

---

---

## Soil quality — In situ caging of snails to assess bioaccumulation of contaminants

*Qualité du sol — Encagement in situ d'escargots pour la mesure de la  
bioaccumulation de contaminants*



## **COPYRIGHT PROTECTED DOCUMENT**

© ISO 2021

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

Page

Foreword.....	iv
Introduction.....	v
<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>2</b>
<b>5 Test organism and equipment.....</b>	<b>2</b>
5.1 Biological material.....	2
5.2 Equipment.....	3
<b>6 Preparation of the organisms for the exposure.....</b>	<b>4</b>
<b>7 Exposure of the test organisms.....</b>	<b>4</b>
7.1 General.....	4
7.2 Beginning of exposure.....	5
7.3 End of the exposure — Starvation.....	6
7.4 Sampling and preparation after exposure.....	7
<b>8 Calculation and expression.....</b>	<b>7</b>
8.1 General.....	7
8.2 For metal(loid)s.....	7
8.2.1 Threshold guide value.....	7
8.2.2 Calculation of the sum of the excess of transfer of metal(loid)s: SET index.....	8
8.3 For other chemicals.....	9
<b>9 Validity of the experiment.....</b>	<b>9</b>
<b>10 Test report.....</b>	<b>9</b>
<b>Annex A (informative) Sources and routes of exposure of snails to contaminants in the field.....</b>	<b>10</b>
<b>Annex B (informative) Main steps of the bioassay in situ.....</b>	<b>11</b>
<b>Annex C (informative) Breeding technique for snails.....</b>	<b>15</b>
<b>Annex D (informative) Example of composition of snail feed.....</b>	<b>22</b>
<b>Annex E (informative) Usual concentrations in the viscera of sub-adult snails before caging.....</b>	<b>23</b>
<b>Annex F (informative) Recommended test systems for in situ exposure to assess bioaccumulation of contaminants in snails.....</b>	<b>25</b>
<b>Annex G (informative) Example of mass of snails before exposure.....</b>	<b>28</b>
<b>Annex H (informative) Results of the international ring test.....</b>	<b>30</b>
<b>Annex I (informative) Ex situ exposure to assess bioaccumulation of chemicals in snails.....</b>	<b>48</b>
<b>Bibliography.....</b>	<b>56</b>

## 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.

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](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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](http://www.iso.org/members.html).

## Introduction

Snails are ubiquitous soil macroinvertebrates living at the interface soil, plants and air. Those pulmonate gastropod molluscs are phytophagous and saprophagous (trophic level of primary consumers and detritivorous). They ingest vegetation and soil, and crawl on the ground where they lay their eggs. Therefore, snails integrate multiple sources and routes of contamination (see [Annex A, Figure A.1](#)). Snails participate in exchanges with soil and are preyed upon by various consumers (invertebrates: glow-worms, ground beetle larvae, or vertebrates: birds, small mammals such as shrews, hedgehogs and humans).

Among snail species, the recommended species is *Cantareus aspersus* O.F. Müller 1774<sup>1)</sup> (synonyms: *Helix aspersa aspersa*, *Cornu aspersum*) also known as common garden snail, brown garden snail, garden snail, land snail, nicked name in French “Petit-Gris” (see [Annex A, Figure A.2](#)). This species is a stylommatophoran pulmonate gastropod molluscs of the Helicidae family, widely distributed across the world<sup>[9],[28]</sup>. This palearctic species can be acclimated to regions with different types of climate: Mediterranean, oceanic temperate, midcontinental temperate and even tropical. *Cantareus aspersus* (Müller, 1774) is of European origin and has been introduced into all parts of the world. It is now on all continents except Antarctica. On the other hand, the species is recognized as an agriculturally harmful snail in some countries and must be treated carefully.

Juvenile snails are already covered in ISO 15952<sup>[1]</sup> that describes how to assess ex situ, i.e. in laboratory conditions, toxic effect of chemicals or contaminated matrix on the survival and growth of juvenile (1 g fw).

Currently there is no standardized in situ bioassay allowing the assessment in the field of the transfer of contaminants from the environment to organisms of the soil fauna. Indeed, despite ISO 19204<sup>[3]</sup> (relative to the TRIAD approach) which recommends the application of three combined lines of evidence (chemistry, ecotoxicology and ecology) and highlights the interest of bioindicators of effect and accumulation as additional tools for site-specific ecological risk assessment, few bioassays are available for this purpose. As described in ISO 19204:2017, Annex A, measurements of bioaccumulation in plants or soil organisms are thus useful to:

- assess the effective bioavailability of soil contaminants to soil organisms;
- approach the food chain transfer and the risk of secondary poisoning of consumers.

In some cases, bioaccumulation can result in toxic effects but this is not always the case (see ISO 17402<sup>[2]</sup>).

Since farming is possible (see ISO 15952:2018, Annex B), snails with a known biological past can be used on the field to analyse bioavailability of contaminants present in the habitats (soil, plants, air) by measuring their accumulation in individuals caged and exposed for a determined period of time.

*C. aspersus* can be used either in the field <sup>[10],[12],[13],[15],[19],[22],[23],[27],[29],[30]</sup> or in the laboratory <sup>[14],[18],[20],[21]</sup> to assess the fate and transfer (i.e. environmental bioavailability, ISO 17402) of chemicals in soils. This soil bioindicator has been applied on numerous field sites<sup>2)</sup> to evaluate habitat and retention function of soils. This bioassay allows determining the bioavailability of chemicals to snails thanks to the measurement of their concentration in their visceral mass (which contain mainly the digestive gland and some other organs as described in Reference <sup>[16]</sup>). The visceral mass is the main site of contaminant accumulation in snails.

This document describes how to expose snails in situ for 28 days and how to prepare them until chemical analysis are performed to assess bioaccumulation in their viscera. This bioassay evaluates the transfer of contaminants from the environment to land snails.

1) Available from: [https://inpn.mnhn.fr/espece/cd\\_nom/199863/tab/taxo](https://inpn.mnhn.fr/espece/cd_nom/199863/tab/taxo).

2) Available from: <https://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur/english/worksheet.php>.

This test is applicable in the field (e.g. contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern and waste materials) by caging snails for 28 days on the studied site/soil/waste. Snails integrate chemicals of all terrestrial sources (soil, plant, air). After exposure, concentrations of chemicals are measured in the visceral mass of snails.

Optionally, the method can be used in the laboratory (ex situ) to evaluate bioaccumulation of chemicals of snails exposed only to soil (see [Annex I](#)).

The results of a ring test performed in situ by six laboratories to assess the method of exposure and by four laboratories from exposure until to chemical analysis are shown in [Annex H](#).

# Soil quality — In situ caging of snails to assess bioaccumulation of contaminants

## 1 Scope

This document describes a method to assess the bioaccumulation of chemicals in snails, i.e. concentrations of metal(loid)s (ME) or organic compounds [e.g. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)] accumulated in their tissues.

This document presents how to prepare snails for caging in situ for 28 days, the in situ test design and then how to collect and prepare the snails until conservation and further analysis. If a kinetic study of accumulation is necessary, sampling of snails at different time-points during exposure is possible as well [13],[19],[22].

This document excludes analytical methods. Preparation (extraction and mineralization) of the samples and quantification of chemicals are not in the scope of the present document.

The method is applicable for soils under different uses (agricultural, industrial, residential, forests, before and after remediation, on potentially contaminated sites, etc.) and waste materials [8],[10], preferably with vegetation and/or humus cover.

The method is applicable subject to certain limits of temperature (frost-free period, i.e. mainly from April to October in temperate region).

Optionally (see [Annex I](#)), the method can be used in the laboratory to evaluate the accumulation of contaminants [and optionally, the sum of excess of transfer (SET) index for ME, PAH, PCB] of snails exposed only to soil.

## 2 Normative references

There are no normative references in this document.

## 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 <https://www.electropedia.org/>

### 3.1

#### **caging**

closed microcosm allowing exposure of snails by various routes and several sources

### 3.2

#### **bioaccumulation**

phenomenon by which a chemical present in the medium accumulates in a living organism

Note 1 to entry: This phenomenon is observed when the rate of absorption exceeds the rate of elimination of the contaminant.