

# SVENSK STANDARD

## SS-EN ISO 11267:2014



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### **Markundersökningar – Reproduktionshämning av Collembola (*Folsomia candida*) på grund av markföroreningar (ISO 11267:2014)**

**Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants (ISO 11267:2014)**



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Denna standard ersätter SS-ISO 11267, utgåva 1.

The European Standard EN ISO 11267:2014 has the status of a Swedish Standard. This document contains the official version of EN ISO 11267:2014.

This standard supersedes the Swedish Standard SS-ISO 11267, edition 1.

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EUROPEAN STANDARD

**EN ISO 11267**

NORME EUROPÉENNE

EUROPÄISCHE NORM

February 2014

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ICS 13.080.30

English Version

## Soil quality - Inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil contaminants (ISO 11267:2014)

Qualité du sol - Inhibition de la reproduction de *Collembola* (*Folsomia candida*) par des contaminants du sol (ISO 11267:2014)

Bodenbeschaffenheit - Hemmung der Reproduktion von Collembolen (*Folsomia candida*) durch Verunreinigungen (ISO 11267:2014)

This European Standard was approved by CEN on 4 January 2014.

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**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

<b>Contents</b>		Page
<b>Foreword</b>		<b>iv</b>
<b>Introduction</b>		<b>v</b>
<b>1 Scope</b>		<b>1</b>
<b>2 Normative references</b>		<b>1</b>
<b>3 Terms and definitions</b>		<b>1</b>
<b>4 Principle</b>		<b>3</b>
<b>5 Reagents and material</b>		<b>4</b>
<b>6 Apparatus</b>		<b>6</b>
<b>7 Procedure</b>		<b>6</b>
7.1	Experimental design	6
7.2	Preparation of test mixture	7
7.3	Addition of the biological material	9
7.4	Test conditions and measurements	9
7.5	Determination of surviving Collembola	9
<b>8 Calculation and expression of results</b>		<b>9</b>
8.1	Calculation	9
8.2	Expression of results	9
<b>9 Validity of the test</b>		<b>10</b>
<b>10 Statistical analysis</b>		<b>10</b>
10.1	General	10
10.2	Single-concentration tests	10
10.3	Multi-concentration tests	11
<b>11 Test report</b>		<b>11</b>
<b>Annex A (informative) Techniques for rearing and breeding of Collembola</b>		<b>13</b>
<b>Annex B (informative) Determination of water-holding capacity</b>		<b>15</b>
<b>Annex C (informative) Guidance on adjustment of pH of artificial soil</b>		<b>16</b>
<b>Annex D (informative) Extraction and counting of Collembola</b>		<b>17</b>
<b>Bibliography</b>		<b>18</b>

## Foreword

This document (EN ISO 11267:2014) has been prepared by Technical Committee ISO/TC 190 "Soil quality" in collaboration with 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 August 2014, and conflicting national standards shall be withdrawn at the latest by August 2014.

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.

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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 [2] and [4]). Reference [2] includes a list and short characterization of recommended and standardized test systems and [4] 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.

Soil-dwelling Collembola are ecologically relevant species for ecotoxicological testing. Springtails are prey animals for a variety of endogeic and epigeic invertebrates and they contribute to decomposition processes in soils. In acidic soils they may be the most important soil invertebrates besides enchytraeids with respect to that function, since earthworms are typically absent.[19] Additionally, Collembola represent arthropod species with a different route and a different rate of exposure compared to earthworms[1] and enchytraeids.[3] Various species were used in bioassays of which four species were used most commonly, *Folsomia candida*, *Folsomia fimetaria*, *Onychiurus armatus*, and *Orchesella cincta*. [20] Numerous soil toxicity tests supported by Environment Canada (EC) resulted in the development and standardization of a biological test method for determining the lethal and sublethal toxicity of samples of contaminated soil to Collembola.[10] The method prepared by EC includes three species, *Orthonychiurus folsomi*, *Folsomia candida*, and *Folsomia fimetaria*. As standardized test systems using Collembola as indicator organisms for the habitat function of soil, another two methods exist. One is designed for assessing the effects of substances on the reproductive output of the Collembola, *Folsomia fimetaria* L. and *Folsomia candida* Willem in soil,[19],[21], and the other method described here, focuses on testing contaminated soil. Optionally the method can be used for testing substances added to standard soils (e.g. artificial soil) for their sublethal hazard potential to Collembola.

This International Standard describes a method that is based on the determination of sublethal effects of contaminated soils to adult Collembola of the species *Folsomia candida* Willem. The species is distributed worldwide. It plays a similar ecological role to *Folsomia fimetaria*. [10],[19] *Folsomia candida* reproduces parthenogenetically and is an easily accessible species as it is commercially available and easy to culture. *Folsomia candida* is considered to be a representative of soil arthropods and Collembola in particular. Background information on the ecology of springtails and their use in ecotoxicological testing is available.[22]



# Soil quality — Inhibition of reproduction of *Collembola (Folsomia candida)* by soil contaminants

## 1 Scope

This International Standard specifies one of the methods for evaluating the habitat function of soils and determining effects of soil contaminants and substances on the reproduction of *Folsomia candida* Willem 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, industrial, agricultural or other sites of concern and waste materials.

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).

This International Standard provides information on how to use this method for testing substances under temperate 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.

NOTE The stability of the test substance cannot be ensured 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, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 10390, *Soil quality — Determination of pH*

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*

## 3 Terms and definitions

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

- 3.1**  
**contaminant**  
substance or agent present in the soil as a result of human activity
- 3.2**  
**EC<sub>x</sub>**  
**effect concentration for x % effect**  
concentration (mass fraction) of a 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 EC<sub>50</sub> 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<sub>x</sub> is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass). When substances are tested, the EC<sub>x</sub> is expressed as mass of the test substance per dry mass of soil in milligrams per kilogram.
- 3.3**  
**ER<sub>x</sub>**  
**effect rate**  
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**  
**limit test**  
single concentration test consisting of at least four replicates each, the soil to be tested without any dilution or the highest concentration of test substance mixed into the control soil and the control
- 3.5**  
**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.
- 3.6**  
**LOER**  
**lowest observed effect rate**  
lowest rate of a soil to be tested in a control soil at which a statistically significant effect is observed
- 3.7**  
**NOEC**  
**no observed effect concentration**  
highest test substance concentration immediately below the LOEC at which no effect is observed
- Note 1 to entry: In this test, the concentration corresponding to the NOEC has no statistically significant effect (probability  $p < 0,05$ ) within a given exposure period when compared with the control.
- 3.8**  
**NOER**  
**no observed effect rate**  
lowest rate of a soil to be tested immediately below the LOER which, when compared to the control, has no statistically significant effect (probability  $p < 0,05$ ) within a given exposure period
- 3.9**  
**reference soil**  
uncontaminated soil with comparable pedological properties (nutrient concentrations, pH, organic carbon content and texture) to the soil being studied

### 3.10

#### **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.

### 3.11

#### **control soil**

reference or standard soil 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.

### 3.12

#### **test mixture**

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

### 3.13

#### **test mixture ratio**

ratio between the soil to be tested and the control soil in a test mixture

## 4 Principle

The effects on reproduction of 10 d to 12 d old Collembola (*Folsomia candida*) exposed to the soil to be tested are compared to those observed in a control soil. If appropriate, effects based on exposure to a test mixture of contaminated soil and control soil or a range of concentrations of a test substance mixed into control soil are determined. Test mixtures are prepared at the start of the test and are not renewed within the test period.

The Collembola are incubated until offspring ( $F_1$ ) emerge from eggs laid by mature adults, and the number of offspring is determined. Usually offspring emerge within 28 d in control experiments. The results obtained from the tests are compared with a control or, if appropriate, are used to determine the concentrations which cause no effects on mortality and reproduction (NOER/NOEC) and the concentration resulting in  $x\%$  reduction of juveniles hatched from eggs compared to the control ( $ER_x/EC_x$ , 28 d) respectively.

If testing a concentration series, all test dilutions/concentrations above the LOER/LOEC have a harmful effect equal to or greater than that observed at the LOER/LOEC. Where there is no prior knowledge of the concentration of the soil to be tested or 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) is carried out, to give an indication of the effect dilution/concentration, and the dilution/concentration giving no mortality (NOER/NOEC). Dilutions/concentrations to be used in the definitive test can then be selected;
- the 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 eggs compared with the control (NOER/NOEC), and the lowest concentration causing effects (LOER/LOEC).

NOTE The use of a reference soil is an essential requirement to demonstrate the present status of the test population, and to avoid misinterpretation of results.