INTERNATIONAL STANDARD

ISO 19820

First edition
2016-03-01

Water quality — Determination of the acute toxicity to the marine rotifer *Brachionus plicatilis*

Qualité de l'eau — Détermination de la toxicité aigue envers le rotifère marin Brachionus plicatilis

Reference number
ISO 19820:2016(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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*.

Introduction

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

Rotifers, and especially the species *Brachionus plicatilis*, are of interest from the ecotoxicological view 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 marine rotifer, *Brachionus plicatilis*, after 24 h or 48 h exposure (depending on the intended use of the results). Prolongation of the exposure to 48 h is advised since it substantially increases the sensitivity of the assay.

Water quality — Determination of the acute toxicity to the marine rotifer *Brachionus plicatilis*

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 plicatilis* after 24 h or 48 h exposure.

The method is applicable to the following:

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) marine or estuarine waters;

d) sediment elutriates/eluates.

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 10523, *Water quality — Determination of pH*

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, 3.1]

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

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

[SOURCE: ISO 6341:2012, 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 and/or 48 h exposure with subsequent calculation of the 24 h LC_{50} and/or the 48 h LC_{50}.

Prolongation of the test to 48 h is recommended since it substantially increases the sensitivity of the assay.

The test is carried out in one or two of the following stages:

— a "range-finding test" to determine the range of concentrations or dilutions needed for calculation of the 24 h LC_{50} or the 48 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} or the 48 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 plicatilis* obtained from a laboratory culture (see References [5],[7], and [8]) or hatched from commercially available cysts.[1)]

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

6.2 Culturing and dilution medium

A natural or an artificial seawater may be used as the water for stock culturing the rotifers or as dilution water for the testing. Natural seawater shall be collected from an unpolluted location and should have salinity between 29 ‰ and 36 ‰. Natural seawater must be filtered (30 µm) and conditioned to test temperature and oxygen saturation prior to use. Natural seawater can be stored cold (4 ± 1) °C for several weeks.

An example of artificial seawater 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 plicatilis* 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.

6.3 Reference substance

Potassium dichromate ($K_2Cr_2O_7$) or copper sulfate ($CuSO_4 \cdot 5H_2O$) are recommended as reference chemicals.

NOTE Since $K_2Cr_2O_7$ is a carcinogenic substance toxic via inhalation, the use of a ready-made solution with a defined concentration of $K_2Cr_2O_7$[2)] for the preparation of the stock solution of the reference substance can reduce the risk of inhalation of the toxic dust in the laboratory.

7 Apparatus

Usual laboratory equipment and, in particular, the following.

7.1 Temperature-controlled room or chamber.

7.2 Petri dishes.

Small Petri dishes (diameter 5 cm) in glass or in inert plastic material.

7.3 Test containers.

Disposable 48 (6 × 8) microplates made from chemically inert material.

7.4 Pipette for sampling rotifers, with a sufficient diameter for capturing the animals while allowing sampling of only a small volume of medium.

For example, single use 1 ml capillary mini-pipettes are suitable.

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

7.6 Light source, providing a range of light intensity in the hatching Petri dish of 3 000 lx to 4 000 lx corresponding to 40 $\mu mol.m^{-2}.s^{-1}$ to 55 $\mu mol.m^{-2}.s^{-1}$.

7.7 Sample collecting bottles, as specified in ISO 5667-16.

8 Treatment and preparation of samples

8.1 Special precautions

Special precautions are required for sampling, transportation, storage, and treatment of seawater or effluent.

Sampling, transportation, and storage of the samples should be performed as specified in ISO 5667-16.

Carry out the toxicity test as soon as possible, ideally within 12 h of collection. If this time interval cannot be met, cool the sample to 0 °C to 5 °C and test the sample within 24 h. If it is not possible to perform the test within 72 h, the sample may be frozen and maintained deep-frozen (below –18 °C) for testing within two months of collection, provided that characteristics are known to be unaffected by freezing. At the time of testing, homogenize the sample to be analysed by shaking manually, and, if necessary, allow to settle for 2 h in a container and sample by drawing off (using a pipette) the required quantity of supernatant maintaining the end of the pipette in the centre of the section of the test tube and halfway between the surface of the deposited matters and the surface of the liquid.

2) Titrisol potassium dichromate solution is an example of a suitable product available commercially. This information is given for the convenience of the users of this document and does not constitute an endorsement by ISO of this product.

If the raw sample of the decanted supernatant is likely to interfere with the test (due to the presence of residual suspended matter, protozoa, microorganisms, etc.), filter or centrifuge the raw or decanted sample.

The sample obtained by either of these methods is the sample submitted to testing.

Measure the dissolved oxygen concentration (as specified in ISO 5814) and record the value (mg/l) in the test report.

8.2 Preparation of the stock solutions of substances to be tested

Prepare the stock solution of the substance to be tested by dissolving a known quantity of substance in a specified volume of test medium (6.2) at the time of use. However, if the stock solution of the substance is stable under certain conditions, it may be prepared in advance and stored under these conditions.

For substances sparingly soluble in the test medium, refer to the specifications given in ISO 5667-16.

9 Procedure

9.1 Selection of test concentrations

The test should comprise at least five concentrations of the sample to be tested. The dilutions shall be selected within a geometric series with a separation factor which depends on the nature of the sample to be analysed (chemical substances, effluents, waters) and of the type of assay (range finding or definitive).

For the range finding test with chemical substances, the separation factor for the serial dilutions is usually ten (one order of magnitude difference between two successive dilutions).

For effluents or waters, a 1:1 dilution factor is normally applied (i.e. dilution of the previous concentration by half).

Dilutions series for the definitive test on chemical substances are prepared with a separation factor not exceeding 3,2, whereas for effluents and waters, a 1:1 dilution factor is normally applied.

The test is carried out with six replicates for each dilution plus a control (i.e. the test medium without sample) also in six replicates.

When using a solvent in order to dissolve or disperse chemical substances, a preliminary test has to be performed to determine whether the highest concentration of the solvent used in the dilution series does not have a negative impact on the test organisms.

9.2 Preparation of the test and control solutions

For testing of samples of lower salinity than seawater (e.g. estuarine water), the salinity of the dilution medium must be adjusted to the salinity of the sample by dilution with pure water.

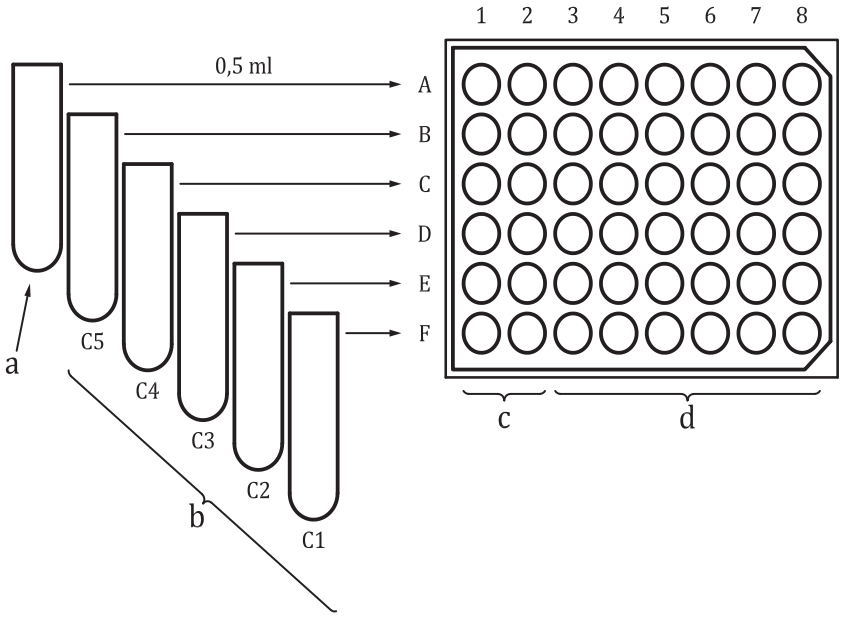
NOTE *Brachionus plicatilis* is a euryhaline species and toxicity tests can even be performed at salinities as low as 5 ‰.

Prepare the test solutions by mixing the appropriate volumes of the sample to be tested (see Clause 8 and 9.1) or of its initial dilution with dilution medium (6.2).

Depending on the purpose of the test, the exposure concentrations used in the test solutions at the start and end of the study should be verified by specific chemical analysis.

Control and test solutions can be prepared in 10 ml containers (e.g. tubes in glass or in inert plastic material).

The containers shall be labelled as control, C1, C2, C3, C4, and C5 in sequence of the highest to the lowest test concentration.



Key

a control medium
b toxicant dilutions
c rinsing wells
d replicates

Figure 1 — Filling of the microplate with control and test solutions

Distribute the test and control solutions in the microplate at the rate of 0,5 ml per well and according to the spatial distribution of the solutions in the wells as shown in Figure 1.

The 48 wells microplate has 6 rows (A to F) and 8 columns (1 to 8).

The 8 wells in the top row (row A) are filled with the control medium [= the dilution medium (6.2)].

The wells of the other rows are filled with the toxicants [test batches (3.3)] as follows: the 8 wells in row B are filled with the lowest toxicant dilution (C5), those of row C with the second lowest toxicant dilution (C4), etc.

Wells 3 to 8 in each row are for the 6 replicates of the control batch columns and the test batch columns, respectively.

The wells in columns 1 and 2 are “rinsing wells” intended to avoid dilution of the toxicant in the test wells during the transfer of the organisms from the Petri dish to the microplate.

9.3 Introduction of the organisms

As indicated in 6.1, rotifers from either laboratory cultures or hatched from cysts can be used for the toxicity test.

If rotifers from live cultures are used, transfer about 300 rotifers in a 5 cm Petri dish (7.2) containing 10 ml natural or artificial fresh water.

In case rotifers hatched from cysts are used, sufficient numbers of neonates for the toxicity test will be present in the hatching Petri dish.