INTERNATIONAL STANDARD

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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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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*.

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.

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*

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 company[1)]. These organisms may be directly used as test organisms.

6.2 Pure water, having a conductivity below 10 µS/cm[2)].

6.3 Test media

6.3.1 General

Two test media are recommended: ELENDT 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.

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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.

6.3.2 ELENDT M4 medium option

Prepare ELENDT M4 test medium in accordance with Annex A. The test medium thus prepared shall have a pH of 8,0 ± 0,3 (measured as specified in ISO 10523), a total hardness of (250 ± 20) mg/l (expressed as $CaCO_3$ and measured as specified in ISO 6059). Aerate the test medium until the dissolved oxygen concentration has reached the air saturation value and until the pH has stabilised. If necessary, adjust the pH to 8,0 ± 0,3 using a diluted solution of sodium hydroxide or hydrochloric acid.

NOTE On account of the high hardness of the test medium and of the presence of EDTA within this medium, the bioavailability of the bivalent metal ions can be reduced, thus resulting in a decrease in the apparent toxicity of these ions.

6.3.3 Moderately hard water medium option

Prepare moderately hard water in accordance with Annex B. The test medium thus prepared shall have a pH of 7,4 to 7,8 (measured as specified in ISO 10523) and a total hardness of (90 ± 10) mg/l (expressed as $CaCO_3$ and measured as specified in ISO 6059).

6.4 Food

6.4.1 Option 1: Fish food and two algae diet

The food is composed of fish food, *Chlorella vulgaris* algae and *Pseudokirchneriella subcapitata*3) algae (formerly known as *Selenastrum capricornutum* and *Raphidocelis subcapitata*) (see NF T90-376[1] and Reference [2]).

Prepare a 5 g/l suspension of fish food4) in the test medium (6.3.2), homogenised with a crusher or any other means allowing particles of a few micrometers to be obtained. Prepare this suspension each day for the daily feeding of cultures or during testing.

Grow the algae separately in any suitable medium (e.g. LC OLIGO, Annex C). Use them when the culture is in the exponential growth phase and has reached a density greater than 5×10^6 cells per millilitre. These cultures may be stored at (4 ± 3) °C, in darkness, for a maximum period of 10 d.

The following constituents should be added to each test solution (9.3) before the transfer of organisms:

a) 12×10^6 cells per litre of *Chlorella vulgaris*;

b) 6×10^6 cells per litre of *Pseudokirchneriella subcapitata*;

c) 500 µl per litre of the fish food suspension.

In any case, the quantity of food shall not constitute more than 10 % of the final volume of each container (9.3).

The use of *Chlorella vulgaris* and/or *Pseudokirchneriella subcapitata* algae immobilised in an inert matrix (gelose), in the form of algae beads5) is possible. In this case, after dissolving the matrix, centrifuge the algae, discard the supernatant, and resuspend the algae by shaking in the test medium (6.3.2 or 6.3.3). Repeat this operation a second time. The algal concentration in the test medium shall be the same as described above.

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3) The Freshwater Biological Association, Ambleside, UK, is an example of a supplier able to provide these algae commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this supplier.

4) Sera Micron is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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

6.4.2 Option 2: Yeast/Cerophyll[6)]/trout chow and one algae diet

A second food combination based on the US EPA, method 1002.0 (Reference [11], p. 141) and Environment Canada (Reference [5]) test methods is recommended (see also References [3] and [4]). Daily feeding with Yeast/Cerophyll/trout chow (YCT) and a single algal species is required for culturing and testing of *Ceriodaphnia*. The algal species most commonly used is *Pseudokirchneriella subcapitata*.

The formula for preparing YCT is given in Annex D. If the YCT/one algae diet is used, mass cultures should be fed at a rate of:

— 7 ml algae concentrate per litre culture;

— 7 ml YCT concentrate per litre culture.

Individual cultures should be fed at the rate of:

— 0,1 ml algae concentrate per 15 ml culture;

— 0,1 ml YCT concentrate per 15 ml culture.

Food should be added to fresh culture medium immediately before or after the transfer of organisms.

Thoroughly mix algal concentrate and YCT by shaking before dispensing. If the YCT is stored frozen, store thawed aliquots at (4 ± 3) °C. Discard unused portions of unfrozen or thawed YCT after 2 weeks. Store unused portions of algal concentrate at (4 ± 3) °C, in darkness, and discard after 10 d.

6.5 Reference substance

Sodium pentachlorophenolate (C_6Cl_5ONa), copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium chloride ($NaCl$), or zinc sulfate ($ZnSO_4$) are acceptable.

CAUTION — If sodium pentachlorophenolate is used as a reference toxicant, the material safety data sheet should be consulted prior to use by laboratory personnel due to the hazardous nature of this substance.

7 Apparatus

Usual laboratory apparatus and in particular the following.

7.1 Temperature-controlled room, chamber or **water bath**.

7.2 Test containers, made from a chemically inert material.

If closed containers are used, make sure that the capacity is sufficient to allow for a gas phase/aqueous phase volume ratio of 1:1.

Prior to use, rinse the containers with the test medium (6.3.2 or 6.3.3) or with pure water (6.2).

7.3 Device for the measurement of algal concentration, e.g. a microscope equipped with a haemocytometer or a particle counter. Indirect methods (e.g. spectrophotometer, turbidimeter, fluorimeter) can be used if an acceptable correlation with the cellular concentration can be established.

6) Cereal Grass Media – Cerophyll is a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

