SLOVENSKI STANDARD
oSIST ISO 14380:2012

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Kakovost vode - Določevanje akutne strupenosti z raki Thamnocephalus platyurus (Crustacea, Anostraca)

Water quality - Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca)

Qualité de l'eau - Détermination de la toxicité aiguë envers Thamnocephalus platyurus (Crustacea, Anostraca)

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Water quality — Determination of the acute toxicity to *Thamnocephalus platyurus (Crustacea, Anostraca)*

Qualité de l'eau — Détermination de la toxicité aiguë envers Thamnocephalus platyurus (Crustacea, Anostraca)

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In accordance with the provisions of Council Resolution 15/1993, this document is **circulated in the English language only**.

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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 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 14380 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

The evaluation of harmful effects on water quality has for several years involved the performance of biological tests. Crustaceans are of interest from the ecotoxicological point of view because they are primary consumers and a major component of the zooplankton in aquatic ecosystems.

The test specified in this International Standard involves determination of the lethal effects on the fresh water fairy shrimp *Thamnocephalus platyurus* after 24 h exposure to the toxicant. A rapid test can also be carried out to determine sublethal effects after a very short exposure time (1 h).

The beavertail fairy shrimp *T. platyurus* is to date already used extensively in toxicity testing for several reasons:

a) this anostracan crustacean has a sensitivity to chemicals which is quite similar to that of the cladoceran crustacean *Daphnia magna* (see References [4][5][6][7]);

b) the assays are performed with neonates hatched from dormant eggs (cysts), which bypasses the need for culturing or maintaining live stock cultures of test organisms;

c) *T. platyurus* neonates are substantially smaller than neonates of *Daphnia magna*, hence the assays require much smaller test containers, and much less bench space and incubation space;

d) *T. platyurus* is very sensitive to cyanotoxins produced by algal blooms in eutrophicated waters (see References [8][9]).

Water quality — Determination of the acute toxicity to *Thamnocephalus platyurus (Crustacea, Anostraca)*

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard 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 International Standard 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 *Thamnocephalus platyurus* test organisms after 24 h exposure. A second method (rapid test) is described in Annex A for the determination of sublethal effects after a very short exposure time (1 h).

The methods are applicable to:

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

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

c) fresh waters;

d) aqueous extracts;

e) toxins of blue-green algae.

This International Standard is not applicable to the testing of unstable chemicals (hydrolysing, absorbing, etc.) in water unless exposure concentration is measured, nor to the testing of aquatic samples from the estuarine or marine environment.

2 Normative references

The following referenced documents are indispensable for the application 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 5667-16, *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

NOTE The role of a control batch in an experimental procedure is to demonstrate the response to the detection system imposed collectively by compounds of the matrix used in the determination, in the absence of the subject of interest.

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

3.3
EC_{50}
concentration or dilution of the sample which gives rise to 50 % effect on the test organisms

3.4
neonate
newly hatched individual

3.5
test batch
series of replicates filled with the same test solution

4 Principle

Freshly hatched *T. platyurus* larvae 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 test organisms are neonates of the beavertail fairy shrimp *T. platyurus*, which are hatched from dormant eggs (cysts) of this crustacean.

Cysts of *T. platyurus* are obtained from laboratory cultures of the crustacean as described in Annex B or can be purchased from a specialized company[1)].

1) MicroBioTests Inc., Mariakerke, Belgium, is an example of a supplier able to provide suitable *Thamnocephalus platyurus* 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.

6.2 Pure water, conductivity below 10 µS/cm.

6.3 Test medium, prepared by dissolving the following mineral substances in 1 l of pure water (6.2):

$NaHCO_3$	96 mg
$CaSO_4 \cdot 2H_2O$	60 mg
$MgSO_4$	60 mg
KCl	4 mg

This test medium corresponds to a synthetic water of moderate hardness, i.e. containing $CaCO_3$ at concentrations of 80 mg/l to 100 mg/l (see Reference [13]). Thus prepared, the medium has a pH of 7,6 ± 0,3.

When stored in a refrigerator at (4 ± 2) °C in the dark, the solution can be used for several months.

Aerate the test medium until the dissolved oxygen concentration has reached the air saturation value and until the pH has stabilized. If necessary, adjust the pH to 7,6 ± 0,3 using sodium hydroxide or hydrochloric acid solutions. The concentration of the acid or base required shall be selected so that the volume to be admixed is as small as possible. Bring the temperature of the test medium up to (25 ± 1) °C prior to use.

6.4 Hatching medium. An eightfold dilution of the test medium (6.3) with pure water (6.2).

6.5 Reference substance. Potassium dichromate ($K_2Cr_2O_7$) is the recommended reference chemical.

7 Apparatus and materials

Usual laboratory apparatus and glassware and in particular the following.

7.1 Temperature-controlled room or **chamber**.

7.2 Hatching Petri dishes, small Petri dishes, diameter 5 cm, in glass or in inert plastic material.

7.3 Test containers. Disposable microplates made from chemically inert material, comprising wells with a capacity >1 ml. For example, 24 (4 × 6) well microplates with a well diameter of approximately 16 mm are suitable.

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

Micropipettes of inert plastic material with a bulb at the end are very suitable for the operations.

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.

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 water, effluent, or aqueous extract samples to be tested.