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Soil quality — Inhibition of reproduction of the soil mite (*Hypoaspis aculeifer*) by soil contaminants

*Qualité du sol — Inhibition de la reproduction de l'acarien prédateur (*Hypoaspis aculeifer*) par des contaminants du sol*

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Foreword

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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).

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

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 (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.

Mites (Acari) are a world-wide and diverse group of arthropods belonging to the class Arachnida with over 40 000 species recorded, divided into two super-orders (Acariformes and Parasitiformes). Due to their relative small size (a few µm to a few cm), they occupy specific ecological niches on plants as well as in soils (see Reference [13]).

Among soil-inhabiting mites, the role of predation is ensured by, for example, *Hypoaspis* sp. (Laelapidae). Because they are exposed to chemical contamination, mites are already considered in the environmental risk assessment of pesticides, as non-target organisms (see Reference [10]). Indeed, among the data required for active substances of pesticides, effects on predatory mites are assessed, i.e. for the plant-inhabitant *Typhlodromus pyri* (Phytoseiidae) and the soil-inhabitant *Hypoaspis aculeifer* (Laelapidae) (see Reference [6]).

The first authors introducing *H. aculeifer* as a test organism in ecotoxicological studies[23][17] were later proposed a two-species test system in the European project SECOFASE (Sublethal Effects of Chemicals on Fauna in the Soil Ecosystem), including the collembolan *Folsomia fimetaria* as prey. In the context of the development of an ecotoxicological test for the assessment of plant protection products on non-target arthropods (see References [5][6]), a protocol on soil predatory mites using *H. aculeifer* was further proposed. More recently, a standard test protocol for the assessment of chemicals was developed for this species by OECD in 2008 and revised in 2016. The results of the associated international ring-test were published in Reference [25].

Among mites, the predator *Hypoaspis aculeifer* is the most studied species in laboratory. The reproduction end point was found in general to be more sensitive than mortality and avoidance. Compared to other soil meso-fauna invertebrates, mites were found in general less sensitive than or as sensitive as other test species, depending on the end points and chemicals studied. Considering semi-field studies, *H. aculeifer* was used as a top predator whereas other soil invertebrates, mainly springtails, were ranked in the grazer group. In these studies, mites showed to be quite tolerant towards anthropogenic contamination. This statement was also corroborated by field surveys. However, the applicability of laboratory test methods for the assessment of environmental samples (contaminated soils, wastes etc.) with mites is emphasized, as to date a limited number of studies are available.

This document describes a method that is based on the determination of lethal and sublethal effects of contaminated soils to adult predatory mites of the species *Hypoaspis aculeifer*. This species is considered to be representative of predatory soil arthropods. Background information on the ecology of these mites and their use in ecotoxicological testing is available in Reference [14].

Soil quality — Inhibition of reproduction of the soil mite (*Hypoaspis aculeifer*) by soil contaminants

1 Scope

This document specifies a chronic test method for evaluating the habitat function of soils and determining effects of soil contaminants and substances on the reproduction of *Hypoaspis aculeifer* by – mainly – alimentary uptake. This method 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 under concern and waste materials (e.g. dredged material, municipal sludge from a wastewater treatment plant, composed material, or manure, especially those for possible land disposal). The reproduction (= number of juveniles) is the measured parameter of the test. The test reflects the bioavailability of a mixture of contaminants in natural soils (contaminated site soils) to a species which represents a trophic level which is not covered by other ISO standards. This test is not intended to replace the earthworm (see ISO 11268-2) or Collembola (see ISO 11267) reproduction tests since this species belongs not only to a different trophic group but also a different taxonomic group (= mites; i.e. arachnids) than those used usually.

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. Depending on 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 document provides information on how to use this method for testing samples (soils or substances) under temperate conditions.

This document is not applicable 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.

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 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 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*

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 terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

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

3.2
effect concentration for *x* % effect
EC_x
concentration (mass fraction) of a test sample that causes *x* % of an effect on a given end point within a given exposure period when compared with a control

EXAMPLE An EC_{50} 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*x* is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass). When substances are tested, the EC*x* is expressed as the mass of the test substance per dry mass of soil in milligrams per kilogram.

3.3
effect rate
ER_x
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
lowest observed effect concentration
LOEC
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
lowest observed effect rate
LOER
lowest rate of a soil to be tested in a control soil at which a statistically significant effect is observed

3.7
no observed effect concentration
NOEC
highest test substance concentration immediately below the *LOEC* (3.5) 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
no observed effect rate
NOER
lowest rate of a soil to be tested immediately below the *LOER* (3.6) 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 type 2.2.

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 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

Adult females are exposed to the soil to be tested and the effects on reproduction measured are compared to those observed for females exposed to a control soil. If appropriate, effects based on exposure to a dilution range 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 test is started with 10 adult females per test vessel. Males are not introduced in the test, because experience has shown that females mate immediately or shortly after hatching from the deutonymph stage, if males are present. As the females are introduced into the test about 7 d after they have reached the adult stage, the females can be considered as already mated (Annex A and Annex E). The test runs until the first offspring have reached the deutonymph stage. At 20 °C the exposure time ends at day 14 after introducing the females (day 0), followed by two days of extraction. The number of surviving females and the number of juveniles per test vessel are determined. The reproductive output of the mites exposed to the test mixtures is compared to that of the controls in order 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) respectively, depending on the experimental design (see 7.1.3).

In case there is no prior knowledge of the dilution/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:

— A range-finding test on reproduction 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).

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.

5 Reagents and material

5.1 Biological material

In this test, *Hypoaspis (Geolaelaps) aculeifer*. adult female mites (7 d to 14 d after becoming adult; 28 d to 35 d after the start of the egg laying in the synchronisation) are required to start the test. The mites shall be selected from a synchronised cohort (see Annex E).

5.2 Test mixtures

5.2.1 Field-collected soil or waste. The field-collected soils used in the test shall be passed through a sieve of 4 mm square mesh to remove coarse fragments and thoroughly mixed. If necessary, soil may be air-dried without heating before sieving. Storage of soil to be tested should be as short as possible. The soil shall be stored in accordance with ISO 18400-206 using containers that minimize losses of soil contaminants sorption to the container walls. If soils or test mixtures have been stored, they should be mixed a second time immediately before use. Soil pH should not be corrected as it can influence bioavailability of soil contaminants.

For interpretation of test results, the following characteristics shall be determined for each soil sampled from a field site:

a) pH in accordance with ISO 10390;

b) texture (sand, loam or silt, clay) in accordance with ISO 11277;

c) water content in accordance with ISO 11465;

d) water-holding capacity according to Annex B;

e) cationic exchange capacity in accordance with ISO 11260;

f) organic carbon in accordance with ISO 10694;

g) percentage of material removed by the 4 mm sieve.

It is important to determine the water holding capacity of all mixtures used in the test.

5.2.2 Control soil, either a) reference soil or b) standard soil that allows the presence of predatory mites. Control soil and soil used for dilution shall not differ in one test [either a) or b)].

a) If reference soils from uncontaminated areas near a contaminated site are available, they should be treated and characterized like the soils to be tested. 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, standard soils (e.g. artificial soil, LUFA standard soil type 2.2.) shall be used as 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 [a particle size of (2 ± 1) mm is acceptable] and with no visible plant remains	5 %
— 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)	74 %

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

NOTE 1 Taking the properties of highly non-polar (log K_{ow} > 2) or ionizing substances into account, 5 % of peat have proven to be sufficient for maintaining the desired structure of the artificial soil.

NOTE 2 It has been demonstrated that *Hypoaspis aculeifer* can comply with the validity criteria even on reproduction when tested in field soils with lower organic carbon content (e.g. 2,7 %), and there is experience that this can be achieved in artificial soil with 5 % peat. Therefore, it is not necessary before using such a soil in a definitive test to demonstrate the suitability of the artificial soil for allowing the test to comply with the validity criteria unless the peat contents lowered more than specified above.

Prepare the artificial soil at least three days prior to start the test, by mixing the dry constituents listed above thoroughly in a large-scale laboratory mixer. A portion of the deionized water required is added while mixing is continued. Allowance should be made for any water that is used for introducing the test substance into the soil. The amount of calcium carbonate required can vary, depending on properties of the individual batch of sphagnum peat and should be determined by measuring sub-samples immediately before the test (see Annex C). Store the mixed artificial soil at room temperature for at least two days to equilibrate acidity. To determine pH and the maximum water holding capacity, the dry artificial soil is pre-moistened one or two days before starting the test by adding deionised water to obtain approximately half of the required final water content of 40 % to 60 % of the maximum water holding capacity.

The total water-holding capacity shall be determined in accordance with Annex B, the pH shall be determined according to ISO 10390.

5.3 Reference substance

5.3.1 General. To ensure the quality of the test system, tests should be performed regularly (once or twice a year) with a reference substance.

The NOEC and/or the EC_x of a reference substance shall be determined to provide assurance that the laboratory test conditions are adequate and to verify that the response of the test organisms did not change over time. The reference substance can be tested in parallel to the determination of the toxicity of each test sample at one concentration, which needs be demonstrated beforehand in a dose response study to result in an effect of about 50 %. In this case, the number of replicates should be the same as that in the controls. Alternatively, the reference substance is tested once or twice a year in a dose-response test. Depending on the design chosen, the number of concentrations and replicates and the spacing factor differ (see 7.1.3), but a response of 10 % to 90 % effect should be achieved (spacing factor of 1,8). Dimethoate as well as boric acid are suitable reference substances that have shown to affect reproduction[25].

The EC_{50} for dimethoate based on the number of juveniles should fall in the range between 3,0 mg a.s. (active substance)/kg soil (dry mass) and 7,0 mg a.s. (active substance)/kg soil (dry mass). Based on the results obtained with boric acid so far, the EC_{50} based on the number of juveniles should fall in the range between 100 mg/kg (dry mass) soil and 300 mg/kg (dry mass) soil.