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SWEDISH STANDARDS INSTITUTE

Soil quality – Avoidance test for determining the quality of soils and effects of chemicals on behaviour –
Part 2: Test with collembolans (Folsomia candida)
(ISO 17512-2:2011, IDT)

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Den internationella standarden ISO 17512-2:2011 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av ISO 17512-2:2011.

The International Standard ISO 17512-2:2011 has the status of a Swedish Standard. This document contains the official version of ISO 17512-2:2011.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.

Denna standard är framtagen av kommittén för Karaktärisering av avfall, mark och slam, SIS/TK 535.

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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.

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 17512-2 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 17512 consists of the following parts, under the general title *Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour*:

— *Part 1: Test with earthworms (*Eisenia fetida *and* Eisenia andrei*)*

— *Part 2: Test with collembolans (*Folsomia candida*)*

Introduction

The use of the avoidance behaviour of soil invertebrates as an indicator of unfavourable conditions allows a preliminary assessment of contaminated soils in a short period of time, with a high degree of sensitivity. Being rapid, cost-effective and ecologically relevant, the avoidance tests with earthworms were proposed to complement conventional chemical analysis. Supporting the results obtained in the chronic tests, the avoidance bioassays can be used as a first screening tool in the assessment of the habitat function of soils. Considering the fact that the avoidance response of soil invertebrates differs between species due to their distinct sensitivity to contaminants and modes of exposure, it is recommended to standardize a second rapid cost-effective and ecologically relevant avoidance bioassay.

Springtails have shown a distinct sensitivity towards several contaminants when compared with earthworms, complementing the information obtained in the avoidance tests with earthworms [1][2]. Until now, the species *Folsomia candida* has been the most commonly used collembolan test species due to a great facility to keep laboratory cultures and due to their high locomotor ability [3]. *Folsomia candida* is considered to be a hemi-edaphic species, meaning that it lives mainly in the soil. Furthermore, this species is already used in ISO 11267.

Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour —

Part 2:
Test with collembolans (*Folsomia candida*)

1 Scope

This part of ISO 17512 specifies a rapid screening method for evaluating the habitat function of soils based on the avoidance behaviour of springtails.

The test is a rapid method that reflects the bioavailability of contaminants in natural soils and substances spiked into soils to *Folsomia candida*. In both cases, it is possible to establish a dose-response-relationship. The avoidance behaviour of the springtails is the measurement endpoint of the test. This test is not intended to replace the Collembola reproduction 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 10390, *Soil quality — Determination of pH*

ISO 11267:1999, *Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants*

ISO 11268-2:1998, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 2: Determination of effects on reproduction*

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soils on the emergence and early growth of higher plants*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 15799, *Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials*

3 Terms and definitions

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

3.1
avoidance behaviour
tendency (of an organism) to avoid the test soil while preferring the control soil

[ISO 17512-1:2008]

3.2
test soil
either a natural or an artificial clean soil that is spiked with the test substance or a contaminated natural soil (a site soil)

3.3
control soil
natural or artificial uncontaminated soil

See 5.3.

3.4
limited habitat function
habitat function is limited if, on average, $>$ 70 % of springtails are found in the control soil (indication of an impact on behaviour) after they were allowed to choose between the control soil and tested soil

3.5
effective concentration
EC_x
concentration at which a specific effect is detected [where x is a percentage (10, 25, 50) of this effect; e.g. avoidance]

NOTE In this part of ISO 17512-2, an EC_{50} means the concentration of a test substance, or dilution of a contaminated test soil, that is estimated to cause an avoidance response of 50 %.

EXAMPLE An avoidance of 50 % occurs when the number of springtails in the test soil is 50 % of the number that should be there in the case of no avoidance: no avoidance (control soil = 10 individuals; test soil = 10 individuals); 50 % avoidance (control soil = 15 individuals; test soil = 5 individuals).

3.6
lowest observed effect concentration
LOEC
lowest tested concentration of a test substance, or dilution of a contaminated test soil, that is observed to cause a statistically significant avoidance response ($p \leq 0{,}05$)

NOTE 1 In the case of a test substance, the concentration is expressed as mass of the test substance per dry mass of test substrate; in the case of a contaminated test soil, the concentration is expressed as the percentage dilution of the test soil.

NOTE 2 All tested concentrations/dilutions above the LOEC should have a harmful effect equal to or greater than those observed at the LOEC. When this condition is not observed, a full explanation should be given for how the LOEC (and hence the NOEC) has been selected.

3.7
no observed effect concentration
NOEC
test concentration/dilution tested immediately below the LOEC, which causes a not statistically significant avoidance response ($p > 0{,}05$)

4 Principle

Springtails (*Folsomia candida*) are exposed at the same time to the control soil and the test soil. These soils are filled into the two sections of the same vessel. After an incubation period of two days, the number of springtails is determined in each section of the vessels.

5 Reagents and materials

5.1 Biological material, only springtails of the species *Folsomia candida* (Willem) (see A.2.6) coming from synchronized cultures should be used.

NOTE Typically, in this test springtails can be used when they are 10 d to 12 d old (or alternatively adult, e.g. 20 d to 22 d old).

5.2 Test substrate, in the case of a natural soil, the substrate to be tested should be sieved (2 mm) and moisture-adjusted to about 40 % to 60 % of the maximum water-holding capacity. If standing water or free water appears when the soil is compressed before achieving the desired percentage of maximum WHC, a lower percentage might be used. The optimum water content is achieved if there is no standing water or free water appearing when the soil is compressed.

NOTE For highly silty and loamy soils, it can be difficult to get the necessary amount of soil sieved to ≤ 2 mm with an acceptable expenditure of work. The holes of the sieves might plug up within several minutes. Frequent cleaning is necessary. In this case, it is acceptable to sieve the amount of soil needed for the test to ≤ 5 mm.

Determine the water content and the pH in the presence of 1 mol/l KCl, in accordance with ISO 11465 and ISO 10390, respectively, immediately before the start of the test. In addition, the maximum water-holding capacity shall be determined according to Annex D.

If testing a substance, a different procedure should be followed (see Annex C).

5.3 Control soil, two choices are possible (see also ISO 15799). Either a) a reference soil or b) a standard soil that allows the presence of springtails.

a) If reference soils from uncontaminated areas near a contaminated site are available, they should be treated and characterized like the test soils. If a toxic contamination or unusual soil properties cannot be ruled out, standard control soils should be preferred.

b) For testing the effects of substances mixed into soil or making dilutions of the test soil, standard soils (e.g. LUFA 2.2) shall be used as the test substrate. The properties of the field-collected standard soil shall be reported.

The substrate called artificial soil can be used as a standard soil and has the following composition:

	Percentage expressed on dry-mass basis
— Sphagnum peat finely ground and with no visible plant remains	10 %
— Kaolinite clay containing not less than 30 % Kaolinite	20 %
— Industrial quartz sand (dominant fine sand with more than 50 % of particle size 0,05 mm to 0,2 mm)	69 %

Approximately 0,3 % to 1,0 % calcium carbonate ($CaCO_3$, pulverized, analytical grade) are necessary to get a pH of 6,0 ± 0,5.

Natural soil should be sieved and the water content should be adjusted according to 5.2.

5.4 Reference substance, having Phenmedipham as the only active ingredient.

6 Apparatus

Usual laboratory equipment and the following.

6.1 Containers (see Annex B).

Cylindrical containers of capacity 200 ml to 300 ml with a cross-sectional area of about 50 cm^2, such that a depth of 3 cm to 4 cm of soil is achieved.

Test containers shall permit gaseous exchange between the medium and the atmosphere and access of light (e.g. by means of a perforated transparent cover), and shall have provisions to prevent springtails from escaping (e.g. by using a tape to fix the cover). To avoid lateral effects of light, test vessels should be made of opaque material, otherwise they should be wrapped with aluminium foil.

NOTE Due to the short test period and the proportionally large volume of soil in the vessels (considering the small amount of soil needed by the springtails), a reduction of the chemical concentration in the soil resulting from sorption to the vessel walls is negligible. Therefore, plastic vessels can be used, although, when available, the use of inert material (e.g. glass or stainless steel) is preferred.

6.2 Divider, made of plastic or thin sheets of metal. It shall divide the test containers vertically into two identical sections.