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Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (*Oppia nitens*) exposed to contaminants in soil

*Qualité du sol — Essai de détermination de l'inhibition de la reproduction chez les acariens oribates (*Oppia nitens*) exposés aux contaminants dans le sol*

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

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Contents

Page

Foreword ... **iv**
Introduction ... **v**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **2**
4 Principle ... **4**
5 Reagents and material ... **4**
6 Apparatus ... **7**
7 Procedure ... **7**
7.1 Experimental design ... 7
7.1.1 General ... 7
7.1.2 Range-finding test (preliminary test) ... 7
7.1.3 Definitive test ... 8
7.2 Preparation of test mixture ... 8
7.2.1 Testing contaminated soil ... 8
7.2.2 Testing substances added to the test substrate ... 9
7.2.3 Preparation of control containers ... 10
7.3 Addition of the mites ... 10
7.4 Test conditions and measurements ... 10
7.5 Determination of adult survival and reproductive output ... 10
8 Calculation and expression of results ... **11**
8.1 Calculation ... 11
8.2 Expression of results ... 11
9 Validity of the test ... **11**
10 Statistical analysis ... **12**
10.1 General ... 12
10.2 Single-concentration tests ... 12
10.3 Multi-concentration tests ... 12
10.3.1 Range-finding test ... 12
10.3.2 Definitive test ... 12
11 Test report ... **13**
Annex A (informative) **Techniques for rearing and breeding of *Oppia nitens*** ... **15**
Annex B (normative) **Determination of water holding capacity** ... **18**
Annex C (informative) **Guidance on adjustment of pH of artificial soil** ... **19**
Annex D (informative) **Extraction and counting of *Oppia nitens*** ... **20**
Annex E (informative) **Performance of the method** ... **24**
Bibliography ... **29**

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).

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For an explanation of 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

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.

Introduction

Ecotoxicological test systems are applied to obtain information about the effects of contaminants in soil and are proposed to complement conventional chemical analysis (see ISO 15799[1] and ISO 17616[2]). ISO 15799 includes a list and short characterization of recommended and standardized test systems and ISO 17616 gives guidance on the choice and evaluation of the bioassays. Aquatic test systems with soil eluate are applied to obtain information about the fraction of contaminants potentially reaching the groundwater by the water path (retention function of soils), whereas terrestrial test systems are used to assess the habitat function of soils with regards to supporting soil biota and interactions within.

Mites (Acari) are a world-wide and diverse group of arthropods belonging to a sub-class of Arachnida with over 40 000 species recorded, divided into two super-orders (Acariformes and Parasitiformes). Due to their relative small size (a few µm to a few cm), they occupy specific ecological niches on plants as well as in soils[5].

In recent years, there has been an increase in the development of biological test methods for assessing contaminated soil, which has historically lagged behind that for other media (e.g., water and sediment). Ecotoxicology tests for soil are challenged, among other things, by the complexity of soil systems (e.g., lack of homogeneity) and the variety of exposure routes (e.g., via alimentary uptake, exposure to pore water or soil vapours, or direct contact with soil particles). A recently developed method (ISO 21285[3]) assesses the effects of contaminated soil on the reproduction of the predatory mite (*Hypoaspis aculeifer*), mainly through alimentary uptake. Oribatid mites represent a different but essential ecological niche than *H. aculeifer* within soil, contributing to carbon mineralization and soil formation, as well as nitrogen and phosphorous release through grazing activities. Oribatid mites are among the most diverse and abundant micro-arthropod species within soil, however, their slower metabolism and development, coupled with low fecundity, long life span, and limited dispersal capacity increase the potential for susceptibility and sensitivity to short- and long-term disturbances[6]. The use of oribatid mites in the context of soil ecotoxicology testing has been thoroughly reviewed[7][8][9][10][11]. Recent research using *Oppia nitens* for soil testing has demonstrated applicability and relative sensitivity of the species for the assessment of contaminated soils from both agronomic regions, and those from the boreal and taiga ecozones[6][12][13][14][15]. Research has also demonstrated its sensitivity to metals[16][17][18][19], pesticides[20][21], and organic compounds[16][17][22]. *Oppia nitens* is an oribatid mite, inhabiting the upper organic layer of soil, and is a member of the largest oribatid family (Oppiidae) with approximately 1 000 species in 129 genera widely distributed throughout Holarctic and Antarctic regions[23]. They are sexually reproducing, polyphagous fungivores that can be easily reared in the laboratory in soil or on plaster of Paris, and on a diet of Baker's yeast[10].

This method outlines procedures for conducting 28-day tests for determining the effects of contaminated soils on the survival and reproduction of the oribatid mite, *Oppia nitens*. Optionally, the method can be used for testing chemicals added to standard soils (e.g., artificial soil) for their lethal and sublethal hazard potential to oribatid mites. The performance of this method has been assessed in an international inter-laboratory investigation[15], as summarized in Annex E. Mites represent communities that cannot be omitted from environmental hazard assessment. This species is considered to be representative of non-predatory soil mites.

Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (*Oppia nitens*) exposed to contaminants in soil

WARNING — Contaminated soils may contain unknown mixtures of toxic, mutagenic, or otherwise harmful chemicals or infectious microorganisms. Occupational health risks may arise from dust or evaporated chemicals during handling and incubation. Precautions should be taken to avoid skin contact.

1 Scope

This document specifies one of the methods for evaluating the habitat function of soils and determining effects of soil contaminants and individual chemical substances on the reproduction of the oribatid mite *Oppia nitens* by dermal and alimentary uptake. This chronic (28-day) test is applicable to soils and soil materials of unknown quality (e.g., contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern and waste materials). This method is not intended to replace the earthworm or Collembola tests since it represents another taxonomic group (= mites; i.e., arachnids), nor the predatory mite test since this species represents a different trophic level and ecological niche.

Effects of substances are assessed using standard soil, preferably a defined artificial soil substrate. For contaminated soils, the effects are determined in the test soil and in a control soil. According to the objective of the study, the control and dilution substrate (dilution series of contaminated soil) should be either an uncontaminated soil with similar properties to the soil sample to be tested (reference soil) or a standard soil (e.g., artificial soil).

Information is provided on how to use this method for testing substances under temperate conditions.

This document is not applicable to substances for which the air/soil partition coefficient is greater than 1, or to substances with vapour pressure exceeding 300 Pa at 25 °C.

NOTE The stability of the test substance cannot be assured over the test period. No provision is made in the test method for monitoring the persistence of the substance under 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 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11260, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

ISO 11265, *Soil quality — Determination of the specific electrical conductivity*

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
contaminant
substance or agent present in the soil as a result of human activity

3.2
effect concentration
EC*x*
concentration (mass fraction) of a test sample or test substance that causes *x* % of an effect on a given end-point within a given exposure period when compared with a control

EXAMPLE An EC50 is a concentration estimated to cause an effect on a test end-point in 50 % of an exposed population over a defined exposure period.

Note 1 to entry: The EC*x* is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass). When substances are tested, the EC*x* is expressed as mass of the test substance per dry mass of soil in milligrams per kilogram.

3.3
effect rate
ER*x*
rate of a soil to be tested that causes an *x* % of an effect on a given end-point within a given exposure period when compared with a control

3.4
lethal concentration
LC*x*
concentration (mass fraction) of a test sample or test substance that causes *x* % mortality within a given exposure period when compared with a control

EXAMPLE An LC50 is a concentration estimated to cause mortality in 50 % of an exposed population over a defined exposure period.

Note 1 to entry: The LC*x* is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass). When substances are tested, the LC*x* is expressed as mass of the test substance per dry mass of soil in milligrams per kilogram.

3.5
limit test
single concentration test consisting of at least five replicates each, the *test soil* (3.14) without any dilution or the highest concentration of test substance mixed into the control soil and the control