International Standard

ISO 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)

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Foreword

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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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This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 14669:1999), which has been technically revised.

The main changes are as follows:

— aqueous extracts (pore water, elutriates, eluates and leachates) have been added to the scope;

— possibility of using copepods from hatcheries has been included;

— informative Annexes A, B and C were amended;

— informative Annex D (culture and test media) was added.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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). This document is applicable to:

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

b) marine or estuarine waters;

c) aqueous extracts (pore water, elutriates, eluates and leachates) from sediments;

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

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 5667-15, *Water quality — Sampling — Part 15: Preservation and handling of samples of sludge, sediment and suspended matter*

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

ISO 5667-19, *Water quality — Sampling — Part 19: Guidance on sampling of marine sediments*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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/

3.1
50 % lethal concentration
LC50
concentration at which 50 % of the test organisms would die in an experiment

[SOURCE: ISO 13073-3:2016, 2.28]

3.2
***x* % confidence interval**
interval of values that theoretically covers the true value of the estimated parameter with *x* % confidence

Note 1 to entry: Standard confidence intervals are based on the assumption that the underlying mathematical model is correct. It does not capture model uncertainty.

Note 2 to entry: A more precise definition is given in ISO 3534-1:2006, 1.28: interval estimator (T_0, T_1) for the parameter θ with the statistics T_0 and T_1 as interval limits and for which it holds that $P[T_0 > \theta > T_1] \geq 1 - \alpha$.

Note 3 to entry: Associated with this confidence interval is the confidence level $100(1 - \alpha)$ % where α is generally a small number. The confidence level is typically 90 % or 95 %. The inequality $P[T_0 > \theta > T_1] \geq 1 - \alpha$ holds for any specific but unknown population value of θ.

Note 4 to entry: A confidence interval does not reflect the probability that the observed interval contains the true value of the parameter (it either does or does not contain it). The confidence reflects the proportion of cases that the confidence interval would contain the true parameter value in a long series of repeated random samples under identical conditions.

[SOURCE: ISO/TS 20281:2006, 3.5]

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 48 h, the concentration at which 50 % of exposed copepods are killed, under the test conditions described above, is determined. This concentration, known as the median lethal concentration, is designated 48 h lethal concentration 50 % (48 h LC50).

NOTE If possible, the concentration which kills 50 % of the exposed copepods in 24 h is also determined (24 h LC50). It is possible to extend the exposure period to 96 h (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 should 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 °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.

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 life stage 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 may be purchased from hatcheries outside the laboratory that performs the acute toxicity test. Only healthy animals from an 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 life stage 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 µm) natural marine water from an unpolluted location. An example of reconstituted salt water suitable for cultivation and testing is given in Annex D (see Tables D.1, D.2, D.3 and D.4). Reconstituted salt water media with a known composition in which the copepods show suitable long-term survival, normal behaviour, development and fecundity may be used as culture and test media, i.e. 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 artificial 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 the copepod species in this document, there is insufficient information on the use of artificial seawater to allow a particular example to be recommended.

The salinity of the dilution water shall be between 25 pratical salinity unit (PSU) and 36 PSU. The use of a lower salinity, which is 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 PSU and *Tisbe battagliai* can be used at salinities down to 20 PSU. Whichever salinity is employed, the test organisms shall be cultured or maintained at the same salinity (±3 PSU) for at least seven 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.

6.3 Reference chemical toxicant, e.g. 3,5-dichlorophenol or a suitable alternative (9.5), of recognized analytical grade.