



SLOVENSKI STANDARD

SIST ISO 14371:2013

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Kakovost vode - Določevanje strupenosti sedimenta celinskih vod s *Heterocypris incongruens* (Crustacea, Ostracoda)

Water quality - Determination of fresh water sediment toxicity to *Heterocypris incongruens* (Crustacea, Ostracoda)

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Qualité de l'eau - Détermination de la toxicité des sédiments d'eau douce envers *Heterocypris incongruens* (Crustacea, Ostracoda)

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ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water
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**Water quality — Determination of fresh
water sediment toxicity to *Heterocypris
incongruens* (Crustacea, Ostracoda)**

*Qualité de l'eau — Détermination de la toxicité des sédiments d'eau
douce envers Heterocypris incongruens (Crustacea, Ostracoda)*

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ISO 14371:2012(E)

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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

ISO 14371 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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Introduction

The evaluation of harmful effects on water quality has for several years involved the performance of biological tests. Historically, toxicity tests have mainly focused on the impact of pollutants present in the water column of aquatic ecosystems, without considering the hazard of toxicants present and accumulating in the sediments.

“Direct contact” tests in which the test organisms are exposed to the whole sediment have been gradually developed with endobenthic species, such as chironomid larvae [*Chironomus riparius* or *Chironomus dilutus* (formerly *C. tentans*)] or epibenthic amphipod crustaceans (*Hyaella azteca*).

The test specified in this International Standard is a direct contact test for determination of the percentage mortality and/or growth inhibition on the fresh water ostracod *Heterocypris incongruens* (Ramdohr, 1808) after 6 d exposure to a whole sediment (see References [1], [2]).

H. incongruens is a cosmopolitan ostracod species, which has to date already been used extensively for toxicity testing not only of whole sediments, but also by extension on sludges and soils (see References [3]–[21]).

The direct contact test with *H. incongruens* has a sensitivity which is quite similar to that of the amphipod crustacean *Hyaella azteca* and the midge larva *Chironomus riparius* (see References [22]–[25]).

The assays are performed with neonates hatched from dormant eggs (cysts), which bypasses the need for culturing or maintaining live stock cultures of test organisms.

H. incongruens neonates (150 µm to 200 µm) are substantially smaller than *Hyaella azteca* and *Chironomus riparius*, and the assays can be performed in much smaller test containers, hence require much less bench space and incubator space.

The effects are evaluated after a shorter exposure time (6 d) than in the assays with the amphipod crustacean (10 d to 28 d) and midge larvae species (10 d to 28 d).

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Water quality — Determination of fresh water sediment toxicity to *Heterocypris incongruens* (Crustacea, Ostracoda)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably qualified staff.

1 Scope

This International Standard specifies a method for the determination of lethal as well as sublethal effects of contaminated sediments on the ostracod crustacean *Heterocypris incongruens* after 6 d exposure.

The method is applicable not only to fresh water sediments, but also by extension to solid wastes and soils after addition of (uncontaminated) water.

The method can also be applied to chemicals or preparations which are spiked into a reference sediment.

This International Standard is not applicable to the testing of sediments from the estuarine or marine environment.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-15:2009, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples*

ISO 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

3 Terms and definitions

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

3.1

neonate

newly hatched individual

3.2

pre-feeding

feeding of the neonates (3.1) with a small amount of dried algae (*Spirulina*) prior to the test

4 Principle

Freshly hatched *Heterocypris incongruens* larvae are exposed to the sediment sample under analysis and the percentage mortality of the test organisms is determined after 6 d exposure.

If the percentage mortality is low (e.g. <30 %), the growth inhibition of the organisms in the sediment sample is determined in comparison to the control sediment, as a sublethal effect criterion.

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5 Test environment

The test shall be carried out in the dark, in a temperature-controlled room or incubator at (25 ± 1) °C in the test containers.

Maintain the atmosphere free from toxic dusts or vapours. The use of control solutions is a double check that the test is performed in an atmosphere free from toxic dusts and vapours.

6 Reagents, test organisms and media

Use only reagents of recognized analytical grade, unless otherwise specified.

6.1 Test organisms. The test organisms are neonates of the fresh water ostracod *Heterocypris incongruens* which are hatched from dormant eggs (cysts) of this crustacean.

Cysts of *H. incongruens* are obtained from laboratory cultures, as described in Annex A, or can be purchased from a specialized company¹⁾.

6.2 Pure water, having a conductivity below 10 µS/cm.

6.3 Test medium, prepared by dissolving the following mineral substances in 1 l of pure water (6.2):

NaHCO ₃	96 mg
CaSO ₄ •2H ₂ O	60 mg
MgSO ₄	60 mg
KCl	4 mg

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This test medium corresponds to a synthetic water of moderate hardness, i.e. containing CaCO₃ at concentrations of 80 mg/l to 100 mg/l (see Reference [26]). Thus prepared, the medium has a pH of $7,6 \pm 0,3$.

The test medium can be used for two weeks when stored in a refrigerator (4 °C to 7 °C) in the dark.

Aerate the test medium until the dissolved oxygen concentration has reached the air saturation value and until the pH has stabilized. If necessary, adjust the pH to $7,6 \pm 0,3$ using a sodium hydroxide or hydrochloric acid solution. The concentration of the acid or base required shall be selected so that the volume to be admixed is as small as possible. Bring the temperature of the test medium up to (25 ± 1) °C prior to use.

6.4 Algal food.

6.4.1 Spirulina suspension. A suspension of powder of the blue-green alga *Spirulina platensis* prepared by mixing 150 mg dry mass *Spirulina* in 10 ml pure water (6.2). *Spirulina* powder is available commercially in health stores.

6.4.2 Green algae. A 25 ml suspension of green algae (e.g. *Scenedesmus* spp.) at a concentration of around $1,5 \times 10^7$ cells/ml, prepared with test medium (6.3).

The algae can also be encapsulated in algal beads, which can be stored for long periods of time in a refrigerator, and “de-immobilized” at the time of use (see ISO 8692:2012^[31], Annexes B and C).

1) MicroBioTests Inc., Mariakerke (Gent), Belgium, is an example of a supplier able to provide suitable *Heterocypris incongruens* cysts. This information is given for the convenience of the users of this document and does not constitute an endorsement by ISO of this supplier.

6.5 Lugol solution, to immobilize and fix the test organisms at the end of the test.

Lugol solution is prepared by mixing 5 g iodine (I₂) and 10 g potassium iodide (KI) with 85 ml pure water (6.2).

6.6 Control sediment. Commercial (not toxic and not calcareous) river sand or marine sand, which is water washed and sieved to eliminate dirt and debris, and air-dried is a suitable control sediment²⁾ provided it is of the category “medium size” sand, with a granulometry 0/2 (i.e. a particle size range between 0 mm and 2 mm).

At the time of testing, the control sediment shall first be “water saturated” as follows.

Fill a small glass beaker with control sediment and pour test medium (6.3) on it until the sediment is completely wet. During this operation, the sediment shall be stirred with a spatula to ensure complete water saturation. Supernatant test medium shall be decanted.

6.7 Reference substance. Copper sulfate pentahydrate (CuSO₄•5H₂O) is the recommended reference chemical.

7 Apparatus and material

Usual laboratory apparatus and, in particular, the following.

7.1 Temperature-controlled room or chamber.

7.2 Petri dishes, diameter 5 cm, glass or inert plastic material for hatching of the cysts and transfers of the test organisms.

7.3 Test containers. Disposable microplates made from chemically inert material, with wells of a capacity of at least 10 ml. Six (2 × 3) well microplates with a well diameter of approximately 35 mm are suitable.

7.4 Micropipette, in glass for sampling the test organisms, with an internal diameter of ~1 mm at the tip, for capturing the animals while allowing sampling of only a small volume of medium.

Micropipettes provided with a bulb at the end are very suitable for the operations.

7.5 “Large mouth” micropipettes, in an inert plastic material and with a wide opening, for transfer of sediment suspensions.

7.6 Spatula scoop, stainless steel or inert plastic material, with a capacity of 500 µl or 1 000 µl.

7.7 Flat spatula, stainless steel or inert plastic material.

7.8 Microsieve, with a 100 µm mesh.

7.9 Stereomicroscope with incident (bottom) illumination, with a magnification of at least 8 times and, if possible, a continuous magnification.

7.10 Calibrated eyepiece for the stereomicroscope, for length measurements of the test organisms.

7.11 Light source, providing a range of light intensity in the hatching Petri dish (7.2) of 3 000 lx to 4 000 lx.

7.12 Sample-collecting containers, in accordance with ISO 5667-15:2009, Clause 7.

2) River sand or marine sand can be obtained in every country from aquarium shops.