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Markundersökningar – Föroreningars effekt på unga marklevande sniglar (Helicidae) – Bestämning av jordföroreningars effekt på tillväxt (ISO 15952:2006)

Soil quality – Effects of pollutants on juvenile land snails (Helicidae) – Determination of the effects on growth by soil contamination (ISO 15952:2006)

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Denna standard ersätter SS-ISO 15952:2006, utgåva 1.

The European Standard EN ISO 15952:2011 has the status of a Swedish Standard. This document contains the official version of EN ISO 15952:2011.

This standard supersedes the Swedish Standard SS-ISO 15952:2006, edition 1.

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 15952

June 2011

ICS 13.080.30

English Version

Soil quality - Effects of pollutants on juvenile land snails (Helicidae) - Determination of the effects on growth by soil contamination (ISO 15952:2006)

Qualité du sol - Effets des polluants vis-à-vis des escargots juvéniles (Helicidae) - Détermination des effets sur la croissance par contamination du sol (ISO 15952:2006)

Bodenbeschaffenheit - Wirkungen von Schadstoffen auf Jungtiere von Landschnecken - Bestimmung der Wirkungen auf das Wachstum durch Bodenverunreinigung (ISO 15952:2006)

This European Standard was approved by CEN on 10 June 2011.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Contents

Page

Foreword

The text of ISO 15952:2006 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 15952:2011 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of an identical text or by endorsement, at the latest by December 2011, and conflicting national standards shall be withdrawn at the latest by December 2011.

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Endorsement notice

The text of ISO 15952:2006 has been approved by CEN as a EN ISO 15952:2011 without any modification.

Introduction

Because of the limited amount of data available concerning toxicity of contaminants on soil organisms, the problems of assessing the ecotoxicity of soils and waste are cause for serious concern at both national and international levels. Currently available tests use soil-fauna organisms restricted to annelid (earthworms and *Enchytraeidae*) and arthropod phyla (insects: Collembola and Coleoptera). Among the latter, two standards assess acute toxicity [earthworms (ISO 11268-1) and coleoptera larvae [5]] and three other standards assess sublethal effects of soil contaminants on reproduction (earthworms [2], Collembola [1], *Enchytraeidae* [3]). In the biological cycles of organisms, it appears that growth is, like reproduction, a fundamental ecophysiological parameter to be taken into consideration for the sustainability of species and ecosystems [33].

Snails are pertinent ecological indicators for assessing the quality of soils [15], as they are characteristic of the soil surface layer (saprophagous and phytophagous) of which a large part of the biological cycle takes place in the soil (egg-laying, hatching, initial stages of development, hibernation, etc.) [6], [17], [26]. During the other phases of their cycle, they eat soil and are in contact with the soil via their moist pedal sole (foot) covered with mucus and participate in the permanent exchanges with the soil (water, mineral salts, excrement and finally shell and organic matter when they die) [6], [17], [28]. In addition, they constitute an important link between plants, fauna and soil microorganisms. They correspond fully to the criteria for a good biological indicator: easy to sample and identify, they are widely distributed; they accumulate contaminants [8, 10 to 14, 16, 17, 19, 21, 26, 27, 35 to 43]; their ecological and physiological characteristics are well-known [6], [9], [29]; and they are now easy to breed under controlled conditions [19], [23, [29]. Their susceptibility to common contaminants of their environment has been demonstrated [10 to 15, 18 to 27, 32, 33, 36 to 42].

This International Standard describes a method for determining the effects on survival and growth of young snails of substances, preparations, soils or waste materials added to an artificial or a natural soil. The described method is thus applicable to test contaminated soils or to compare different uncontaminated soils. The recommended species is *Helix aspersa aspersa* Müller (also commonly called: common garden snail, brown garden snail, garden snail, land snail, “Petit-Gris”). Among land snails (stylommatophoran pulmonate gastropod molluscs of the *Helicidae* family), *Helix aspersa aspersa* Müller is the most ubiquitous. This palearctic species can be acclimated to regions with different types of climate: Mediterranean, oceanic temperate, midcontinental temperate and even tropical. *Helix aspersa aspersa* Müller is of European origin and has been introduced into all parts of the world. They are now on all continents except Antarctica [9].

Indeed, in their natural environment, snails integrate the contaminants by contact (with various substrates such as soil, soil leachates, plant litter), by ingestion (of plants and soil), as well as through the respiratory tract [6], [26]. So, for specific testing purposes (evaluation of the toxicity of a pesticide, for example), another test design, which is focussed on exposure via food uptake, is optionally available (Annex F and Reference [4]).

Soil quality — Effects of pollutants on juvenile land snails (*Helicidae*) — Determination of the effects on growth by soil contamination

1 Scope

This International Standard specifies a semi-static method for the determination of the effects of contaminants on growth and survival of young snails, usually *Helix aspersa aspersa* Müller. The animals are exposed via the cutaneous and digestive route using a test substrate (artificial or natural soil according to the objective of the study) to which defined amounts of the following are added:

— substances or preparations;

— soils (contaminated or of unknown quality) or waste materials.

A static method may be implemented in addition to the semi-static method (optional). This method is described in Annex A.

This method does not apply to volatile substances, i.e. substances for which the Henry constant, H, or the air/water partition coefficient is over 1, or for which the vapour pressure is over 0,013 3 Pa at 25 °C.

This test takes into account the possible change in the test substance, preparation, soil or waste material because the test mixture is prepared and renewed every 7 days during the 28-day test period.

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 for the assessment of aerobic microbial processes 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 11268-1, *Soil quality — Effects of pollutants on earthworms (*Eisenia fetida*) — Part 1: Determination of acute toxicity using artificial soil substrate*

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

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

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

EN 14735, *Characterization of waste — Preparation of waste samples for ecotoxicity tests*

3 Terms and definitions

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

3.1
test substrate
artificial soil or natural soil used as control and dilution substrate

3.2
matrix
soil or waste material under test

3.3
test mixture
mixture of the test substance, preparation or matrix with the test substrate

3.4
growth
increase in the biomass, i.e. in the total fresh mass (body and shell) of the organisms and increase in the maximum shell diameter, between the start and completion of the test

NOTE It is expressed in the form of a growth coefficient.

3.5
effect concentration
EC_x
concentration at which a specific effect is detected; x is the percentage (10, 25, 50) of this effect, e.g. growth inhibition

EXAMPLE EC_{50} means the concentration estimated to reduce growth at the end of the test to 50 % compared to the control.

3.6
median lethal concentration
LC_{50}
concentration of the substance, of the test preparation initially present, or the concentration of the matrix causing the death of 50 % of the snails submitted to testing

3.7
lowest observed effect concentration
LOEC
lowest tested concentration at which the test substance is observed to have a statistically significant effect ($p < 0{,}05$) when compared with the control

NOTE All test concentrations above the LOEC have a harmful effect equal to or greater than those observed at the LOEC. When these two conditions cannot be satisfied, a full explanation should be given for how the LOEC (and hence the NOEC) has been selected.

3.8
no observed effect concentration
NOEC
test concentration immediately below the LOEC, which, when compared with the control, has no statistically significant effect ($p > 0{,}05$) within a given exposure time

NOTE 1 The NOEC is the concentration just below the LOEC.

NOTE 2 For 3.5, 3.6, 3.7 and 3.8, results are given:

— in dry mass of test substance or preparation per dry mass of the test substrate;

— in mass percentage of the tested matrix in the test mixture (expressed in dry mass).

4 Principle

Juvenile land snails (usually *Helix aspersa aspersa* Müller) are exposed during a period of 28 days to a test mixture containing the test substance, preparation or matrix at different concentrations. The test mixture is freshly prepared and renewed every 7 days.

According to the objectives, the test mixture may be prepared with artificial soil (6.3.2) or with a suitable natural soil (6.3.3).

The snails are fed during the test with uncontaminated food.

The effects on growth (fresh mass and shell diameter) and on survival are measured after 28 days of exposure (optionally, effects could be measured every 7 days during 28 days).

The results obtained during testing are compared with those of a control to determine the NOEC or LOEC and to allow the estimation of the concentration which reduces the growth of the snails by 50 % within 28 days with respect to the fresh mass [$EC_{50,m}$ (28 days)] and to the shell diameter [$EC_{50,d}$ (28 days)] or other values of EC_x.

If the concentrations selected result in lethal effects, the results obtained during testing are compared with those of a control and used for estimating the concentration which causes the death of 50 % of the snails [LC_{50}(28 days)].

For particular applications, various parameters (EC_x, NOEC, LOEC, LC_{50}) can be assessed (optional) after exposure periods lower than 28 days (7 days, 14 days or 21 days).

The test is conducted in two stages:

— a preliminary test intended to indicate both the non-observed effect concentration, NOEC, and the complete growth inhibition. The resulting dose-response relationship is important for the proper design of the definitive test;

— a definitive test specifying the concentrations which cause between 10 % and 90 % of growth inhibition. It is not necessary to perform a final test where the preliminary test has not revealed any inhibitory effects at the maximum concentration tested.

5 Test environment

The test shall be carried out at a temperature of (20 ± 2) °C under a day-night photoperiod of 18 h to 6 h. The illumination intensity (artificial light of daylight type), without any natural light in the test containers shall be 50 lux to 100 lux.

6 Reagents

6.1 Water, of purity at least deionized

6.2 Biological material

Test organisms shall be juvenile snails. The recommended species is *Helix aspersa aspersa* Müller which shall be 3 to 5 weeks old, having a mean fresh mass of (1 ± 0,3) g and a shell diameter of (15,5 ± 1) mm.

NOTE The use of some other genus and/or species of *Helicidae* is possible (see examples and conditions in Annex G).

The snails shall be selected from synchronous breeding in order to form a population as homogeneous as possible with respect to size, mass and age. The breeding techniques for snails are described in Annex B.