# SVENSK STANDARD
# SS-EN ISO 15952:2018

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**Markundersökningar – Föroreningars effekt på unga marklevande sniglar (Helicidae) – Bestämning av jordföroreningars effekt på tillväxt (ISO 15952:2018)**

**Soil quality – Effects of pollutants on juvenile land snails (Helicidae) – Determination of the effects on growth by soil contamination (ISO 15952:2018)**

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Europastandarden EN ISO 15952:2018 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 15952:2018.

Denna standard ersätter SS-EN ISO 15952:2011, utgåva 1

The European Standard EN ISO 15952:2018 has the status of a Swedish Standard. This document contains the official version of EN ISO 15952:2018.

This standard supersedes the SS-EN ISO 15952:2011, edition 1



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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

# EN ISO 15952

July 2018

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Supersedes EN ISO 15952:2011

English Version

# Soil quality - Effects of pollutants on juvenile land snails (Helicidae) - Determination of the effects on growth by soil contamination (ISO 15952:2018)

Qualité du sol - Effets des polluants vis-à-vis des escargots juvéniles (Helicidae) - Détermination des effets sur la croissance par contamination du sol (ISO 15952:2018)

Bodenbeschaffenheit - Wirkungen von Schadstoffen auf Jungtiere von Landschnecken - Bestimmung der Wirkungen auf das Wachstum durch Bodenverunreinigung (ISO 15952:2018)

This European Standard was approved by CEN on 15 June 2018.

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



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# European foreword

This document (EN ISO 15952:2018) has been prepared by Technical Committee ISO/TC 190 "Soil quality" in collaboration with Technical Committee CEN/TC 444 “Test methods for environmental characterization of solid matrices” 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of an identical text or by endorsement, at the latest by January 2019, and conflicting national standards shall be withdrawn at the latest by January 2019.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 15952:2011.

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, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

**Endorsement notice**

The text of ISO 15952:2018 has been approved by CEN as EN ISO 15952:2018 without any modification.

# Introduction

Because of the limited amount of data available concerning toxicity of contaminants on soil organisms, 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[6]] and three other standards address sublethal effects of soil contaminants on reproduction (earthworms[3], Collembola[2], *Enchytraeidae*[4]). 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[38].

Snails are relevant ecological indicators for assessing the quality of soils(See References [16][18] to [20][32][33][40] to [42]), 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.)[7][18][29]. 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)[7][18][31]. 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 (See References[9],[11] to [15],[17][18][22][24][29][30],[33] to [48]); their ecological and physiological characteristics are well-known[7][10][32]; and they are now easy to breed under controlled conditions[22][26][32]. Their susceptibility to common contaminants of their environment has been demonstrated (See References [11] to [16],[19] to [28],[30],[33] to [38],[37] to [48]).

This International Standard describes a method for determining the effects on survival and growth of young snails of substances, preparations (i.e. a mixture or solution composed of two or more substances), 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”; synonyms: *Cantareus aspersus, Cornu aspersum*[56]). 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[10].

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[7][29]. So, for specific testing purposes (evaluation of pesticide toxicity, for example), another test design, which is focussed on exposure via food uptake, is optionally available (Annex F and Reference [6]).

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

## 1 Scope

This document specifies a semi-static method for determining 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, mixtures or preparations;

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

This test takes into account the possible changes in the test substance, preparation, soil or waste material because the test mixtures are prepared and renewed every week during the 28-day test period.

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 substances for which the air/soil partition coefficient is greater than one, or to substances with vapour pressure exceeding 300 Pa, at 25 °C.

## 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 18400-206, *Soil quality — Sampling — Part 206: Guidance on the collection, handling and storage of soil for the assessment of biological functional and structural endpoints in the laboratory*

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

ISO 11269-2:2012, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early 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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

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

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

**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* (3.1)

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

Note 1 to entry: It is expressed as a biomass growth coefficient $k_{GC,m}$.

**3.5**
**growth**
<shell> increase in the maximum shell diameter, between the start and completion of the test

Note 1 to entry: It is expressed as a shell diameter growth coefficient $k_{GC,d}$.

**3.6**
**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 ($EC_{50,m}$ and $EC_{50,d}$ for biomass growth and shell growth respectively).

**3.7**
**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

**3.8**
**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 1 to entry: 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.

**3.9**
**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 to entry: The NOEC is the concentration just below the LOEC.

Note 2 to entry: For 3.6, 3.7, 3.8 and 3.9, results are given:

— in dry mass of test substance or preparation per dry mass of the *test substrate* (3.1);

— 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 d to test mixtures containing the test substance, preparation or matrix at different concentrations. The test mixtures are freshly prepared and renewed every 7 d.

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

The snails are fed during the test with uncontaminated food.

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

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 d with respect to the fresh mass [$EC_{50,m}$ (28 d)] 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 d)].

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

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 to 100 lx.

## 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 (also known as *Cantareus aspersus* and *Cornu aspersum*) 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).