwelling amphipod species listed in Annex B should be used as test organism for the method in this International Standard. The species identification should be conducted using taxonomic keys[18] and confirmed by a qualified taxonomist familiar with identifying marine or estuarine amphipods.

4.1.2 Life stage and size

Amphipods of uniform age and size shall be used for testing and shall not be larger than the maximum allowable species size listed in Annex B. Do not use mature females bearing embryos, nor individuals longer than the maximum length (including antennae) identified in Annex B, as they might be senescent.

4.1.3 Source

All amphipods used in a test shall be derived from the same population and source. Test organisms can be either recently collected from an area in which contaminants are at or below background levels, or organisms can be cultured in a laboratory [11], [12], [48].

4.1.4 Collection, handling and transport

Depending on species and/or collection site conditions, collect amphipods using a benthic grab[1)], a small biological dredge or, in inter-tidal zones, a shovel. If a dredge is used, a short haul ($<$ 10 m) minimizes damage to the animals[39]. Collect at least one-third more individuals than are required for the test. Choose a collection site for which the presence of abundant organisms of the correct size and age has been demonstrated previously, or by pre-collection sieving of the sediment at the site[31]. The organisms to be used as the test species should be confirmed taxonomically (e.g. references [4], [5], [32]).

Measure and record the salinity, temperature and dissolved oxygen content of water near the sediment at the collection site. Sieve sediment samples at the time of collection through a sieve of mesh 0,5 mm to 1,0 mm. The choice of sieve size depends on the size of the species to be collected and is important for determining the number of amphipods recovered. The sieve shall be made of non-toxic materials.

Use collection-site water for sieving sediment in the field, and to cover the sediment in the container(s) during specimen collection and transport. Discard detritus and predators recovered by sieving. Transport the collected amphipods either at a cool temperature, with only moist inert material such as seaweed in the transport container, or with overlying water and the amphipods returned to the sieved sediment in the transport container(s). Aerate overlying water during transport. Deliver an additional portion of the sieved sediment to the laboratory to use for holding amphipods and as control sediment. Reserve a portion of sediment for physical analysis (e.g. grain-size) and chemical analyses. Alternatively, collect and transport amphipods in bulk sediment without sieving at the field site. However, predatory organisms shall be removed by hand-picking from containers before shipment.

Efforts should be made to maintain site-water collection temperature and salinity during transport. Temperature in the transport container shall not rise above the optimum range for specific amphipod species, as outlined in Annex B. Overlying seawater shall be aerated during transit.

4.1.5 Holding and acclimation

If necessary, field-collected specimens may be re-sieved upon return to the laboratory (0,5 mm to 1,0 mm screen, depending on size of amphipods to be used in the test), if one wishes to assess their survival and condition, to confirm species, and to select and count numbers of amphipods of a size suitable for testing. However, it shall be noted that re-sieving of field-collected amphipods in the laboratory puts an additional stress on the organisms. Use seawater from the collection site, another field site or reconstituted seawater, as overlying water in the transport container, maintaining the original salinity (within $\pm$ 2 g/kg) and temperature (within $\pm$ 2 °C) of the collection-site seawater during transport.

In the laboratory, slowly agitate a sieve immersed in seawater to isolate organisms and move them using a wide-bore pipette, spoon, or fine net. Ensure that sieved organisms are submersed in seawater at all times. To minimize stress, handle organisms carefully and quickly. Amphipods that are dropped, injured, or in contact with dry surfaces shall be discarded. Only active and apparently healthy amphipods shall be transferred into the

1) Smith-McIntyre and van Veen are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

holding/acclimation containers. Depending on the species, any individuals that fail to burrow or that emerge from the sediment at any time during the holding/acclimation period and appear dead or inactive when gently prodded shall be discarded. On the day of a test, select amphipods that are active and apparently healthy, and which have an appearance and behaviour typical of that species. Discard any animals that appear or behave atypically.

Count the amphipods selected for use in tests as they are transferred into holding containers (e.g. plastic trays or glass finger bowls). Place at least 2 cm to 4 cm of previously re-sieved control sediment (free of small amphipods and other organisms) and at least 2 cm to 5 cm of overlying seawater in these containers. The density of macroorganisms in the sediment should not exceed either that observed in the field or one amphipod per cm^2 to avoid crowding.

Place holding containers with organisms in one of the following:

a) a tank or trough with flowing seawater;

b) a large aquarium (e.g. 60 l to 100 l) containing reconstituted seawater or natural, clean seawater held under static conditions;

c) a smaller aquarium (e.g. 20 l to 40 l) containing seawater held under semi-static conditions (e.g. with daily renewal of 50 % of the seawater), unless a recycled water system with proper water treatment is used, in which case daily renewal of seawater is not required; or

d) a separate room with the appropriate temperature and lighting conditions.

A photoperiod of 16 h light and 8 h dark is recommended during amphipod holding/acclimation. The seawater in which holding containers are submersed should be aerated.

Field-collected or cultured amphipods shall be acclimated to test temperature and salinity conditions for a minimum of 3 d. Upon their arrival in the laboratory, acclimate amphipods from the field salinity conditions to the test salinity conditions by changing the salinity in the holding container at a rate of 5 g/(kg $\times$ d) (or slower depending on the species to be used). Acclimation of amphipods to test temperature conditions, within the holding/acclimation container, should not occur at a rate of temperature increase greater than 3 °C per day. Once test salinity conditions have been reached, hold organisms at that salinity for at least 24 h before testing.

Temperature, salinity, pH and dissolved oxygen content shall be monitored and recorded daily during the initial acclimation period, when the amphipods are acclimating to the test conditions. Thereafter temperature, salinity, pH and dissolved oxygen content should be measured during the remaining acclimation period, and shall be measured and recorded at the end of the acclimation period. Replace the overlying water continuously or periodically (i.e. daily or every second day) with air-saturated, fresh seawater adjusted to the required temperature and salinity. While the minimum duration of the holding/acclimation period for amphipods is 3 d, the holding period for field-collected test organisms shall not exceed 14 d before use in a test. The maximum holding time does not apply to laboratory-cultured test organisms. Amphipods shall not be fed during their period of acclimation or under test conditions.

4.2 Overlying water

4.2.1 General

Amphipods are to be held and acclimated using either an uncontaminated supply of natural seawater, or reconstituted seawater. The seawater supply used should be monitored and assessed as frequently as required to document its quality. Measure the salinity, pH, dissolved oxygen content, ammonia nitrogen, nitrite, relevant pesticides and metals of the seawater used.

De-ionized or distilled water is preferred for preparing reconstituted seawater. Dechlorinated municipal drinking water, natural surface water or groundwater may also be used.

Seawater used for holding, acclimating and testing amphipods shall be free of suspended matter. It is recommended that seawater be filtered ($< 5\ \mu m$) before use to ensure the removal of suspended particles and organisms. If stored, hold natural seawater within the range of temperature appropriate for the test species (see

Annex B) and use within a few days. For laboratories that have water treatment systems such as sand-bed filters, seawater may be held for longer periods as long as the quality of the water is closely monitored.

Prepare reconstituted seawater by adding hypersaline brine (HSB) or by direct addition of dry salts to a suitable fresh water, in quantities sufficient to provide the desired salinity[22]. HSB may also be prepared using commercially available dry ocean salts or reagent-grade salts[47], or by using reagent-grade chemicals to produce reconstituted salt water (Annex A). Reconstituted water should be homogeneous and aged for 1 week to 2 weeks [1], [2], then filtered ($\leqslant$ 5 μm) shortly before use to remove suspended particles, and should be used within 24 h of filtration[46].

Reconstituted seawater is prepared by adding specified amounts of a suitable salt reagent to high-purity distilled or de-ionized water[47]. Suitable salt reagents can be reagent grade chemicals or commercial sea salts. Pre-formulated brine (e.g. 60 % to 90 %) prepared with dry ocean salts or heat-concentrated natural seawater can also be used.

4.2.2 Salinity

The choice of the appropriate test salinity conditions depends on the salinity of the pore water of the test sediment, the range of salinity tolerance for the test species[10] and the test objectives. For evaluations of marine or estuarine sediments, the acclimation and test salinity can range between 1 g/kg and 35 g/kg, depending on the test species chosen (see Annex B). Salinity can be adjusted by the addition of dry ocean salts or brine (if too brackish), or distilled water (if too saline).

4.2.3 Dissolved oxygen content

The dissolved oxygen content of the seawater overlying the sediment shall be 85 % of the air-saturation value or higher during the test organism holding or acclimation period, at test initiation and throughout the 10-d test. Maintain this level of dissolved oxygen by gentle aeration of the seawater, using filtered, oil-free compressed air, but the rate of aeration should not suspend the sediment.

5 Apparatus

Use ordinary laboratory apparatus and the following for organism holding or culturing and testing[3], [18], [31], [46]. Before initiating a test, ensure all test vessels and associated labware are clean and free of all contaminants from previous use[1], [28].

5.1 Environmental controls, apparatus to control temperature and light intensity.

5.2 Measuring apparatus and/or instruments for measuring dissolved oxygen content, pH, salinity, total organic carbon, ammonia, nitrate, light intensity and temperature.

5.3 Containers

Containers and accessories, such as sieves, that might contact the organisms, control or test sediment, and seawater during sorting, handling, holding and acclimation shall be made of non-toxic materials (e.g. glass, stainless steel, polyolefin, nylon, porcelain, polyethylene, polypropylene, fibreglass) cleaned and rinsed with distilled water, de-ionized water, dechlorinated laboratory water, reconstituted seawater or natural seawater from the collection site or an uncontaminated source.

Materials such as copper, zinc, brass, galvanized metal, lead and natural rubber shall not come in contact with this apparatus and equipment, or with samples of control, reference or test sediment, seawater, or test vessels.

1-litre glass containers (beakers or wide-mouthed jars) with internal diameter of approximately 10 cm are recommended for use as test vessels. Cover each vessel with a glass or a plastic lid to reduce the possibility of contamination of the contents and to reduce evaporation.

6 Treatment and preparation of samples

6.1 General

Collect sediment from reference, control and test sites following established practices[1], [3], [18], [19], [20] or, if required, add a test chemical or preparation to a sample of control sediment[1], [18], [20]. Similar sediment collection and handling procedures for both test and reference sediments should be used within the same testing programme. Store collected sediment in a sealed container in darkness at 4 °C ± 2 °C until required for the toxicity test. Drying, freezing and cold storage all affect toxicity and bioavailability of chemicals in sediment. Initiate sediment tests as soon as possible to maintain chemical integrity, but preferably within 5 d and not after 30 d unless chemical stability can be assured. Analysis of known chemical contaminants may be conducted on sediment samples from the field, and results may be compared to analysis of sediment at the beginning and end of the test to quantify any changes in chemical concentration or form.

6.2 Control or reference sediment

Control sediment obtained from the amphipod-collection site can be used as the negative-control sediment for a test, as a clean material for spiking a test chemical, or for organism culture. Clean reference sediment can be used as an additional experimental control.

6.3 Test sediment

Collect test sediment from the site to be evaluated using apparatus such as coring[2)] or grab[3)] devices. Sediment is taken from the middle of the sampler that has not been in contact with the apparatus. Typically, the top 2 cm to 4 cm of sediment representing the oxic zone is collected and composited from sufficient samples of the site to meet the needs of the test. A deeper depth of sediment may be collected for testing depending on the objective of the study. Transfer the sediment with a non-reactive, pre-cleaned scoop to an inert vessel, and mix the composited sample until colour and texture are uniform. Store the composited sample in a clean brown glass container (if organics are suspected contaminants), or in a clean high-density polyethylene or polycarbonate container (if metals are suspected contaminants). Fill containers to capacity and transport to the laboratory at 4 °C ± 2 °C.

Apparatus should be cleaned between sites to prevent cross-contamination (see Clause 5). Retain any solvent cleaning wastes and return to the laboratory for disposal.

6.4 Preparation of sediment samples

Remove large particles (> 1 cm) and indigenous organisms by hand sorting using tweezers or a similar instrument. Sieving the sediment for this purpose is not recommended, because water-soluble contaminants and fine non-settling clay particles could be lost. Adjust the water to the test temperature and test salinity appropriate for the test species (see Annex B) and aerate to a dissolved oxygen content of 85 % saturation or higher. If control/reference sediment is to be used at the completion of the test for determining the ability of surviving amphipods to rebury, then re-seal and refrigerate the required sediment.

Chemical and physical characterization of the sediment sample is helpful in the interpretation of results. Allow the sieved sediment to settle for at least 4 h to recover fines, before submitting it for particle size and chemical analyses. Analyse a sub-sample of the sediment for the following: total organic carbon and particle size distribution (percentage gravel, coarse and fine sands, and silt and clay), and pore water salinity (before sieving in the laboratory). Further characterization can include total volatile residue, acid-volatile sulfides (AVS)/simultaneously extracted metals (SEM), percent water content, biochemical and/or sediment oxygen

2) A Phleger box is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) Ekman, Ponar, van Veen, Petersen, Shipek and Kajak-Brinkhurst are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.