

<b>STN</b>	<b>Kvalita pôdy. Vplyvy znečisťujúcich látok na dážďovky. Časť 1: Určenie akútnej toxicity pomocou Eisenia fetida alebo Eisenia andrei (ISO 11268-1: 2012).</b>	<b>STN EN ISO 11268-1</b>  46 5272
------------	---	--

Soil quality - Effects of pollutants on earthworms - Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei (ISO 11268-1:2012)

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. 11/15

Obsahuje: EN ISO 11268-1:2015, ISO 11268-1:2012

**121926**

---

Úrad pre normalizáciu, metrológiu a skúšobníctvo SR, 2015  
Podľa zákona č. 264/1999 Z. z. v znení neskorších predpisov sa môžu slovenské technické normy rozmnožovať a rozširovať iba so súhlasom Úradu pre normalizáciu, metrológiu a skúšobníctvo SR.

EUROPEAN STANDARD

**EN ISO 11268-1**

NORME EUROPÉENNE

EUROPÄISCHE NORM

August 2015

ICS 13.080.30

English Version

**Soil quality - Effects of pollutants on earthworms - Part 1:  
Determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei*  
(ISO 11268-1:2012)**

Qualité du sol - Effets des polluants vis-à-vis des vers de terre - Partie 1: Détermination de la toxicité aiguë vis-à-vis de *Eisenia fetida*/*Eisenia andrei* (ISO 11268-1:2012)

Bodenbeschaffenheit - Wirkungen von Schadstoffen auf Regenwürmer - Teil 1: Bestimmung der akuten Toxizität auf *Eisenia fetida*/*Eisenia andrei* (ISO 11268-1:2012)

This European Standard was approved by CEN on 6 August 2015.

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, 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

The text of ISO 11268-1:2012 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 11268-1:2015 by Technical Committee CEN/TC 345 “Characterization of soils” the secretariat of which is held by NEN.

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 February 2016, and conflicting national standards shall be withdrawn at the latest by February 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] 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, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

### Endorsement notice

The text of ISO 11268-1:2012 has been approved by CEN as EN ISO 11268-1:2015 without any modification.

---

---

**Soil quality — Effects of pollutants on  
earthworms —**

Part 1:  
**Determination of acute toxicity to *Eisenia  
fetida*/*Eisenia andrei***

*Qualité du sol — Effets des polluants vis-à-vis des vers de terre —  
Partie 1: Détermination de la toxicité aiguë vis-à-vis de Eisenia fetida/  
Eisenia andrei*





**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2012

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
Case postale 56 • CH-1211 Geneva 20  
Tel. + 41 22 749 01 11  
Fax + 41 22 749 09 47  
E-mail [copyright@iso.org](mailto:copyright@iso.org)  
Web [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

Page

Foreword .....	iv
Introduction .....	v
1 Scope .....	1
2 Normative references .....	1
3 Terms and definitions .....	2
4 Principle .....	3
5 Reagents and material .....	3
6 Apparatus .....	5
7 Procedure .....	5
7.1 Experimental design .....	5
7.2 Preparation of test mixture .....	6
7.3 Addition of the earthworms .....	8
7.4 Test conditions and measurements .....	8
7.5 Reference substance .....	8
8 Calculation and expression of results .....	8
8.1 Calculation .....	8
8.2 Expression of results .....	8
9 Validity of the test .....	9
10 Statistical analysis .....	9
10.1 General .....	9
10.2 Single-concentration tests .....	9
10.3 Multi-concentration tests .....	10
11 Test report .....	11
Annex A (informative) Determination of the acute toxicity of chemicals (in particular pesticides) on earthworms under tropical test conditions .....	12
Annex B (informative) Culturing of <i>Eisenia fetida</i> and <i>Eisenia andrei</i> .....	14
Annex C (informative) Determination of water holding capacity of artificial soil .....	15
Annex D (informative) Background information on the acute effect of boric acid on earthworms .....	16
Bibliography .....	17

## 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 11268-1 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 11268-1:1993), which has been technically revised.

ISO 11268 consists of the following parts, under the general title *Soil quality — Effects of pollutants on earthworms*:

- *Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei*
- *Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei*
- *Part 3: Guidance on the determination of effects in field situations*



## 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 <sup>[33]</sup> and ISO 17616 <sup>[34]</sup>). 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. As standardized test systems using earthworms as indicator organisms for the habitat function of soil, an acute test for survival and a chronic test for reproduction are available.

This part of ISO 11268 describes a method that is based on the determination of the acute toxicity of contaminated soils to 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 a standard soil (e.g. artificial soil) for their acute toxic potential to earthworms. Finally, information is provided on how to use this method for testing chemicals under tropical conditions (see Annex A).

*Eisenia fetida* and *Eisenia andrei* are considered to be representatives of soil fauna and earthworms in particular. Background information on the ecology of earthworms and their use in ecotoxicological testing is available. Other species, e.g. *Aporrectodea caliginosa*, *Lumbricus rubellus* and *Lumbricus terrestris*, have also been used as test organisms. These or other species have not been proven to be more sensitive in general, and the database and experience in testing soils is small <sup>[15][16][23]</sup>.

This part of ISO 11268 has been drawn up taking into consideration test procedures adopted by the Organization for Economic Cooperation and Development <sup>[26][27]</sup> and by the European Union <sup>[9]</sup>.



# Soil quality — Effects of pollutants on earthworms —

## Part 1:

## Determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei*

**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 part of ISO 11268 specifies one of the methods for evaluating the habitat function of soils and determining the acute toxicity of soil contaminants and chemicals to *Eisenia fetida*/*Eisenia andrei* by dermal and alimentary uptake. It 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.

Effects of substances are assessed using a standard soil, preferably a defined artificial soil substrate. For contaminated soils, the effects on survival 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 comparable 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 chemicals under temperate as well as under tropical conditions.

The method is not applicable to volatile substances, i.e. substances for which  $H$  (Henry's constant) or the air/water partition coefficient is greater than 1, or for which the vapour pressure exceeds 0,013 3 Pa at 25 °C.

This method does not take into account the possible degradation of the substances or contaminants during the test.

### 2 Normative references

The following referenced documents are indispensable for the application 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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

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 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*

**koniec náhľadu – text ďalej pokračuje v platenej verzii STN**