

STN	Kvalita vody Stanovenie inhibície rastu Spirodela polyrhiza vplyvom odpadových vôd, prírodných vôd a chemických látok Metóda nezávislého mikrobiotestu so zásobnou kultúrou (ISO 20227: 2017)	STN EN ISO 20227 75 7748
------------	--	--

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 microbionest (ISO 20227:2017)

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 01/18

Obsahuje: EN ISO 20227:2017, ISO 20227:2017

125539

Úrad pre normalizáciu, metrológiu a skúšobníctvo Slovenskej republiky, 2018
Podľa zákona č. 264/1999 Z. z. o technických požiadavkách na výrobky a o posudzovaní zhody a o zmene a doplnení niektorých zákonov v znení neskorších predpisov sa slovenská technická norma a časti slovenskej technickej normy môžu rozmnožovať alebo rozširovať len so súhlasom slovenského národného normalizačného orgánu.

EUROPEAN STANDARD

EN ISO 20227

NORME EUROPÉENNE

EUROPÄISCHE NORM

July 2017

ICS 13.060.70

English Version

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 20227:2017)

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 20227:2017)

Wasserbeschaffenheit - Bestimmung der wachstumshemmenden Wirkung von Abwässern, natürlichen Wässern und Chemikalien auf die Wasserlinsenart *Spirodela polyrhiza* - Verfahren mittels Stammkultur unabhängigem mikrobiologischem Test (ISO 20227:2017)

This European Standard was approved by CEN on 9 May 2017.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents	Page
European foreword.....	3

European foreword

This document (EN ISO 20227:2017) has been prepared by Technical Committee ISO/TC 147 "Water quality" in collaboration with Technical Committee CEN/TC 230 "Water analysis" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2018 and conflicting national standards shall be withdrawn at the latest by January 2018.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 20227:2017 has been approved by CEN as EN ISO 20227:2017 without any modification.

**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 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*





COPYRIGHT PROTECTED DOCUMENT

© ISO 2017, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Test organisms	3
6 Growth medium	3
6.1 Preparation of stock solutions	4
6.2 Preparation of the final concentration of modified Steinberg medium	4
7 Apparatus	4
8 Reference chemicals	5
9 Procedure	5
9.1 Germination of the <i>Spirodela polyrhiza</i> turions	5
9.2 Tests on effluents (and waste waters)	5
9.2.1 Addition of concentrated growth medium to the effluent sample	5
9.2.2 Preparation of the effluent dilutions	6
9.2.3 Procedure	6
9.3 Tests on chemical compounds	7
9.3.1 Range finding test	7
9.3.2 Definitive test	8
9.4 Filling of the test plate with the toxicant dilutions	9
9.4.1 General	9
9.4.2 Procedure	9
9.5 Transfer of the germinated turions in the test cups	10
9.6 Photo of the multiwell at the start of the toxicity test	10
9.7 Incubation of the multiwell	10
9.8 Photo of the multiwell at the end of the toxicity test	11
9.9 Measurement of the area of the first fronds	11
10 Data treatment — Calculation of the growth inhibition	11
11 Validity criterion	12
12 Test sensitivity	12
13 Test with reference chemicals	12
14 Test report	15
Annex A (informative) <i>Spirodela polyrhiza</i> stock culturing for turion production	16
Annex B (informative) Sensitivity of the <i>Spirodela polyrhiza</i> microbiotest	17
Annex C (informative) Performance data	19
Bibliography	20

ISO 20227:2017(E)

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 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.

This document was prepared by Technical Committee 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 [6] to [11] and in particular plant protection products, see Reference [12]).

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 document 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:

- a) the assay does not require culturing or maintenance of live stocks of the test species, and can be performed “anytime, anywhere” by the use of stored turions;
- b) stored turions have a shelf life of several months with a high germination success;
- c) the microbiotest requires minimal bench and incubation space, and minimal equipment;
- d) the area measurements of the first fronds do not need to be made immediately and can be postponed to an appropriate timing;
- e) the area measurements by image analysis are very rapid and precise, and take less than 1 h 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 document 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 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 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

koniec náhľadu – text ďalej pokračuje v platenej verzii STN