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Soil quality — Effects of contaminants on *Enchytraeidae* (*Enchytraeus* sp.) — Determination of effects on reproduction

Qualité du sol — Effets des contaminants sur les Enchytraeidae *(*Enchytraeus *sp.) — Détermination des effets sur la reproduction*

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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).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 16387:2014), which has been technically revised.

The main changes are as follows:

— correction of the vapor pressure value to update Clause 1 according to the recommendations of ECHA/OECD;

— addition (in Annex D) of an extraction method of enchytraeids with colloidal silica, suitable for *Enchytraeus crypticus*.

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.

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Introduction

Ecotoxicological test systems are applied to obtain information about the effects of contaminants in soil and are proposed to complement conventional chemical analysis. ISO 15799[38] includes a list and short characterization of recommended and standardized test systems. 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. For the latter, a standardized test system using *Enchytraeidae* (a chronic test with end-point reproduction) is proposed.

This document describes a method that is based on the determination of acute and sublethal effects of contaminated soils to adult *Enchytraeidae* of the genus *Enchytraeus*. Optionally, the method can be used for testing substances added to standard soils (e.g. artificial soil) for their sublethal hazard potential to *Enchytraeidae*.

Soil-dwelling annelids of the genus *Enchytraeus* are ecologically relevant, i.e. they are abundant in many soils where earthworms are scarce but can also reach high population densities in soils well inhabited by earthworms. *Enchytraeidae* can be used in laboratory tests as well as in semi-field and field studies. From a practical point of view, many *Enchytraeus* species are easy to handle and breed, and their generation time is significantly shorter than that of earthworms [the test duration for a reproduction test with *Enchytraeidae* is four weeks to six weeks, compared to eight weeks (12 weeks including synchronization) with earthworms]. In addition, a much smaller volume of soil is needed in the enchytraeid test compared to the amount needed in earthworm tests.

This document has been drawn up taking into consideration test procedures recommended by the Organization for Economic Co-operation and Development (OECD[24],[25]).

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Soil quality — Effects of contaminants on *Enchytraeidae* (*Enchytraeus* sp.) — Determination of effects on reproduction

1 Scope

This document specifies one of the methods for evaluating the habitat function of soils and determining effects of soil contaminants and substances on the reproduction of *Enchytraeus* sp. by dermal and alimentary uptake in a chronic test. It is applicable to soils and soil materials of unknown quality, for example, from contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern and waste materials.

This document provides information on how to use this method for testing substances under temperate conditions.

The method is not applicable to substances, for which the air/soil partition coefficient is greater than 1, or to substances for which the vapour pressure exceeds 300 Pa at 25 °C.

NOTE 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, treated biowaste and sludge – 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 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 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/

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3.1
reproduction
mean number of offspring per test vessel after incubation under the specified test conditions

Note 1 to entry: The test period for the determination of the reproduction (definitive test) is six weeks.

Note 2 to entry: Offspring refers to hatched juvenile enchytraeids.

3.2
EC*x*
effect concentration for *x* % effect
concentration (mass fraction) of a test substance that causes *x* % of an effect on a given endpoint within a given exposure period when compared with a control

EXAMPLE An EC_{50} 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
LOEC
lowest observed effect concentration
lowest test substance concentration that has a statistically significant effect (probability $p < 0,05$)

Note 1 to entry: In this test, the LOEC is expressed as a mass of test substance per dry mass of the soil to be tested. All test concentrations above the LOEC should usually show an effect that is statistically different from the control.

3.4
NOEC
no observed effect concentration
highest test substance concentration immediately below the *LOEC* (3.3) at which no effect is observed

Note 1 to entry: In this test, the concentration corresponding to the NOEC, has no statistically significant effect ($p < 0,05$) within a given exposure period when compared with the control.

3.5
test mixture
mixture of contaminated soil or test substance (e.g. chemical, biosolid, waste) with *control soil* (3.10)

3.6
test mixture ratio
ratio between the soil to be tested and the *control soil* (3.10) in a *test mixture* (3.5)

3.7
contaminant
substance or agent present in the soil as a result of human activity

3.8
reference soil
uncontaminated soil with comparable pedological properties (nutrient concentrations, pH, organic carbon content and texture) to the soil being studied

3.9
standard soil
field-collected soil or artificial soil whose main properties (pH, texture, organic matter content) are within a known range

EXAMPLE Euro soils, artificial soil, LUFA Standard soil.

Note 1 to entry: The properties of standard soils can differ from the soil to be tested.

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3.10
control soil
reference (3.8) or *standard soil* (3.9) used as a control and as a medium for preparing dilution series with soils to be tested or a reference substance, which fulfils the validity criteria

Note 1 to entry: In the case of natural soil, it is advisable to demonstrate its suitability for a test and for achieving the test validity criteria before using the soil in a definitive test.

4 Principle

The effects on survival and reproduction of adult *Enchytraeidae* (*Enchytraeus* sp.) exposed to a dilution range of contaminated soil or range of concentrations of a test substance are determined. Test mixtures are prepared at the start of the test and are not renewed within the test period.

The test can be divided into two distinct steps: a short (two weeks) test in which the range of toxic effects (mainly mortality) is determined, and a long-term (six weeks) definitive test in which the survival of parental worms and the fecundity (number of juveniles) are measured. The results obtained from the tests are compared with a control and are used to determine the dilutions or concentrations which cause no effects on survival and reproduction (NOEC) and the concentration (dilution) resulting in x % reduction of juveniles hatched from cocoons compared to the control (ECx, 42 d).

All test dilutions/concentrations above the LOEC have a harmful effect equal to, or greater than that observed at the LOEC. Where there is no prior knowledge of the dilution/concentration of the test substance likely to have an effect, then it is useful to conduct the test in two steps:

— an acute toxicity test (range-finding test) to give an indication of the effect dilution/concentration, and the dilution/concentration giving no mortality (NOEC). Dilutions/concentrations to be used in the definitive test can then be selected;

— a definitive test on reproduction to determine sublethal effects of (dilutions of) contaminated soil or the concentration of a substance which, when evenly mixed into the standard soil, causes no significant effects on numbers of offspring hatched from cocoons compared with the control (NOEC), and the lowest concentration causing effects (LOEC).

NOTE A suitable reference soil is used to demonstrate the appropriate status of the test population and to avoid misinterpretation of results.

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

5 Reagents and material

5.1 Biological material, recommended test species is *Enchytraeus albidus* Henle 1837 (white potworm; *Enchytraeidae*, subclass: *Oligochaeta*, phylum: *Annelida*). *E. albidus* is one of the largest enchytraeid species, measuring 15 mm to 40 mm, and has a world-wide distribution (see, for example, References [22] and [32]). It can be easily recognized by two characteristics: four setae per bundle ventrally, and the very long seminal duct in the clitellum region as well as some segments behind it. The species can be found in marine, limnic and terrestrial habitats, mainly in decaying organic matter (seaweed, compost) and only rarely in meadows. This broad ecological tolerance and some morphological variations indicate that the species probably consists of several races (or ecotypes). *E. albidus* can be obtained commercially, since it is sold as food for fish. It should be verified whether such a culture is contaminated by other, usually smaller species (see, for example, References [7], [10] and [33]). If contamination of the culture occurs, all worms are washed in water in a Petri dish. With the help of a stereomicroscope, large adult specimens of *E. albidus* are selected to start a new culture. All other worms of the original culture are discarded. *E. albidus* can be bred easily in a wide range of

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