BRITISH STANDARD

BS
6068-5.24:1999
ISO 14669:1999

Water quality —

Part 5: Biological methods —

Section 5.24: Determination of acute lethal toxicity to marine copepods (*Copepoda*, *Crustacea*)

ICS 13.060.70

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British Standards

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National foreword

This British Standard reproduces verbatim ISO 14669:1999 and implements it as the UK national standard.

The UK participation in its preparation was entrusted to Technical Committee EH/3, Water quality, which has the responsibility to:

— aid enquirers to understand the text;
— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

This is one of a series of standards on water quality, others of which have been, or will be, published as sections of BS 6068. The various sections of BS 6068 are comprised within Parts 1 to 7, which, together with Part 0, are listed below.

Part 0 *Introduction*
Part 1 *Glossary*
Part 2 *Physical, chemical and biochemical methods*
Part 3 *Radiological methods*
Part 4 *Microbiological methods*
Part 5 *Biological methods*
Part 6 *Sampling*

NOTE The tests described in this British Standard should only be carried out by suitably qualified persons with an appropriate level of biological expertise. Standard biological procedures should be followed throughout.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Standards Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Find” facility of the BSI Standards Electronic Catalogue.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, the ISO title page, pages ii and iii, a blank page, pages 1 to 16, an inside back cover and a back cover.

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INTERNATIONAL STANDARD

ISO 14669

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Water quality — Determination of acute lethal toxicity to marine copepods *(Copepoda, Crustacea)*

Qualité de l'eau — Détermination de la toxicité létale aiguë vis-à-vis de copépodes marins (Copepoda, Crustacea)



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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 3.

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.

International Standard ISO 14669 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods.*

Annexes A, B and C of this International Standard are for information only.

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Water quality — Determination of acute lethal toxicity to marine copepods *(Copepoda, Crustacea)*

1 Scope

This International Standard describes a method for the determination of the acute toxicity to one of three specified species of marine copepod (*Copepoda, Crustacea*) of

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

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

c) marine or estuarine waters.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 5667-2, *Water Quality — Sampling — Part 2: Guidance on sampling techniques.*

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

3 Principle

Copepods are exposed to a range of concentrations in seawater of a chemical substance, effluent or water sample. Mortality of the copepods is recorded after 24 h and 48 h.

The concentration which, in 48 h, kills 50% of exposed copepods under the conditions defined in this International Standard is determined. This concentration, known as the median lethal concentration, is designated 48 h LC50.

NOTE If possible, the concentration which kills 50% of the exposed copepods in 24 h is also determined. This concentration is designated 24 h LC50. It may be appropriate for certain purposes to extend the exposure period to 96 h and to determine the 96 h LC50.

An indication of the lowest concentration tested which kills all the copepods and the highest concentration tested which does not kill any of the copepods is desirable and provides useful information in cases where the 48 h LC50 cannot be determined.

The test is carried out in one or two stages:

— a preliminary test which determines the range of concentrations to be tested in the definitive test and gives an approximate value of the 48 h LC50 (and where appropriate, the 24 h LC50).

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— a definitive test, conducted when the approximate value given by the preliminary test is not sufficient, which permits calculation of the 48 h LC50 (and where appropriate, the 24 h LC50) and determines concentrations corresponding to 0 and 100% mortality.

If the method described in this International Standard is used for chemical substances, a limit test can be performed at 100 mg/l or at a lower concentration which is the maximum at which the substance is soluble or is in stable dispersion under the conditions of the test.

4 Test environment

The procedure described in this International Standard shall be carried out in a room, incubator or water-bath controlled at 20 °C ± 2 °C and under a 16 h/8 h light/dark photoperiod. The atmosphere shall be free from vapours or dusts toxic to copepods.

5 Reagents and materials

5.1 Test organism, one of the following species of marine copepod:

a) *Acartia tonsa* Dana;

b) *Tisbe battagliai* Volkmann-Rocco;

c) *Nitocra spinipes* Boeck.

Obtain the test organisms from laboratory cultures. Guidance on identification and culture methods for each species are given in Annex B. After hatching of eggs, the lifecycle of copepods consists of naupliar, copepodid and adult stages. The age and lifestage at the start of the test shall be indicated in the test report and are as follows:

— *Acartia tonsa*: large copepodids (Stage 5) or adults;

— *Tisbe battagliai:* copepodids 6 ± 2 days old;

— *Nitocra spinipes:* adults 3 to 4 weeks old.

5.2 Dilution water.

A natural or a synthetic seawater may be used as the dilution water. If natural seawater is used, it shall be collected from a location as distant as possible from known sources of pollution and filtered to remove indigenous organisms. If synthetic seawater is used, it shall be prepared by dissolving reagents of recognized analytical grade, or a commercially available formulation, in distilled or deionized water. However, for these copepod species, there is insufficient information on the use of synthetic seawater to allow a particular example to be recommended.

The salinity of the dilution water shall be between 29×10^{-3} and 36×10^{-3}. The use of a lower salinity, which may be more appropriate for tests concerning estuarine or brackish water situations, shall be justified in the test report. *Nitocra spinipes* can be used at salinities down to 1×10^{-3} and *Tisbe battagliai* can be used at salinities down to 20×10^{-3}. Whichever salinity is employed, the test organisms shall be cultured or maintained at the same salinity ($\pm 3 \times 10^{-3}$) for at least 7 days before the start of the test. The dilution water shall have a dissolved oxygen concentration above 80 % of the air saturation value, and a pH of 8,0 ± 0,3 before being used to prepare the test solutions.

The dilution water shall permit survival of the copepods for at least 48 h and should be from the same source as water that has been found to support culture of the organisms through at least two generations.

5.3 Reference chemical toxicant, e.g. 3,5-dichlorophenol or a suitable alternative (8.5), of recognized analytical grade.

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