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Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions

*Qualité du sol — Guide relatif aux essais en laboratoire pour la
biodégradation de produits chimiques organiques dans le sol sous
conditions aérobies*

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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.

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.

International Standard ISO 11266 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Annexe A of this International Standard is for information only.

Introduction

Organic chemicals may be introduced into the soil both intentionally and accidentally, after which they may, or may not, degrade biologically. For chemicals which do degrade, the rate of degradation can vary considerably, depending not only on the molecular structure of the chemical, but also on soil conditions such as temperature, water and oxygen availability which influence microbial activity. The activity of micro-organisms often plays a major role in degradative processes.

It is necessary to have laboratory tests available to estimate the rate and extent of biodegradation and thereby the persistence of organic chemicals in soil. Numerous laboratory methods are available for the estimation of aerobic biodegradation, but these differ considerably according to the specific circumstances, for example, soil type, temperature and incubation times.

This International Standard provides general guidelines for the selection and conduct of tests for determining the biodegradation of organic chemicals in aerobic soils.

At the time of writing, there is insufficient agreement on methodology for testing biodegradability in anaerobic soils for guidelines to be prepared.

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Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions

1 Scope

This International Standard provides guidance on the selection and conduct of appropriate test methods for the determination of biodegradation of organic chemicals in aerobic soils. It does not describe any specific test method.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 9408:1991, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by determining the oxygen demand in a closed respirometer*.

ISO 10381-6:1993, *Soil quality — Sampling — Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory*.

ISO 10390:1994, *Soil quality — Determination of pH*.

ISO 10694:—¹⁾, *Soil quality — Determination of organic and total carbon after dry combustion ("Element analysis")*.

ISO 11260:1994, *Soil quality — Determination of cation exchange capacity and base saturation — Method using barium chloride solution*.

ISO 11261:—¹⁾, *Soil quality — Determination of total nitrogen — Kjeldahl method using titanium dioxide as catalyst*.

ISO 11274:—¹⁾, *Soil quality — Determination of the water retention characteristic — Laboratory methods*.

ISO 11277:—¹⁾, *Soil quality — Determination of particle size distribution*.

ISO 11461:—¹⁾, *Soil quality — Determination of soil water content calculated on a volume basis — Gravimetric method*.

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 biodegradation: The molecular degradation of an organic substance resulting from the complex actions of living organisms.

3.2 primary biodegradation: The degradation of a substance to an extent sufficient to remove some characteristic property of the parent molecule. In practice, this will be determined by analysis as a loss of parent compound or some specific function of the parent compound.

3.3. ultimate biodegradation: The breakdown of an organic compound to carbon dioxide, water, the oxides or mineral salts of any other elements present, and products associated with the normal metabolic processes of microorganisms.

3.4 persistence: The residence time of a chemical species in a specifically defined compartment of the environment.

3.5 the disappearance time DT-50: The time taken for the concentration of a given compound to decrease by 50 % of its original value.

1) To be published.

3.6 the disappearance time DT-90: The time taken for the concentration of a given compound to decrease by 90 % of its original value.

3.7 bound residues; non-extractable residues: Chemical species in plants and soils, originating from, for example, organic molecules that are not extracted by methods which do not significantly change the chemical nature of these residues. These non-extractable residues are considered to exclude fragments recycled through metabolic pathways leading to natural products. (For examples and further information, see [3] in annex A.)

3.8 mineralization: The complete degradation of an organic substance to inorganic products.

4 Principle

After addition of the test compound to a selected soil (5.1), biodegradation is measured under aerobic conditions (see ISO 9408). Use of a radiolabelled compound allows determination of the rate of disappearance of the test compound and the formation of metabolites, carbon dioxide, other volatiles and non-extractable residue. The metabolites should be identified using appropriate analytical methods. The disappearance of the test compound can also be followed by specific analysis.

5 Materials

5.1 Soil

If practicable, soils selected for testing should come directly from the site where chemical contact is anticipated. However, if it is not possible to obtain clean samples owing to contamination which has already been introduced, the soil selected should have comparable properties.

The field history of the soil used should be considered and recent amendments, such as tillage practices and pesticide applications, noted. Precise data should be provided on the sampling site, its location, the presence of plants or previous crops, the date of removal of the sample from the field, and the sampling depth.

5.1.1 Soil characteristics

A knowledge of soil characteristics is essential for full interpretation of the results of the study. It is therefore recommended that at least the following tests are performed on the selected soil.

a) Physical properties:

- 1) particle size analysis in accordance with ISO 11277;

- 2) field water content in accordance with ISO 11461;

- 3) total water holding capacity and/or water retention characteristic in accordance with ISO 11274.

b) Chemical properties:

- 1) pH of the soil in accordance with ISO 10390, or the pH in KCl solution or CaCl₂ solution;

- 2) organic matter content in accordance with ISO 10694;

- 3) cation exchange capacity (CEC) in accordance with ISO 11260;

- 4) nitrogen content in accordance with ISO 11261.

c) Biological properties:

The microbial activity should be determined by either using an appropriate biodegradable reference compound or by determining active biomass in accordance with an International Standard which will be published later.

NOTE 1 It may be useful to determine the microbial activity before conducting a biodegradation test, and to determine whether any changes in microbial activity have occurred during the test.

5.2 Test material

Ideally, substances to be tested should be pure compounds (chemical purity > 98 %). The influence of any carriers or formulation ingredients should also be considered.

The following data on compounds are important for the interpretation of results:

- name (IUPAC);
- structure;
- relative molecular mass;
- data on purity;
- stability in water and in organic solvents;
- solubility in water;
- vapour pressure;
- octanol/water partition coefficient;
- sorption constant;
- acid dissociation constant;
- for radiolabelled chemicals:
 - the nature and position of the label,
 - specific activity,
 - radiochemical purity.