
**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).

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 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.

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]}).

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/>