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Soil quality — Effects of pollutants on earthworms —

Part 2: Determination of effects on reproduction of *Eisenia fetida/Eisenia andrei* and other earthworm species

Qualité du sol — Effets des polluants vis-à-vis des vers de terre —

Partie 2: Détermination des effets sur la reproduction de Eisenia fetida/Eisenia andrei *et d'autres espèces de vers de terre*

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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 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,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 11268-2:2012), which has been technically revised.

The main changes are as follows:

— modification of the concentration for the reference substance (boric acid);

— inclusion of alternative species of earthworms – *Dendrodrilus rubidus, Aporrectodea caliginosa* – in informative annexes; information on their taxonomy and ecology as well as their specific testing requirements have also been added.

A list of all the parts in the ISO 11268 series can be found on the ISO website.

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 (see ISO 15799 and ISO 17616). 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.

This document describes a method that is based on the determination of sublethal effects of contaminated soils on adult earthworms of the species *Eisenia fetida* (Savigny 1826) and *Eisenia andrei* (André 1963). Optionally, the method can be used for testing chemicals added to standard soils (e.g. artificial soil) for their sublethal hazard potential to earthworms. Finally, information is provided on how to use this method for testing chemicals or test soil under tropical conditions (see Annex A).

Eisenia fetida and *Eisenia andrei* are considered to be representatives of soil fauna and earthworms in particular in temperate regions. Background information on their earthworm ecology and their use in ecotoxicological testing is available. However, these species do not occur regularly in agricultural lands (crop sites and grasslands) or forests in these regions. In addition, they are not representative of boreal or tropical regions. Therefore, other species such as *Dendrodilus rubidus* (an epigeic litter inhabitant in boreal regions) and *Aporrectodea caliginosa* (an endogeic mineral dweller in temperate regions) have been added as potential alternative test species (see Annexes B and C). These alternative earthworm species have been used as ecotoxicological test species for some time, however, testing experience has been limited to specific countries.

This document has been drawn up taking into consideration test procedures adopted by the Organization for Economic Cooperation and Development[45][46] and by the European Union[29].

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Soil quality — Effects of pollutants on earthworms —

Part 2: Determination of effects on reproduction of *Eisenia fetida/Eisenia andrei* and other earthworm species

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 the effects of soil contaminants and chemicals on the reproduction of *Eisenia fetida/Eisenia andrei* by dermal and alimentary uptake. This chronic test is applicable to soils and soil materials of unknown quality, e.g. from contaminated sites, amended soils, soils after remediation, agricultural or other sites concerned, and waste materials.

This method is designed mainly for determining the effects of soil contaminants and chemicals on the reproduction of *Eisenia fetida/Eisenia andrei*. Technical information is also provided on how to use *Eisenia fetida/andrei* for testing chemicals under tropical conditions (see Annex A). Finally, this method also includes technical information on how to use it with other environmentally relevant earthworm species: e.g. *Dendrodrilus rubidus* and *Aporrectodea caliginosa* (see Annexes B and C).

This method does not apply to substances for which the air/soil partition coefficient is greater than one, or to substances with vapour pressure exceeding 300 Pa, at 25 °C. This method does not take into account the persistence of the substance during the 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*

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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/

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

[SOURCE: ISO 15176:2019, 3.2.6]

3.2
growth
increase in biomass (i.e. the fresh mass of organisms)

Note 1 to entry: It is expressed as a percentage of the fresh mass of organisms at the start of the test.

3.3
reproduction
mean number of offspring per test container after eight weeks' incubation under the specified test conditions

3.4
ER_x
EC_x
effective rate
effective concentration
x % effect rate or concentration of the test sample or test substance at which *reproduction* (3.3) is reduced by x % compared to the control

3.5
limit test
single concentration test consisting of at least four replicates each, the test sample without any dilution or the highest concentration of test substance mixed into the *control soil* (3.10) and the control

3.6
LOER
LOEC
lowest observed effect rate
lowest observed effect concentration
lowest tested percentage of a test sample in a *control soil* (3.10) or concentration of a substance at which a statistically significant effect is observed

Note 1 to entry: The LOEC is expressed as a percentage of test-soil dry mass per test-mixture dry mass. All *test mixtures* (3.11) above the LOEC have a harmful effect equal to or greater than that observed at the LOEC. If this condition cannot be satisfied, an explanation should be given for how the LOEC and *NOEC* (3.7) have been selected.

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3.7
NOER
NOEC
no observed effective rate
no observed effect concentration
test soil percentage immediately below the LOER/LOEC or, highest tested concentration of a test substance which, when compared to the control, has no statistically significant lethal or other effect such as reduced *reproduction* (3.3) or mass alteration (error probability: $p < 0,05$)

Note 1 to entry: The NOEC is expressed as a percentage of test-soil dry mass per test-mixture dry mass.

3.8
reference soil
uncontaminated site-specific soil (e.g. collected in the vicinity of a contaminated site) with similar properties (nutrient concentrations, pH, organic carbon content and texture) to the test soil

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

EXAMPLE Euro-Soils,[29] artificial soil,[46] LUFA standard soil[40].

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

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 test samples 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.

3.11
test mixture
mixture of contaminated soil or the test substance with a *control soil* (3.10)

Note 1 to entry: Test mixtures are given in percent of contaminated soil based on soil dry mass.

4 Principle

The effects on reproduction of adult earthworms (species: *Eisenia fetida* or *Eisenia andrei*) exposed to the test soil are compared to those observed for samples exposed to a control soil. If appropriate, effects based on exposure to a dilution range of contaminated soil or range of concentrations of a test substance are determined. In addition, observations on growth and survival of adult earthworms are recorded. Test mixtures are prepared at the start of the test and are not renewed within the test period.

After four weeks, adult worms are removed from the test containers and effects on mortality and biomass are measured by counting and weighing. The effect on reproduction as the definitive end point is measured by counting the number of offspring hatched from the cocoons after an additional period of four weeks. The results obtained from the tests are compared with a control soil or, if appropriate, are used to determine the dilutions or concentrations which cause no effects on biomass, mortality and reproduction (NOER/NOEC) and the dilution (concentration) resulting in x % reduction of juveniles hatched from cocoons compared to the control (ER_x/EC_x, 56 d), respectively.

If testing a dilution or concentration series, all test dilutions/concentrations above the LOER/LOEC shall have a harmful effect equal to, or greater than that observed at the LOER/LOEC. Where there is

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