SLOVENSKI STANDARD
oSIST prEN ISO 20227:2016

01-julij-2016

Kakovost vode - Določevanje vplivov odpadne vode, naravne vode in kemikalij na zaviranje rasti vodne leče Spirodela polyrhiza (ISO/DIS 20227:2016)

Water quality - Determination of the growth inhibition effects of waste waters, natural waters and chemicals on the duckweed Spirodela polyrhiza - Method using a stock culture independent microbiotest (ISO/DIS 20227:2016)

Wasserbeschaffenheit - Bestimmung der wachstumshemmenden Wirkung von Abwässern, natürlichen Wässern und Chemikalien auf die Wasserlinsenart Spirodela polyrhiza - Verfahren mittels Stammkultur unabhängigem mikrobiologischem Test (ISO/DIS 20227:2016)

Qualité de l'eau - Détermination des effets d'inhibition sur la croissance de la lentille d'eau Spirodela polyrhiza par les eaux usées, les eaux naturelles et les produits chimiques - Méthode utilisant un bioessai miniaturisé indépendant d'une culture mère (ISO/DIS 20227:2016)

Ta slovenski standard je istoveten z: **prEN ISO 20227**

ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

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DRAFT INTERNATIONAL STANDARD

ISO/DIS 20227

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Water quality — Determination of the growth inhibition effects of waste waters, natural waters and chemicals on the duckweed *Spirodela polyrhiza* — Method using a stock culture independent microbiotest

Qualité de l'eau — Détermination des effets d'inhibition de la croissance des eaux usées, des eaux naturelles et des produits chimiques sur la lentille d'eau Spirodela polyrhiza *— Méthode utilisant un microbiotest indépendant de la culture mère*

ICS: 13.060.70

ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

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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.

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

Duckweeds are free-floating higher water plants commonly used in ecotoxicological research for the assessment of the toxicity of waste waters, natural waters and chemicals (see ISO 20079 and References [1] to [6] and in particular of plant protection products see Reference [7]).

Duckweeds are fast growing plants, many of which have a cosmopolitan distribution, and they are hence well suited as primary producers for hazard assessment of pollutants in freshwater environments.

Contrary to terrestrial plants, for which bioassays can be started from the "dormant" life stages (seeds), toxicity tests with duckweeds require continuous culturing and maintenance of live stocks, with the inherent biological, technical and financial costs.

A few duckweed species, however, produce "dormant vegetative buds" (turions) which can be stored for long periods of time, and which can be germinated "on demand" at the time of performance of the bioassay.

One of the duckweeds producing turions is *Spirodela polyrhiza*, and this species was eventually selected for a simple and practical microbiotest which is independent of the stock culturing and maintenance of live stocks.

Spirodela polyrhiza was found to be as sensitive to toxicants as the conventional bioassays with duckweeds.

The microbiotest procedure for this International Standard involves a 3 d germination of the turions, followed by a 3 d toxicity test in a multiwell test plate, with determination of the growth inhibition of the first fronds via image analysis.

The *Spirodela polyrhiza* microbiotest is very simple and easy to perform and has several advantages over the conventional duckweed tests:

1) the assay does not require culturing or maintenance of live stocks of the test species, and can be performed "anytime, anywhere" by use of stored turions;

2) stored turions have a shelf life of several months with a high germination success;

3) the microbiotest requires minimal bench and incubation space, and minimal equipment;

4) the test does not require manipulation of the organisms during or at the end of the test;

5) the area measurements of the first fronds do not need to be made immediately and can be postponed to an appropriate timing;

6) the area measurements by image analysis are very rapid and precise, and take less than one hour for a complete test.

Water quality — Determination of the growth inhibition effects of waste waters, natural waters and chemicals on the duckweed *Spirodela polyrhiza* — Method using a stock culture independent microbiotest

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 according to this document be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of the inhibition of the growth of the first fronds of *Spirodela polyrhiza* germinated from turions, by substances and mixtures contained in water or waste water, including treated municipal waste water and industrial effluents.

The test is also applicable to pure chemicals and in particular plant protection products and pesticides.

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 20079, *Water quality — Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) — Duckweed growth inhibition test*

ISO/TS 20281, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

3 Terms and definitions

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

3.1
EC*x*
calculated concentration (of a substance) or dilution (of an aqueous sample, in %) for which an effect of x% is expected compared to the control

3.2
frond
leaf-like structure which develops from a germinated turion

3.3
growth
increase in size of the first frond developing from a germinated turion

3.4
growth medium
nutrient medium used for the germination of the turions and the growth of the fronds

3.5
inoculation
transfer of a germinated turion with its small frond in all the test wells at the start of the toxicity test

3.6
pure water
deionized or distilled water with a conductivity below 10 µS/cm

3.7
root
part of the plant which develops underneath a frond

3.8
stock culture
laboratory culture of the duckweed for the production of the turions

3.9
test dilution medium
growth medium which is also used as test dilution medium

3.10
test sample
portion of the collected material or the dissolved chemical to be used for the preparation of the dilution series

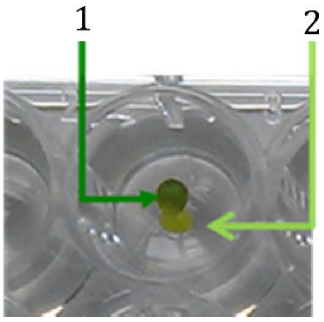
3.11
turion
small vegetative bud which develops from a colony of the duckweed under specific environmental conditions

4 Principle

Turions produced by culturing *Spirodela polyrhiza*, or taken from test tubes in which they are stored (see Annex A) are transferred to a Petri dish containing growth medium, and incubated for 3 d at 25 °C with continuous illumination of at least 6 000 lx (corresponding approximately to 85 µE m^{-2} s^{-1}).

During this time the turions germinate and produce a small (first) frond (see Figure 1).

One germinated turion with its first frond is then taken from the Petri dish and inoculated into each cup of a 6 × 8 multiwell test plate which contains the toxicant dilutions and the negative control (each of which is prepared in growth medium).



Key

1 turion
2 first frond

Figure 1 — Enlargement of a germinated turion with its first frond, in a cup of the test plate

On completion of the inoculations, a photo of the multiwell is taken (= at t0h) with a digital camera and transferred to a computer file.

The multiwell is subsequently incubated for 3 d at (25 ± 1) °C with continuous illumination of minimum 6 000 lx, after which a photo is again taken (= at t72h) and transferred to a computer file.

The area of the first frond in each test cup is measured with the aid of an image analysis programme, on the 2 photos of the multiwell (i.e. taken at t0h and at t72h).

The growth of the first fronds in the controls and in the test concentrations or dilutions is calculated as the difference between the t72h areas and the t0h areas, after which the growth inhibition and the 72 h EC50 or EC*x* values are determined.

5 Test organisms

The test species used in this International Standard is the duckweed *Spirodela polyrhiza* (L.) Schleid.

The test organisms are obtained by germination of (stored) turions.

Turions can be produced in the laboratory according to the procedure described in Annex A.

They can also be purchased from a commercial source [1]

6 Growth medium

The growth medium (3.4) used for the germination of the turions and the growth of the duckweeds during the toxicity test is the "modified STEINBERG medium" which is described and used in ISO 20079 and OECD guideline for testing chemicals (see Reference [1]).

This medium will also be used to prepare the toxicant dilutions.

[1] The turions supplied by MicroBioTests Inc. Mariakerke-Gent, Belgium are an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

The growth medium is composed of "macroelements" and "microelements" of which stock solutions are prepared according to Table 1 and Table 2 respectively.

6.1 Preparation of stock solutions

Prepare the 8 stock solutions by adding the prescribed weight of the chemicals to 1 l of pure water (3.6).

Table 1 — Macroelements stock solutions

Macroelements (50-fold concentrated)		g/l
Stock solution 1	KNO_3	17,50
	KH_2PO_4	4,5
	K_2HPO_4	0,63
Stock solution 2	$MgSO_4 \cdot 7H_2O$	5,00
Stock solution 3	$Ca(NO_3)_2 \cdot 4H_2O$	14,75

Table 2 — Microelements stock solutions

Microelements (1 000-fold concentrated)		mg/l
Stock solution 4	H_3BO_3	120,00
Stock solution 5	$ZnSO_4 \cdot 7H_2O$	180,00
Stock solution 6	$Na_2MoO_4 \cdot 2H_2O$	44,0
Stock solution 7	$MnCl_2 \cdot 4H_2O$	180,00
Stock solution 8	$FeCl_3 \cdot 6H_2O$	760,00
	EDTA disodium-dihydrate	1 500,00

Stock solutions 2 and 3, and 4 to 7 may be pooled (taking into account the required concentrations).

6.2 Preparation of the final concentration of modified STEINBERG medium

Add 20 ml each of stock solutions 1, 2 and 3 to about 900 ml pure water (3.6) in a 1 l volumetric flask.

Then add 1,0 ml each of stock solutions 4, 5, 6, 7 and 8.

Fill the volumetric flask to 1 000 ml with pure water.

The pH of the growth medium should be 5,5 ± 0,2 and can be adjusted with either HCl or NaOH.

Once prepared, the growth medium has a relatively short shelf life and should be used within 2 weeks after preparation.

7 Apparatus

Usual laboratory equipment and in particular the following.

7.1 Temperature-controlled cabinet or room, or incubator, with white fluorescent light providing continuous uniform illumination of at least 6 000 lx at the surface of the turion germination Petri dish and the multiwell test plate.

