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Water quality — Determination of the acute toxicity to the freshwater rotifer *Brachionus calyciflorus*

Qualité de l'eau — Détermination de la toxicité aigue envers le rotifère d'eau douce Brachionus calyciflorus

Reference number
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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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Introduction

The evaluation of harmful effects on chemicals and pollutants on the biota in freshwater environments has for several years involved the performance of biological tests.

Rotifers, and especially the species *Brachionus calyciflorus*, are of interest from the ecotoxicological standpoint, because they are often an important component of the zooplankton and serve as prey for small fish and larger invertebrates.

The test specified in this International Standard involves determination of the lethal effects of toxicants to the freshwater rotifer *Brachionus calyciflorus*, after 24 h exposure.

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Water quality — Determination of the acute toxicity to the freshwater rotifer *Brachionus calyciflorus*

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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 document be carried out by suitably qualified staff.

1 Scope

This International Standard specifies a method for the determination of the lethal effects of toxicants to *Brachionus calyciflorus* after 24 h exposure.

The method is applicable to:

a) chemical substances which are soluble, or which can be maintained as a stable suspension of dispersions under the conditions of the test;

b) industrial or sewage effluents, treated or untreated, if appropriate after decantation, filtration or centrifugation;

c) freshwaters;

d) aqueous extracts.

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

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

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
control batch
series of replicates containing control solution

[SOURCE: ISO 6341:2012, definition 3.1]

3.2
LC_{50}
concentration of dilution of the test sample which gives rise to 50 % mortality of the test organisms

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3.3
test batch
series of replicates filled with the same test solution

[SOURCE: ISO 6341:2012, definition 3.6]

3.4
pure water
deionized or distilled water with a conductivity below 10 µS/cm

4 Principle

The test organisms are exposed to a range of concentrations of the sample under analysis and the percentage mortality of the test organisms is determined after 24 h exposure, with subsequent calculation of the 24 h LC_{50}.

The test is carried out in one or two stages:

— a "range-finding test" to determine the range of concentrations or dilutions needed for calculation of the 24 h LC_{50};

— a "definitive test" conducted when the data of the range-finding test are not sufficient or adequate for calculation of the 24 h LC_{50}.

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 tests organisms are females of the species *Brachionus calyciflorus*, obtained from a laboratory culture (see References [2],[3],[4]), or hatched from commercially available cysts[1)].

The procedure for hatching of *Brachionus calyciflorus* from cysts is described in Annex A.

6.2 Culturing and dilution medium

A natural or an artificial freshwater may be used as the water for stock culturing the rotifers, or as dilution water for the testing. Natural freshwater shall be collected from an unpolluted location; it must be filtered (30 µm) and conditioned to test temperature and oxygen saturation prior to use. Natural freshwater can be stored cold (4 ± 1) °C for several weeks.

An example of artificial freshwater suitable for culturing and testing is given in Annex B.

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

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