



SLOVENSKI STANDARD
SIST ISO 20665:2010

01-september-2010

Kakovost vode - Določevanje kronične strupenosti s Ceriodaphnia dubia

Water quality - Determination of chronic toxicity to Ceriodaphnia dubia

Qualité de l'eau - Détermination de la toxicité chronique vis-à-vis de Ceriodaphnia dubia

Ta slovenski standard je istoveten z: ISO 20665:2008

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ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water
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en,fr

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INTERNATIONAL
STANDARD

ISO
20665

First edition
2008-12-15

**Water quality — Determination of chronic
toxicity to *Ceriodaphnia dubia***

*Qualité de l'eau — Détermination de la toxicité chronique vis-à-vis de
Ceriodaphnia dubia*

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Reference number
ISO 20665:2008(E)

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Published in Switzerland

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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.

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 20665 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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Introduction

The highlighting of harmful effects for water quality has for several years involved the carrying out of biological tests. The Cladocera, *Ceriodaphnia dubia*, is recognised as being representative of the zooplankton species widely used in aquatic toxicity tests.

The shortness of the chronic toxicity test, (7 ± 1) d, and the low volumes used are major assets for obtaining relevant results on samples that may be subject to changes during the storage period.

The user should be aware that particular problems could require the specifications of additional marginal conditions.

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Water quality — Determination of chronic toxicity to *Ceriodaphnia dubia*

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International 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 according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of the chronic toxicity to *Ceriodaphnia dubia* (Cladocera, Crustacea), based on reproduction inhibition after (7 ± 1) d.

The method is applicable to:

- a) chemical substances which are soluble or which can be maintained as stable suspensions or dispersions under the conditions of the test;
- b) industrial or sewage effluents, if appropriate after decantation, filtration or centrifugation;
- c) fresh waters;
- d) aqueous extracts.

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

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 5667-16:1998, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

ISO 6059, *Water quality — Determination of the sum of calcium and magnesium — EDTA titrimetric method*

ISO 10523, *Water quality — Determination of pH*

ISO/TS 20281, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

ISO 20665:2008(E)

3 Terms and definitions

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

3.1 brood
group or cohort of sibling offspring, consisting of two or more neonates in any test container, during any given day of the test, released from the adult female during an inter-moult period (i.e. before the carapace is shed by that female during moulting)

3.2 brood organism
healthy adult female daphnid that produces and releases multiple broods of live neonates

3.3 control batch
series of replicates containing **control solution** (3.4)

NOTE In this International Standard, 10 replicates constitute the control batch.

3.4 control solution
mixture of test medium and of food without sample under test

3.5 effective concentration producing x % reproduction inhibition
 EC_x
estimated concentration of a test sample giving rise to x % **reproduction inhibition** with respect to the **control batch** (3.3), which represents a point of the test sample concentration that is estimated to cause a designated percent impairment in a quantitative biological function

3.6 neonate
newly born or newly hatched individual

NOTE In this International Standard, a neonate is a first-instar daphnid, < 24 h old.

3.7 reproduction inhibition
comparison between the number of living offspring born from all adults at the end of the test between the **control batch** (3.3) and the **test batch** (3.8)

3.8 test batch
series of replicates containing the same **test solution** (3.9)

NOTE In this International Standard, 10 replicates constitute a test batch.

3.9 test solution
mixture of test medium, of food and of sample under test

4 Principle

Ceriodaphnia dubia, less than 24 h old at the beginning of the test, are exposed individually to a range of concentrations of the sample under test for a period of (7 ± 1) d. The test typically ends after 7 d when 60 % of the control organisms have produced their third brood. The mortality of the adult females and their reproduction are monitored throughout the exposure time. All other relevant biological parameters can also be studied.

The data obtained allow, using a suitable model, the calculation of the concentration which gives rise to x % reproduction inhibition, EC_x , e.g. EC_{10} , EC_{20} or EC_{50} .

5 Test environment

Carry out the test in a temperature-controlled room or chamber at (25 ± 2) °C in the test containers. Ensure that, within one test, the temperature does not vary by more than 2 °C.

Adjust the day/night test cycle (photoperiod) to 16 h of daylight and 8 h of darkness. In the test containers (7.2), a range of lighting intensity at the air/water interface of 100 lx to 600 lx (7.8) is recommended. Do not shake or aerate 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 being performed in an atmosphere free from toxic dusts and vapours.

6 Reagents, test organisms and media

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

6.1 Test organisms

Ceriodaphnia dubia neonates are obtained by parthenogenesis from adult females for at least three generations under the conditions of temperature, photoperiod and food identical to those in the test.

The *Ceriodaphnia dubia* used for the test shall be less than 24 h old and shall have been taken from a brood comprising at least eight newly born animals.

The day before the test, isolate from the culture a dozen or more adults that are over 6 d and less than 14 d old. Isolate each one in a separate container containing food (6.4.1 or 6.4.2) and test medium (6.3.2 or 6.3.3). Before the test, remove the adults from their containers and count the offspring. Discard all vessels containing less than eight live offspring.

The *Ceriodaphnia dubia* may also derive from the hatching of ephippia purchased from a specialised company¹⁾. These organisms may be directly used as test organisms.

6.2 Pure water, having a conductivity below 10 $\mu\text{S}/\text{cm}^2$.

6.3 Test media

6.3.1 General

Two test media are recommended: ELENDET M4 (6.3.2) or moderately hard water (6.3.3). Alternative test media may be used as long as validity criteria (Clause 11) are met.

For alternative test media, supply either a reference to a publication, or for natural waters (in case of effluent testing) the date of collection, details of storage, handling, and additions, as well as physical chemistry data relating to major ions [Na(I), K(I), Ca(II), Mg(II), carbonates, chloride, sulfate], pH and dissolved organic carbon.

1) Microbiotest, Deinze, Belgium, is an example of a supplier able to provide suitable ephippia commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this supplier.

2) 1 mS/m.