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Introduction

This draft standard is based on national and international discussions. Your comments on this draft are invited and will assist in the preparation of the consequent standard.

For international standards, comments will be reviewed by the relevant UK national committee before sending the consensus UK vote and comments to the international committee, which will then decide appropriate action. If the international standard is approved, it is usual for the text to be published as a British Standard.

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Please indicate whether you consider the UK should submit a negative (with supporting technical reasons) or positive vote on this draft. Please indicate if you are aware of any reason why this draft standard should not be published as a British Standard.

Submission of Comments

- **Annotated drafts are not acceptable and will be rejected.**
- All comments should be submitted online at <https://standardsdevelopment.bsigroup.com/>. You will need to register in order to comment.

Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/ Figure/Table/Note	Type of comment	Comment (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	



DRAFT International Standard

ISO/DIS 14669

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)

ICS: 13.060.70

ISO/TC 147/SC 5

Secretariat: **DIN**

Voting begins on:
2025-05-19

Voting terminates on:
2025-08-11

This document is circulated as received from the committee secretariat.

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Contents

Page

Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Test environment	2
6 Reagents and materials	2
7 Apparatus	3
8 Sampling, treatment and preparation of samples Special precautions for sampling and transportation of samples of water or effluent	3
8.1 Sampling and storage of samples	3
8.2 Preparation of solutions of substances to be tested	4
8.2.1 Preparation of stock solutions	4
8.2.2 Preparation of aqueous extracts from sediments	4
8.2.3 Preparation of test solutions	4
9 Procedure	4
9.1 Preliminary test	4
9.2 Definitive test	4
9.3 General procedure	5
9.4 Limit test	5
9.5 Check of sensitivity of copepods and conformity with the procedure	5
10 Calculation of the LC50	6
11 Validity of results	6
12 Expression of results	6
13 Performance data	7
14 Test report	7
Annex A (informative) Example of determination of acute lethal toxicity of a chemical substance to a marine copepod	8
Annex B (informative) Culture methods for marine copepods <i>Acartia tonsa</i>, <i>Tisbe battagliai</i> and <i>Nitocra spinipes</i>	10
Annex C (informative) Precision data	15
Annex D (informative) Defined culture and test media	16
Bibliography	18

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).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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.

This document 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.

The main changes are as follows:

- addition of aqueous extracts (pore water, elutriates, eluates, and leachates) in the scope of the standard.
- possibility of using copepods from hatcheries.

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

1 Scope

This document 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;
- d) aqueous extracts (pore water, elutriates, eluates, and leachates) from sediments.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

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

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org>

4 Principle

Copepods are exposed to a range of concentrations of chemical substances in seawater, effluent, water samples or aqueous extracts from sediments. Mortality of the copepods is recorded after 24 h and 48 h.

In 48h, the concentration at which, 50 % of exposed copepods are killed under the conditions defined in this document 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);
- a definitive test, if toxicity is observed, 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 document 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.

5 Test environment

The procedure described in this document shall be carried out in a room, incubator or water-bath controlled at $20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and under a 16 h/8 h light/dark photoperiod. The atmosphere shall be free from vapours or dusts toxic to copepods.

6 Reagents and materials

6.1 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.

Suitable organisms can be purchased from hatcheries outside the laboratory that performs the acute toxicity test. Only healthy animals from unpolluted source should be used.

If individuals are received at an early stage, they should be maintained in cultured conditions similar to the hatchery (sea water, temperature) and fed with appropriate algae mix (similar to the hatchery) until they reach the proper development stage. Organisms should be tested after confirming that the above developmental stage has been reached.

If individuals are received at the life stage mentioned above, the test should be conducted the day of reception or the day after in order to guarantee the developmental stage certified by the hatchery. In this case, feeding should be added at the start of the test with the same algae mix that in the hatchery and the test conditions should be similar to the hatchery water characteristics (water salinity and temperature).

In any case, the copepods shall have the age and lifestage mentioned above and be in a good health at the beginning of the test.

6.2 Dilution water:

A natural or an artificial seawater may be used as the dilution water. Culture and test media are prepared from either reconstituted salt water or filtered ($0,2\text{ }\mu\text{m}$) natural marine water from an unpolluted location. An example of reconstituted salt water suitable for cultivation and testing is given in [Annex D](#). Reconstituted