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Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials

Qualité du sol — Lignes directrices relatives à la caractérisation écotoxicologique des sols et des matériaux du sol

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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ISO 15799 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

Introduction

The majority of existing ecotoxicological test methods (biotests) being internationally harmonized were developed to describe the ecotoxic potential of a test substance when added to a soil or soil material. These methods can be used, with some modification, for the ecotoxicological characterization of soils and soil materials with respect to their function and depending on the intended use. However, in such cases, users of the methods need to be aware that the validation of the methods is not complete.

For substances with properties resulting in toxic effects, biotests are a complement to conventional chemical analysis. Results from chemical analysis can be used for ecotoxicological assessments based on information on the substances identified, including properties of the chemicals, e.g. their bioaccumulation potential. This information is often scarce (if it exists at all) and does not include possible interactions (synergy/antagonism) between chemicals and the complex soil matrix. Furthermore, an exhaustive identification and quantification of substances is impractical. Therefore, ecotoxicological testing of soils can be used for investigating the potential toxicity of complex chemical mixtures. The extrapolation from laboratory tests to field conditions requires adequate consideration of important environmental factors within the test conditions and the selection of suitable ecotoxicological endpoints.

This International Standard is one of a series providing guidance on soils and soil materials in relation to certain functions and uses, including wildlife conservation, and ought to be read in conjunction with those other standards.

Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials

1 Scope

This International Standard provides guidance on the selection of experimental methods for the assessment of the ecotoxic potential of soils and soil materials (e.g. excavated and remediated soils, refills, embankments) with respect to their intended use and possible adverse effects on aquatic and soil-dwelling organisms, and habitat maintenance and the retention function of the soil.

It does not cover tests for bioaccumulation. Genotoxicity tests using eukaryotic organisms in soils are not yet available. It is not applicable to the ecological assessment of uncontaminated soils with a view to natural, agricultural or horticultural use, such soils being of possible interest where they can serve as a reference for the assessment of soils from contaminated sites. Nor is the interpretation of the results gained by application of the proposed methods within its scope.

2 Terms and definitions

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

2.1 Types of soil and other soil materials

2.1.1
soil
upper layer of the Earth's crust composed of mineral particles, organic matter, water, air and organisms

[ISO 11074-1:1996, definition 5.4]

2.1.2
soil material
excavated soil, dredged materials, manufactured soils, treated soils and fill materials

[ISO 15176:2002, definition 3.1.4]

2.1.3
excavated soil
any natural material excavated from the ground, including top soil, sub soil, altered parent rock and parent rock itself

NOTE Excavated soil typically arises during construction works.

[ISO 15176:2002, definition 3.1.5]

2.1.4
standard soil
field-collected soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

EXAMPLE Eurosoils[34].

2.2 Terms relating to soil characteristics

2.2.1
habitat function
ability of soils/soil materials to serve as a habitat for micro-organisms, plants, soil-living animals and their interactions (biocenoses)

2.2.2
retention function
ability of soils/soil materials to adsorb pollutants in such that they cannot be mobilized via the water pathway and translocated into the food chain

NOTE The habitat and retention functions include the following soil functions according to ISO 11074-1:

— control of substance and energy cycles as components of ecosystems;

— basis for the life of plants, animals and humans;

— carrier of genetic reservoir;

— basis for the production of agricultural products;

— buffer inhibiting movement of water, contaminants or other agents into the ground water.

2.2.3
pollutant
substance or agent present in the soil which due to its properties, amount or concentration causes adverse impact on soil functions or soil use

cf. **contaminant** (2.2.4), **potentially harmful substance** (2.2.5)

[ISO 15176:2002, definition 3.2.7]

NOTE See Introduction to ISO 11074-1:1996.

2.2.4
contaminant
substance or agent present in soil as a result of human activity

cf. **pollutant** (2.2.3), **potentially harmful substance** (2.2.5)

NOTE There is no assumption in this definition that harm results from the presence of the contaminant.

[ISO 15176:2002, definition 3.2.6]

2.2.5
potentially harmful substance
substance which, when present in sufficient concentration or amount, may be harmful to humans or the environment

NOTE It may be present as a result of human activity [**contaminant** (2.2.4)] or naturally.

[ISO 15176:2002, definition 3.2.8]

2.3 Land and sites

2.3.1
re-use
useful and harmless utilization of soil materials

NOTE In the context of this International Standard the re-use means the transfer of soil materials to another location for use in agriculture, horticulture, forestry, gardens, recreational areas and construction sites.

[ISO 15176:2002, term 3.4.1]

3 Field of application

3.1 Soils and areas of soil use where ecotoxicological tests should be considered:

— assessment of the ability of a soil to sustain a natural biocenosis or agriculture;

— assessment of the combined ecotoxicity of all bioavailable contaminants present in soils or soil materials;

— assessment of the ecotoxicity of potentially harmful substances in cases where the soil or soil material can affect the ground and surface water;

— identification of soils or soil materials (refills, embankments) having a low degree of contamination — usually within a depth of 1 m — and which can remain at the site without further treatment;

— detection of potential ecotoxicity which could not be traced by chemical analysis;

— monitoring and control of the success of soil treatment (off-site, on-site, *in situ*);

— monitoring and control of soils/soil materials that have been decontaminated and are to be applied at the surface.

3.2 Soils and areas of soil use where ecotoxicological tests are not necessary (provided groundwater contamination can be excluded):

— contaminated soils classified as hazardous waste or which can be characterized clearly by chemical/analytical parameters, in which cases ecotoxicological testing could be useful for a final investigation after remediation and for process control during biological remediation;

— commercially/industrially used areas with no prospect of horticultural/agricultural use;

— soil materials or backfilled materials in an area to be effectively sealed by covering with buildings or other forms of low permeability cover such as concrete or tarmacadam or asphalt.

4 Selection of tests according to use/re-use of soils and soil materials and soil functions

4.1 Use of ecotoxicity tests

Toxicants can affect different species (and in some cases genotypes) present within ecosystems at different concentrations. The ideal approach for the precise ecotoxicological characterization of the soil toxicity is to use a battery of tests with several species belonging to different taxonomic and trophic groups, in order to avoid false negative results owing to an adaptation of a test system (genotypic shift) to a specific contaminant as compared to uncontaminated soils. Studies using field or semi-field investigations are rarely carried out and can be very expensive.

The ideal scheme can be rendered more practicable by the adoption of simpler testing strategies and the application of safety factors to the results obtained. If, however, testing is performed on one species or function only, the high diversity in the sensitivity of species to toxicants will result in a high level of uncertainty. It is therefore recommended to test at least a microbial process, a species from the plant kingdom, and one from the animal kingdom, usually a saprophagous/detritivorous species; if more than one animal species is tested, a predatory species should be included in the test battery. The minimum number of species to be tested depends on the regulations to which the test strategy must comply. This International Standard only gives the basic principles for their use. Further considerations to the selection of tests using soil organisms are given in 4.3.

4.2 General criteria for selection of tests

Criteria for the selection of ecotoxicity tests have been established in the context of hazard assessment and classification of chemicals. These criteria should also apply for the ecotoxicological characterization of contaminated soils. Criteria reviewed were: scientific validity, ecological significance, practicability and acceptability. See [30] and [31].

The basic requirements that test protocols must meet in order to be established in international standards include reproducibility, statistical validity, general acceptance and performance.

The importance of a criterion is relative to the specific situation. Decisions have to be made as to which criteria are the most important or which tests may have to be modified, and choices made between these and more practical considerations — for example, easy culturing of test organisms in the laboratory or the availability of life stages required for a test throughout the year.

The test methods recommended here were originally designed for hazard assessment of chemicals and were in most cases internationally harmonized (e.g. by OECD, EU or ISO). In most of the methods, provisions have been made to adapt the test design to come within the scope of this International Standard. Nevertheless, in many cases experience still has to be gained using the test methods for characterizing soil quality. In addition, the selection of ecotoxicological test methods for the assessment of soils or soil materials depends on their intended use or re-use and on the soil functions to be protected, in particular the retention and habitat functions.

Table 1 gives an example of a decision scheme based on the relevant function.

Table 1 — Relevance for ecotoxicological testing to the intended re-use of the soil

Re-use of soils	Soil function		
	Retention function	Habitat function	
	Aquatic organisms	Plant growth	Soil biocenoses
	Detection of biological effects		
Below sealed areas	low[a]	low	low
Commercially and industrially used unsealed areas	high	low	low
Landfill covering	high	high	low
Green areas, parks and recreation areas	high	high	high
Areas used in horticulture or agriculture	high	high	high

[a] Applies only to the unsaturated soil zone.

4.3 Considerations for the examination of soil functions

4.3.1 Retention function

Transport via water of soluble, colloidal or particle fractions plays a dominant role in the risk assessment of contaminated soils. This is true not only because water may mobilize contaminants, but also because contaminants and metabolites in the water phase potentially have a severe effect on micro-organisms, plants and soil fauna.

Aqueous eluates are useful for testing ecotoxic effects on organisms exposed via the water-mediated transport. It should be taken into account that substances mobilized via water could be subjected to different types of changes, (e.g. metabolism or hydrolization) when transported into the groundwater and from there into surface waters, and that their concentrations are reduced by dilution. Moreover, substances may be mobilized over time due to environmental changes (e.g. pH, chemical and biological transformation). Eluates

may serve as early indicators for the contamination of pore and ground water prior to the exposure of surface and drinking water.

With these aspects, the investigation of groundwater and eluates is of the utmost importance — regardless of the proposed soil use.

4.3.2 Habitat function

4.3.2.1 Representativeness of organisms and processes

The suitability of the soil for living organisms can best be examined by means of test methods selected to include organisms and processes representative of different taxonomic groups.

4.3.2.2 Soil material used as control for bioassays on solid matrices

As a general principle in ecotoxicological testing, any end points measured in a treatment are compared with those measured in the control or controls.

When evaluating the suitability of the soil for soil-dwelling organisms, it is a prerequisite to compare the contaminated soil or soil material with a control material, which may also be used for preparing dilution series with the contaminated sample.

Several types of control material can be used:

— an uncontaminated soil with comparable pedological properties to the sample being tested;

— an inert material (e.g. quartz sand);

— a certified natural soil (e.g. standard soil);

— a standardized artificial soil (see ISO 11267, ISO 11268-1 and ISO 11268-2).

The choice between these control materials should be made depending entirely on the aims of the ecotoxicological assessment, the type of biological test being carried out and the requirements of the test organism. This recommendation cannot be generalized for all biological tests. Adding sand to a soil or a soil material can create a compact mixture incompatible with the growth and development of many organisms (e.g. plant growth tests). It is preferable to use a more complex control material (such as artificial soil) for dilution where this would have the advantage of reproducing more closely the natural environment of the organisms and even if it may interact with pollutants. Placing an organism in a medium that does not match the most important characteristics of its natural habitat may cause stress.

— If a dose-response curve is needed, one of the control materials mentioned above may be used to dilute the contaminated substrate.

— If the aim is to classify each sample of soil or soil materials in terms of ecotoxicity hazard, it is preferable to use an inert material (e.g. quartz sand), which will not interact with the pollutants present in the sample and whose composition and granulometry can be rigorously standardized.

The requirements of the control material must take into account the different soil uses and the type and origin of the soil (e.g. undisturbed soil, refilling material, excavated soil, remediated soil). Nutrient deficiency, as well as physical conditions, can cause differences in plant growth and animal behaviour that need not necessarily be caused by the pollutant situation and the hazard potential.

— If the aim is to evaluate the ecotoxicity of a soil or soil material sample from a contaminated site, the preferred method would be to use an uncontaminated control material similar to the sample being tested.

— If the aim is to evaluate the ecotoxicity of soils or soil materials which may be re-employed for certain specific uses, the preferred method would be to use as a control material any material which may in future be mixed with the soil or soil material.

4.3.2.3 Soil as substrate (medium) for soil micro-organisms

The soil microflora comprises on average 80 % of the mass of organisms living in soil. In combination with the microfauna, the main functions of the microflora are the decomposition and degradation of complex organic substances to easily available nutrients, thereby maintaining the natural substance cycles of carbon, nitrogen, phosphorus and sulfur.

Substrate-induced respiration provides an indicator of the microbial population density.

Nitrifying bacteria, which are responsible for the oxidation of ammonium to nitrite and from nitrite to nitrate, are a very sensitive group of micro-organisms. Reduced nitrification need not necessarily lead to significant changes in the ecosystem but can be used as a sensitive indicator for the inhibition of an essential soil process.

The purpose of determining the microbial biomass or other microbial processes in soils is to allow assessment of the continued maintenance of soil fertility, the potential ability to degrade organic compounds, and the effects of added materials on the soil microbial community.

4.3.2.4 Soil as substrate for plant growth

After micro-organisms, plant roots constitute the largest biological surface in soil. Their contact area with soil particles is increased by the presence of root hairs and mycorrhizal associations (VA-mycorrhiza with cultivated plants and additional ectomycoorhizae with woody plants).

As with the other bioassays proposed, tests with higher plants are designed to assess the bioavailability and effects of pollutants detected or not detected by chemical analysis, respectively. By applying a test period of at least 14 days, short-term changes in the soil by the test plant itself are included.

The accumulation of pollutants in plants, their metabolism and their effects on consumers are not investigated in these tests. They do not apply to the assessment of soil fertility and productivity.

4.3.2.5 Soil as substrate for soil living fauna

Soil animals generally fulfil the following four functions:

a) mechanical activities (drainage, aeration, mixing, mechanical comminution);

b) chemical changes (enhanced availability of nitrate and phosphate from excrements and accelerated formation of clay-humus complexes, after the substrate has passed the gut);

c) biological changes (distribution of micro-organisms in the soil matrix, synergistic effects through stimulation of microbial activity and organic matter decomposition);

d) significant links in the food web.

Short-term and long-term tests are available for examination of the effects of pollutants on soil fauna. For testing the habitat function, characterization by sublethal test parameters is particularly recommended.

Since a single test method cannot adequately represent the vast number of very diverse invertebrates, a test battery should be used. When selecting the individual test species, the following criteria should be considered:

a) trophic level — *e.e.* saprophagous and predatory species should be included;

b) taxonomic/physiological groups — in order to cover the biodiversity of soil communities, at least representatives of annelids and arthropoda have to be selected;

c) size class/exposure pathway: species of the micro-, meso- and macrofauna do not only represent various size classes but also different life-styles and therefore exposure routes (e.g. pore water versus food uptake);

d) ecological role — at least soil-dwelling and litter-inhabiting species are important to consider.

Only internationally standardized methods should be used.

5 Sampling, transport, storage and sample preparation

Before soil quality is assessed by any of the methods proposed, soil samples need to be collected from the site under investigation. Soil sampling should be carried out by trained personnel with sufficient knowledge of sampling, handling of samples and safety measures at contaminated sites and sampling locations. The sampling strategy and handling should be determined by the site to be investigated, the kind of contamination and the aim of the biological tests (e.g. quantities of soil samples could vary between 100 mg and 100 kg, depending on the tests selected).

Record all data concerning sampling, transport and sample preparation. For instructions on the design of sampling programmes, sampling techniques, safety, investigations of natural, cultivated, urban and industrial sites and on the collection, handling and storage of soil for the assessment of aerobic/anaerobic microbial processes in the laboratory, see ISO 10381-6.

6 Limitations of proposed biotests for soils/soil materials

Biological test systems are suitable for volatile pollutants only to a limited extent. Other methods should be developed for this purpose. Similarly, the impact of organic contaminants, which are easily degradable under aerobic conditions, may be detected incompletely by the methods described. In this case, alternative methods for sampling and sample preparation should be applied.

NOTE The proposed terrestrial and aquatic test methods in A.1 and A.2 were developed to assess the ecotoxic potential of chemicals. The characterization of soils or soil eluates was not their primary goal. Therefore, the methods need to be adapted to the specific requirements of soil and site assessment.

Annex A
(informative)

Standardized forms of recommended test systems

A.1 Terrestrial test methods

A.1.1 Soil fauna

A.1.1.1 Collembola — Effects on reproduction

See Table A.1.

Table A.1

1. Title of test	Soil quality — Inhibition of reproduction of Collembola *(Folsomia candida)* by soil pollutants
2. Harmonization	International
3. Reference	ISO 11267
4. Principle	Determination of the effect on reproduction of springtails incubated over a 4 weeks test period
5. Test type	Static subchronic
6. Test organism	Springtails
Breeding stocks	*Folsomia candida* Willem 1902
Age	10 d to 12 d
Feeding	Dry yeast
7. Test substrate	Artificial soil, contaminated soil
Volume	30 g (wet mass)/container
8. Test conditions	
Test chamber	Enclosures
Temperature	20 °C ± 2 °C
pH	6 ± 0,5
Light intensity/quality	Between 400 lx and 800 lx
Photoperiod	12 h:12 h or 16 h:8 h
Soil moisture	40 % to 60 % of total water holding capacity
9. No. replicates	4
10. Test duration/incubation	28 d
11. Neg. control/ dilution soil	Artificial soil
12. Validity criteria	Control: mortality < 20 %, min. reproduction 100 juveniles, CV ⩽ 30 %
13. Pos. control/reference toxicant Mean EC50, CV	E 605 forte (a.i. 507,5 g/l) Betanal plus (a.i. 160 g/l) LOEC: 0,18 mg/kg to 0,32 mg/kg; 100 mg/kg to 200 mg/kg
14. Statistics	Multiple t-test, u-test, regression analysis
15. Test parameter(s)	Mortality of adults, inhibition of reproduction
16. End points	EC_x (x = % effect level, e.g. 10, 50), NOEC
17. Limitations/comments	
The test was originally designed for testing substances added to an artificial soil. To compare or to monitor soil quality, the method has to be adapted. Care should be taken that any control soil used meets the biological requirements of the test species. The number of replicates might have to be increased because of the heterogeneity of field samples.	

A.1.1.2 Earthworms — Acute toxicity

See Table A.2.

Table A.2

1. Title of test	Soil quality — Effects of pollutants on earthworms *(Eisenia fetida)* — Determination of acute toxicity using artificial soil substrate
2. Harmonization	International
3. Reference	ISO 11268-1
4. Principle	Determination of the percentage mortality of adult earthworms placed in a defined substrate containing the test substance
5. Test type	Acute, static
6. Test organism	Earthworm
Breeding stocks	*Eisenia fetida* Savigny*, E. andrei* Bouché
Age	$>$ 2 months
Feeding	No
7. Test substrate	Artificial soil
Volume	500 g (dry mass)
8. Test conditions	
Test chamber	Enclosure capable of being controlled.
Temperature	20 °C $\pm$ 2 °C
pH	6 $\pm$ 0,5
Light intensity/quality	400 lx to 800 lx
Photoperiod	Between 12 h:12 h or 16 h:8 h
Soil moisture	40 % to 60 % water holding capacity
9. No. replicates	4
10. Test duration/incubation	14 d
11. Neg. control/ dilution soil	Artificial soil
12. Validity criteria	Control: mortality $<$ 10 %, biomass loss $\leqslant$ 20 %
13. Pos. control/reference toxicant Mean EC50, CV	Chloroacetamide, LC50 20 mg/kg to 80 mg/kg
14. Statistics	Multiple *t*-test
15. Testparameter(s)	Mortality, biomass
16. End points	LC50 — 14 d
17. Limitations/comments	
Same as for ISO 11267.	
Also available as a test method in ASTM E1676-97 and as OECD Test Guideline 207.	

A.1.1.3 Earthworms — Effects on reproduction

See Table A.3.

Table A.3

1. Title of the test:	Soil quality — Effects of pollutants on earthworms *(Eisenia fetida)* — Determination of effects on reproduction
2. Harmonization	International
3. Reference	ISO 11268-2
4. Principle	Determination of the percentage mortality, effects on growth and reproduction of adult earthworms placed in a defined substrate containing the test substance
5. Test type	Subchronic, static
6. Test organism	Earthworm
Breeding stocks	*Eisenia fetida* Savigny*, E. andrei* Bouché
Age	$>$ 2 months $<$ 1 year
Feeding	Cow dung
7. Test substrate	Artificial soil
Volume	500 g to 600 g dry mass
8. Test conditions	
Test chamber	Enclosure capable of being controlled.
Temperature	20 °C $\pm$ 2 °C
pH	6 $\pm$ 0,5
Light intensity/quality	400 lx to 800 lx
Photoperiod	Between 12 h:12 h or 16 h:8 h
Soil moisture	40 % to 60 % water holding capacity
9. No. replicates	4
10. Test duration/incubation	8 weeks
11. Neg. control/dilution soil	Artificial soil
12. Validity criteria	Control: 30 juveniles/container, CV $\leqslant$ 30 %, adult mortality $\leqslant$ 10 %
13. Pos. control/reference toxicant Mean EC50, CV	Carbendazim LOEC 1 mg/ai to 5 mg/ai Carbendazim
14. Statistics	Multiple t-test, u-test, regression analysis
15. Test parameter(s)	Mortality, growth, reproduction
16. End points	EC50, NOEC
17. Limitations/comments	
Same as for ISO 11268-1.	

A.1.1.4 Enchytraeid — Effects on reproduction

See Table A.4.

Table A.4

1. Title of test	Enchytraeid reproduction test
2. Harmonization	International ring test protocol, OECD Guideline and ISO Standard in preparation
3. Reference	[38]
4. Principle	Adult enchytraeid worms are exposed to a test substance mixed in artificial soil. After a test period of 6 weeks, the effect on the sublethal parameter reproduction is determined. The test design includes the investigation of possible lethal effects (mortality) on the parental enchytraeids.
5. Test type	Subchronic, static
6. Test organism	Enchytraeids
Breeding stocks	*Enchytraeus albidus* Henle 1837 and other *Enchytraeus sp*.
Age	Adult worms with eggs in the clitellum region
Feeding	Rolled oats
7. Test substrate	Artificial soil
Volume	20 g dry mass/container
8. Test conditions	
Test chamber	Enclosure capable of being controlled.
Temperature	20 °C ± 2 °C
pH	6 ± 0,5
Light intensity/quality	400 lx to 800 lx
Photoperiod	Preferably 16 h:8 h
Soil moisture	40 % to 60 % water holding capacity
9. No. replicates	2 to 4 depending on the test design (NOEC/EC_x)
10. Test duration/incubation	6 weeks (final test)
11. Neg. control/dilution soil	Artificial soil
12. Validity criteria	Control: mort. ⩽ 20 %, min. no. of juveniles 25/vessel, CV ⩽ 50 %
13. Pos. control/reference toxicant Mean EC50, CV	Carbendazim EC50 1,2 mg a.i./kg ± 0,8 mg a.i./kg
14. Statistics	Multiple *t*-test, regression analysis, probit analysis
15. Test parameter(s)	Mortality, reproduction
16. End points	LC50, NOEC, EC_x
17. Limitations/comments	
Same as for ISO 11267.	

A.1.1.5 *Oxythyrea funesta* — Acute effects

See Table A.5.

Table A.5

1. Title of test:	Soil quality — Effects of pollutants on insect larvae (*Oxythyrea funesta*) — Determination of acute toxicity using artificial soil substrate
2. Harmonization	International
3. Reference	ISO 20963
4. Principle	Determination of the percentage mortality of *Cetoniidae larvae* placed in a defined substrate containing the test substance
5. Test type	Acute, static
6. Test organism	*Cetoniidae larvae (*species *Oxythyrea funesta)*
Breeding stocks	*Oxythyrea funesta (Scarabaedoidae, Cetoniidae)*
Age	15 d
Feeding	Finely ground cow dung
7. Test substrate	Artificial soil
Volume	300 g (dry mass)
8. Test conditions	
Test chamber	Enclosure capable of being controlled.
Temperature	26 °C ± 1 °C
pH	6 ± 0,5
Light intensity/quality	Darkness
Photoperiod	—
Soil moisture	50 % water holding capacity
9. No. replicates	3
10. Test duration/incubation	10 d
11. Neg. control/dilution soil	Artificial soil
12. Validity criteria	Control: mortality ⩽ 10 %, biomass loss ⩽ 20 %
13. Pos. control/reference toxicant Mean EC50, CV	Mercuric chloride, LC50 15 mg/kg to 45 mg/kg
14. Statistics	Multiple *t*-test
15. Test parameter(s)	Mortality, biomass
16. End points	LC50 — 10 d
17. Limitations/comments	
Same as for ISO 11267.	

A.1.2 Soil flora

A.1.2.1 Soil flora — Inhibition of root growth

See Table A.6.

Table A.6

1. Title of test	Soil quality — Determination of the effects of pollutants on soil flora — Method for the measurement of inhibition of root growth
2. Harmonization	International
3. Reference	ISO 11269-1
4. Principle	Growth of pregerminated seeds under controlled conditions. Differences in the root lengths of seedlings grown in any test medium compared to the controls is indicative of an effect.
5. Test type	Acute, static
6. Test organism	Barley (*Hordeum vulgare L.)*
Breeding stocks	Variety CV Triumph or other varieties
Age	Seeds
Feeding	No
7. Test substrate	Test soil, control soil, sand
Volume	500 g dry mass/container
8. Test conditions	
Test chamber	Growth cabinet
Temperature	20 °C ± 2 °C
Light intensity/quality	25 000 lm/m^2
Photoperiod	12 h:12 h or 16 h:8 h day/night
Soil moisture	70 % ± 5 % water holding capacity
9. No. replicates	3
10. Test duration/incubation	< > 7 d
11. Neg. control/dilution soil	Soil, sand
12. Validity criteria	Not mentioned.
13. Pos. control/reference toxicant Mean EC50, CV	Not mentioned.
14. Statistics	Multiple *t*-test
15. Test parameter(s)	Root elongation
16. End points	NOEC
17. Limitations/comments	
The method is applicable to all soils, soil materials, waste or chemicals which can be applied to soil, except where the contaminant is highly volatile or only affects photosynthesis. The method may be used to compare soils to monitor changes in their activity or to determine the effect of added substances. The method is not intended for use as a measure of the ability of the soil to support sustained plant growth. In the case of contaminated soil, it might be necessary to dilute with uncontaminated soil or sand before testing. The proposed plant test is not suitable for soil samples with a very disturbed structure (e.g. mixtures of soil and rubble). In these cases, an inhibition may result without relevant contamination.	

A.1.2.2 Soil flora — Effects on emergence and growth

See Table A.7.

Table A.7

1. Title of test	Soil quality — Determination of the effects of pollutants on soil flora — Effects of chemicals on the emergence and growth of higher plants
2. Harmonization	International
3. Reference	ISO 11269-2
4. Principle	Emergence and early growth response of a variety of terrestrial plant species to various concentrations of a chemical added to the test soil
5. Test type	Subchronic, static
6. Test organism	Monocotyledonous and dicot. plants
Breeding stocks	Various species
Age	Seeds
Feeding	Does not apply to this test. See under 17.
7. Test substrate	Soil
Volume	500 g
8. Test conditions	
Test chamber	Phytotron, plant growth room, green house
Temperature	—
pH	Suitable for normal growth.
Light intensity/quality	—
Photoperiod	—
Soil moisture	—
9. No. replicates	4
10. Test duration/incubation	14 d to 21 d after 50 % emergence in the control pots
11. Neg. control/dilution soil	Soil
12. Validity criteria	5 healthy seedlings per control pot
13. Pos. control/reference toxicant Mean EC50, CV	Sodium trichloroacetate
14. Statistics	Multiple *t*-test
15. Test parameter(s)	Emergence, growth
16. End points	NOEC, LOEC
17. Limitations/comments	
Same as for ISO 11269-1 (see A.1.2.1)	
As with other bioassays proposed, tests with higher plants are designed to consider the pollutant situation and bioavailability of pollutants not detected by chemical analysis. By applying a test period of at least 14 days, short-term changes in soil by the test plant itself are included.	
The accumulation of pollutants in soils, their metabolism and effects on consumers are not investigated in the test. They also do not apply for assessment of soil fertility and productivity.	
The requirements of the control soil must take into account the different soil uses and the type and origin of the soil (e.g. undisturbed soil, refilling material, excavated soil, remediated soil). Different soil compaction and nutrient deficiency as well as differences in the water-holding capacity and pore volume can cause differences in plant growth that need not necessarily be caused by the pollutant load or hazard potential.	
Also available as a test method: ASTM E 1598-94.	

A.1.3 Soil micro-organisms

A.1.3.1 Mineralization and nitrification

See Table A.8.

Table A.8

1. Title of test	Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes
2 Harmonization	International
3. Reference	ISO 14238
4. Principle	The rates or extent of N-mineralization in aerobic soils are determined by measuring the concentrations of ammonium, nitrite and nitrate released during mineralization of nitrogen contained in the soil organic matter, or during mineralization of an added nitrogenous organic compound.
5. Test type	—
6. Test organism	Microbial organisms present in a test soil
Breeding stocks	Does not apply to this test.
Age	Does not apply to this test.
Feeding	Does not apply to this test.
7. Test substrate	Field soil treated according to ISO 10381-6.
Volume	50 g to 100 g recommended; or bulk incubation with sub-sampling
8. Test conditions	
Test chamber	Appropriate container; soil layer < 3 cm.
Temperature	(20 ± 2) °C
pH	Intrinsic pH of the soil
Light intensity/quality	Dark (toxicity test)
Photoperiod	—
Soil moisture	40 % to 60 % water holding capacity or approximately 0,02 MPa suction pressure (toxicity test)
9. No. replicates	3
10. Test duration/incubation	28 d
11. Neg. control/dilution soil	Soil
12. Validity criteria	Not mentioned.
13. Pos. control/reference toxicant Mean EC50, CV	Not mentioned.
14. Statistics	Regression analysis
15. Test parameter(s)	Mineralization rate, nitrification rate
16. End points	Concentration of mineral N; Inhibitory dose (ID %)
17. Limitations/comments	
ISO 14238 describes laboratory procedures in different soils, or for comparison of N-mineralization in one soil collected at different times of the year.	
To determine the influence of chemicals on N-mineralization, a simplified test design can be used allowing for the establishment of dose-response relationships.	
The experience of monitoring the soil quality of polluted soils is limited. Care should be taken to collect unpolluted control soil.	

A.1.3.2 Biomass — SIR method

See Table A.9.

Table A.9

1. Title of test	Soil quality — Determination of soil microbial biomass — Substrate-induced respiration method
2. Harmonization	International
3. Reference	ISO 14240-1
4. Principle	Soil is amended with a series of increasing concentrations of glucose until a maximum respiration rate is reached. From this rate, the active biomass is estimated.
5. Test type	—
6. Test organism	Microbial organisms present in a test soil.
Breeding stocks	Does not apply to this test.
Age	Does not apply to this test.
Feeding	Does not apply to this test.
7. Test substrate	Field soil treated according to ISO 10381-6.
Volume	Not specified.
8. Test conditions	
Test chamber	An appropriate container of a respirometer
Temperature	(22 ± 1) °C
pH	Intrinsic pH of the test soil.
Light intensity/quality	Not specified.
Photoperiod	—
Soil moisture	Intrinsic soil moisture of the test soil
9. No. replicates	3
10. Test duration/incubation	6 h
11. Neg. control/dilution soil	Does not apply to this test.
12. Validity criteria	None.
13. Pos. control/reference toxicant Mean EC50, CV	None.
14. Statistics	None.
15. Test parameter(s)	Respiration/CO_2 evolution
16. End points	Soil microbial carbon
17. Limitations/comments	
The International Standard for the determination of microbial biomass offers different incubation systems.	
ISO 14240-1 gives a method for the estimation of active microbial biomass in soil.	
Methods for the determination of substrate-induced respiration are described in ISO 16072.	

A.1.3.3 Biomass — FE method

See Table A.10.

Table A.10

1. Title of test	Soil quality — Determination of soil microbial biomass — Fumigation-extraction method
2. Harmonization	International
3. Reference	ISO 14240-2
4. Principle	Through fumigation of the soil sample, intact microbial cells are analysed and the microbial organic matter released. The organic carbon extracted is determined for fumigated and unfumigated samples. The difference is used to determine microbial biomass.
5. Test type	—
6. Test organism	Microbial organisms present in a test soil.
Breeding stocks	Does not apply to this test.
Age	Does not apply to this test.
Feeding	Does not apply to this test.
7. Test substrate	Field soil treated according to ISO 10381-6.
Volume	25 g to 50 g (dry mass)
8. Test conditions	
Test chamber	Glass beaker or petri dish
Temperature	25 °C ± 2 °C
pH	Intrinsic pH of the test soil
Light intensity/quality	Not specified.
Photoperiod	—
Soil moisture	Minimum 30 % water holding capacity
9. No. replicates	3
10. Test duration/incubation	22 h to 24 h
11. Neg. control/dilution soil	Does not apply to this test.
12. Validity criteria	None.
13. Pos. control/reference toxicant Mean EC50, CV	None.
14. Statistics	None.
15. Test parameter(s)	Extractable organic carbon
16. End points	Soil microbial carbon
17. Limitations/comments	
ISO 14240-2 gives a method for the estimation of microbial biomass of soils by measurement of total biomass of extractable organic material mainly from freshly killed micro-organisms. The $CHCl_3$ fumigation also affects soil fauna. But the contribution of carbon from these organisms can be neglected (< 5 %) and therefore it is referred to as microbial biomass. The method is applicable to aerobic and anaerobic (e.g. water-logged or paddy) soils over the whole range of soil pH. Biomass can also be measured in soils containing actively decomposing substrates and soils supersaturated with K_2SO_4 solution.	

A.1.3.4 Ammonium oxidation — Rapid test

See Table A.11.

Table A.11

1. Title of test	Soil quality — Determination of potential nitrification — Rapid test by ammonium oxidation
2. Harmonization	International
3. Reference	ISO 15685
4. Principle	Autotrophic ammonium oxidizsing bacteria in soil are exposed to ammonium sulphate in a soil slurry buffered at pH 7,2. The accumulation rate of the nitrite during 6 h of incubation is taken as an estimate of the activity.
5. Test type	—
6. Test organism	Autotrophic ammonium oxidizing bacteria present in the test soil.
Breeding stocks	Does not apply to this test.
Age	Does not apply to this test.
Feeding	Does not apply to this test.
7. Test substrate	Soil slurry; soil treated according to ISO 10381-6
Volume	25 g moist soil in 100 ml medium
8. Test conditions	
Test chamber	Glass flasks (of appropriate volume) on an oscillating table
Temperature	25 °C
pH	Approx. 7,2
Light intensity/quality	Not specified.
Photoperiod	—
Soil moisture	Not applicable.
9. No. replicates	2
10. Test duration/incubation	6 h
11. Neg. control/dilution soil	None.
12. Validity criteria	Ammonium-oxidizing activity of soil 200 ngN/g soil/hour to 800 ngN/g soil/hour
13. Pos. control/reference toxicant Mean EC50, CV	None.
14. Statistics	Mean, standard deviation
15. Test parameter(s)	Rate of ammonium oxidation
16. End points	In tests of chemicals EC10, EC50
17. Limitations/comments	
The test is a rapid method for determining the potential rate of ammonium oxidation, the first step in the autotrophic nitrification in nitrifying soils. The measurement can be taken as an assessment of the potential activity of nitrifying populations at the time of sampling. It can be used as a rapid screening test for monitoring soil quality, and is suitable for testing the effects of both chemical substances in soil and the effects of cultivation methods. Test substances with limited water solubility need special attention.	

A.1.3.5 Soil respiration

See Table A.12.

Table A.12

1. Title of test	Soil quality — Determination of abundance and activity of soil microflora using respiration curves
2. Harmonization	International
3. Reference	ISO 17155
4. Principle	The CO_2 production or O_2 consumption (respiration rate) from unamended soils as well as the decomposition of an easily degrading substrate (glucose + ammonium + phosphate) is monitored regularly (e.g. every hour). From the CO_2 production or O_2 consumption data the different microbial parameters (basal respiration, substrate-induced respiration, lag time) can be calculated.
5. Test type	—
6. Test organism	Micro-organisms present in the test soil.
Breeding stocks	Does not apply to this test.
Age	Does not apply to this test.
Feeding	Does not apply to this test.
7. Test substrate	Field soil treated according to ISO 10381-6.
Volume	The sub-samples should contain 1 g of organic matter. If mineral soils are used the sub-samples should not be less than 20 g.
8. Test conditions	
Test chamber	Appropriate container of a respirometer
Temperature	20 °C
pH	Intrinsic pH of the soil
Light intensity/quality	Not specified.
Photoperiod	—
Soil moisture	< 400 % of the organic matter content
9. No. replicates	3 for each level of contamination
10. Test duration/incubation	ca. 5 d
11. Neg. control/dilution soil	Does not apply to this test.
12. Validity criteria	Not mentioned.
13. Pos. control/reference toxicant Mean EC50, CV	Not mentioned.
14. Statistics	Mean values for each sample. The microbial parameters should be plotted against the concentration of the contaminating substance and evaluated by regression analysis.
15. Test parameter(s)	Basal respiration, substrate-induced respiration, lag time
16. End points	In tests of chemicals EC10, EC50
17. Limitations/comments	
The test can be used in field- and laboratory-contamination studies. It is suitable for the A_O or more layer of podzolic forest soils and arable soils. For the use of mineral soils, complementary studies of suitable moisture content and sample size will have to be made. The test can also be used for soils of unknown quality and for soils sampled along contamination gradients. In contaminated soils, the quotient of basal respiration/substrate-induced respiration is much higher than in uncontaminated soils. Contaminated soils show much longer lag times.	

A.2 Aquatic test methods

A.2.1 *Daphnia magna* Straus — Inhibition of mobility

See Table A.13.

Table A.13

1. Title of test	Water quality — Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera, Crustacea*) — Acute toxicity test
2. Harmonization	International
3. Reference	ISO 6341
4. Principle	Determination of the effect of toxicants on mobility of young daphnids
5. Test type	Acute, static/semi-static
6. Test organism	Daphnids
Breeding stock	*Daphnia magna* Straus
Age of test organism	< 24 h to 28 h
Feeding	None.
7. Test substrate	Freshwater or restored synthetic medium
Volume	10 ml
8. Test conditions	
Test chamber size	20 ml
Temperature	20 °C ± 2 °C
pH	7,8 ± 0,2
Light intensity/quality	Darkness
Photoperiod	—
9. No. container, no. replicates	5 daphnids per vessel, 4 rep.
10. Test duration	48 h
11. Neg. control dilution soil	Water
12. Validity criteria	a) Dissolved oxygen concentration at the end of the test is greater than or equal to 2 mg/l; b) Control mortality is less than or equal to 10 %; c) 24 h – EC50 of the potassium dichromate is within the range of 0,6 mg/l to 1,7 mg/l.
13. Positive control/reference toxicant, EC50 (and CV)	$K_2Cr_2O_7$ LC50 0,6 mg/l to 1,7 mg/l
14. Statistics	Regression
15. Test parameter(s)	Immobilization
16. End points	EC50
17. Limitations/comments	—

A.2.2 Freshwater algal growth inhibition test

See Table A.14.

Table A.14

1. Title of test	Water Quality — Freshwater algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*
2. Harmonization	International
3. Reference	ISO 8692
4. Principle	Effect on unicellular algae growth, inoculum from a culture in exponential growth phase
5. Test type	Chronic, static
6. Test organism	Unicellular algae
Breeding stock	*Scenedesmus subspicatus* or *Pseudokirchneriella subcapitata capricornuthum* (formerly known as *Selenastrum capricornuthum*)
Age of test organism	Inoculum from culture
Feeding	Mineral culture medium
7. Test substrate	Water
Volume	≈ 100 ml (alternatives on small volumes)
8. Test conditions	
Test chamber size	250 ml erlenmeyer flasks
Temperature	23 °C
pH	8,3
Light intensity/quality	35 to 70 × 10^{18} photons/m^2/s (400 nm to 700 nm)
Photoperiod	Continuous light
9. No. container, no. replicates	at least 3 replicates × 5 concentrations + 6 replicates of control
10. Test duration	72 h
11. Neg. control.	None.
12. Validity criteria	Control population increase > 16 within 72 h
13. Positive control/reference toxicant, Mean EC50	$K_2Cr_2O_7$: EC50 growth rate *Scenedesmus* 0,84 mg/l
14. Statistics	Multisample comparison or regression
15. Test parameter(s)	Growth rate or biomass integral
16. End points	NOEC or EC_x (x = 10, 20, 50)
17. Limitations/comments — Chemicals absorbing light in the range 400 nm to 700 nm can interfere with algal growth for physical reasons rather than by toxic action. — Metals may not be bioavailable by complexation with EDTA from the test medium. — Volatile substances may be stripped by aeration in the tests flasks. See ISO 14442.	

A.2.3 *Lemna minor* — Growth inhibition test

See Table A.15.

Table A.15

1. Title of test	Water quality — Determination of toxic effect of water constituents and waste water to duckweed (*Lemna minor*) — Duckweed growth inhibition
2. Harmonization	International
3. Reference	To form the subject of a future ISO 20079.
4. Principle	Determination of effect on growth of the aquatic plant *Lemna minor*
5. Test type	Chronic, static
6. Test organism	Monocotyledonous, free-floating angiosperm
Breeding stock	*Lemna minor*
Age of test organism	Inoculum from culture at least 7 to 10 days adaptation to test conditions; quality criteria: growth rate $\geqslant$ 0,275 d^{-1},
Feeding	Nutritive mineral medium (mod. Steinberg)
7. Test substrate	Water
Volume	100 ml
8. Test conditions	
Test chamber size	250 ml cylindrical vessels
Temperature	24 °C $\pm$ 1 °C
pH	5,5 $\pm$ 0,2
Light intensity/quality	85 to 125 µE $m^{-2}s^{-1}$ (400 nm to 700 nm) $\pm$ 15 %, at level of water. Light from side (under water surface) and bottom excluded, Neutral white
Photoperiod	Continuous light
9. No. container, no. replicates	$\geqslant$ 10 fronds (2 or 3 per plant)container 3 replicates per concentration, 6 controls
10. Test duration	7 d
11. Neg. control.	Dilution water
12. Validity criteria	Growth rate within 0,25 to 0,35 d^{-1} Growth rate of frond number growth rate $\geqslant$ 0,275 d^{-1}
13. Positive control/reference toxicant, Mean EC50 (and CV)	3,5-Dichlorophenol E_RC_{50} (growth rate, frond number) within 1,8 mg/l to 3,6 mg/l
14. Statistics	Regression
15. Test parameter(s)	Growth; obligatory observation parameter: a) frond number; b) frond area or dry weight or chlorophyll.
16. End points	E_rC_x, lowest ineffective dilution (LID, informative annex B)
17. Limitations/comments	
No interference with light-absorbing substances (400 nm to 700 nm) due to exclusion of reflection light. Special considerations for substances enriched at the water surface. EDTA minimized in nutrient medium to minimise complexation of metals. ISO-Test (Steinberg medium) validated for soil eluates (soil:water 1:2) and respective dilutions. OECD medium not appropriate for soil elutriates due to reduced sensitivity and large stimulation. Also available: AFNOR XP T90-337; OECD Guideline under preparation.	

A.2.4 Freshwater fish acute toxicity test

See Table A.16.

Table A.16

1. Title of test	Water quality — Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (*Teleostei*, *Cyprinidae*)]
2. Harmonization	International
3. References	ISO 7346 (all parts)
4. Principle	Effect on survival of *Danio rerio*
5. Test type	Acute, (Part 1 = static, 2 = semi-static, 3 = continuous renewal).
6. Test organism	Zebra fish
Breeding stock	*Brachydanio rerio* Hamilton-Buchanan
Age of test organism	Adults
Feeding	None.
7. Test substrate	Freshwater
Volume	1 l per g of fish
8. Test conditions	
Test chamber size	Up to 10 l
Temperature	23 °C ± 1 °C
pH	7,8 ± 0,2
Light intensity/quality	Normal laboratory illumination
Photoperiod	12 h to 16 h day light
9. No. container, no. replicates	At least 7 fish per vessel, 1 vessel per concentration
10. Test duration	96 h
11. Neg. control. dilution	Water
12. Validity criteria	Dissolved > 60 % saturation, control fish mortality < 10 %, no abnormal behaviour
13. Reference toxicant	$K_2Cr_2O_7$
14. Statistics	Regression
15. Test parameter(s)	Mortality
16. End points	LC50
17. Limitations/comments	
Danio rerio is the new name of zebra fish and replaces *Brachydanio rerio*.	

A.2.5 Marine algal growth inhibition test

See Table A.17.

Table A.17

1. Title of test	Water quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*
2. Harmonization	International
3. Reference	ISO 10253
4. Principle	Algal population growth inhibition
5. Test type	Chronic, static
6. Test organism	Unicellular algae; inoculuml from a culture in exponential growth phase
Breeding stock	*Skeletonema costatum* or *Phaeodactylum tricornutum*
Age of test organism	Inoculum from a population
Feeding	Nutritive medium
7. Test substrate	Sea water
Volume	approximately 100 ml
8. Test conditions	
Test chamber size	250 ml
Temperature	20 °C ± 1 °C
pH	6 to 8,5
Light intensity/quality	35 to 70 × 10^{18} photons/m^2/s (400 nm to 700 nm)
Photoperiod	Continuous light
9. No. container, no. replicates	3 replicates per concentration, 6 replicates for control
10. Test duration	72 h
11. Neg. control. dilution	Sea water
12. Validity criteria	Control growth rate 0,1 h^{-1}
13. Positive control/reference toxicant, Mean EC50 (and CV)	$K_2Cr_2O_7$, 3,5-dichlorophenol
14. Statistics	Comparison and regression
15. Test parameter(s)	Population growth inhibition
16. End points	NOEC and EC_x
17. Limitations/comments — Chemicals absorbing light in the range 400 nm to 700 nm could interfere with algal growth for physical reasons rather than by toxic action. — Metals may not be bioavailable by complexation with EDTA from the test medium. — Volatile substances could be stripped by aeration in the tests flasks. See ISO 14442 for information on difficult substances management.	

A.2.6 *Daphnia magna* reproduction test

See Table A.18.

Table A.18

1. Title of test	Water quality — Determination of long term toxicity of substances to *Daphnia magna* Straus *(Cladocera crustacea)*
2. Harmonization	International
3. Reference	ISO 10706
4. Principle	Inhibition of reproduction and survival of *Daphnia magna*
5. Test type	Chronic, static/semi-static
6. Test organism	*Daphnia magna* at least third generation obtained by acyclical parthenogenesis
Breeding stock	*Daphnia magna* Straus
Age of test organism	$<$ 24 h
Feeding	Unicellular algae (*Chlorella* sp., *Pseudokirchneriella subcapitata* or *Scenedesmus subspicatus*). 0,1 mg to 0,2 mg carbon/animal/day
7. Test substrate	Aqueous test medium
Volume	50 ml to 100 ml
8. Test conditions	
Test chamber size	100 ml to 200 ml beakers
Temperature	Within 18 °C to 22 °C, variations within less than 2 °C
pH	7,8 $\pm$ 0,2
Light intensity/quality	$<$ 1200 lx
Photoperiod	16 h light
9. No. container, no. replicates	5 concentrations $\times$ 10 replicates (one animal per vessel is recommended)
10. Test duration	21 d
11. Neg. control. dilution	Water
12. Validity criteria	Mortality of adults or living males $<$ 20 % in the control, mean number of offspring per parent $>$ 60 in the control
13. Positive control/reference toxicant, Mean EC50 (and CV)	The Daphnid culture may be controlled using acute $K_2Cr_2O_7$ test.
14. Statistics	Dunnett or Williams test and regression
15. Test parameter(s)	Mortality of adults, inhibition of reproduction or growth
16. End points	EC_x, NOEC
17. Limitations/comments	
This test is mainly used for pure substances; short term alternatives exist, for example using *Ceriodaphnia dubia*. Also available as OECD Test Guideline 211.	

A.2.7 *Vibrio fischeri* — Luminescent bacteria test

See Table A.19.

Table A.19

1. Title of test	Water quality — Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test)
2. Harmonization	International
3. References	ISO 11348 (all parts)
4. Principle	Short term inhibition of effect of toxicants on bacterial luminescence
5. Test type	Acute, static
6. Test organism	*Vibrio fisheri* (saltwater luminescent bacteria)
Breeding stock	*Vibrio fisheri* NRRL B-11177 Freshly prepared 1, liquid-dried 2, freeze-dried 3
Age of test organism	Innoculum from culture
Feeding	None.
7. Test substrate	Salt water
Volume	1 ml
8. Test conditions	
Test chamber size	Test tubes
Temperature	15 °C ± 1 °C
pH	7,0 ± 0,2
Light intensity/quality	Obscurity
Photoperiod	None.
9. No. container, no. replicates	5 concentrations × 3 replicates
10. Test duration	15 min and 30 min
11. Neg. control. dilution soil	—
12. Validity criteria	—
13. Positive control/reference toxicant, Mean EC50 (and CV)	3,5-dinitrophenol, $ZnSO_4$, $K_2Cr_2O_7$
14. Statistics	Regression
15. Test parameter(s)	Inhibition of luminescence
16. End points	EC50
17. Limitations/comments	
Coloured substances can interfere with luminescence.	
This test can be performed with bacteria from different origins. ISO 11348 is divided into three parts for that purpose.	

A.2.8 Marine copepods — Acute toxicity test

See Table A.20.

Table A.20

1. Title of test	Water quality — Determination of acute lethal toxicity to marine copepods *(Copepoda, crustacea)*
2. Harmonization	International
3. Reference	ISO 14669
4. Principle	Determination of effects of toxicants on survival of marine copepods
5. Test type	Acute, static/semi-static
6. Test organism	Marine copepods
Breeding stock	*Acartia tonsa* (Dana), *Tisbe battagliai* (Volkmann-Rocco), *Nitocra spinipes* (Boeck)
Age of test organism	*A.t.* Stage 5 or adults, *T.b.* copepodids 6 ± 2 days, *N.s.* Adults 3 to 4 weeks
Feeding	None.
7. Test substrate	Natural or synthetic sea water
Volume	*A.t.* 5 ml per animal, others 0,5 ml per animal
8. Test conditions	
Test chamber size	Depending on the number of animals per vessel
Temperature	20 °C ± 0,2 °C
pH	8,0 ± 0,3
Light intensity/quality	Not specified.
Photoperiod	16 h daylight
9. No. container, no. replicates	5 replicates of 5 animals per concentration
10. Test duration	48 h
11. Neg. control. dilution soil	Dilution sea water
12. Validity criteria	Dissolved oxygen at end of test > 4 mg/l, control mortality < 10 %
13. Positive control/reference toxicant, Mean EC50 (and CV)	$K_2Cr_2O_7$
14. Statistics	Regression
15. Test parameter(s)	Mortality of animals
16. End points	LC50
17. Limitations/comments	—

A.3 Genotoxicity tests

A.3.1 *Pleurodeles waltl*

See Table A.21.

Table A.21

1. Title of test	Water quality — Evaluation of genotoxicity with larvae of amphibians (*Xenopus laevis*, *Pleurodeles waltl*)
2. Harmonization	National
3. Reference	AFNOR NF T90-325
4. Principle	Induction of micronuclei within erythrocytes of batracian
5. Test type	Genotoxicity to eucaryotes, semi-static
6. Test organism	Pleurodele
Breeding stock	*Pleurodeles waltl*
Age of test organism	Not specified, stage 52 b of development table (size approx. 35 mm).
Feeding	Daphnids, chironomids and tubifex or artemia
7. Test substrate	Water
Volume	1,5 to 2 l
8. Test conditions	
Test chamber size	25 l to 50 l
Temperature	20 °C ± 0,5 °C
pH	7 ± 1
Light intensity/quality	Soft daylight
Photoperiod	Yes.
9. No. container, no. replicates	15 to 20 animals per concentration
10. Test duration	12 d
11. Neg. control. dilution	Water
12. Validity criteria	—
13. Positive control/reference toxicant, Mean EC50 (and CV)	Cyclophosphamide, significant effect with 2 mg/l
14. Statistics	Comparison tests (Mac Gill)
15. Test parameter(s)	Significant increase of micronuclei ratio
16. End points	Positive or negative
17. Limitations/comments	—

A.3.2 umu-test

See Table A.22.

Table A.22

1. Title of test	Water quality — Determination of the genotoxicity of water and waste water using the umu-test
2. Harmonization	International
3. Reference	ISO 13829
4. Principle	The test organisms are exposed to the test sample with and without metabolic activation system using microplates. After 4 h of incubation, the genotoxin-dependent induction of the umuC-gene is compared with the spontaneous activation of the untreated, control culture.
5. Test type	Genotoxicity to procaryotes, static
6. Test organism	*Salmonella typhimurium*
Stock culture	*Salmonella typhimurium* TA 1535/pSK 1002 is preserved in 150 µl culture medium with 10 % dimethyl sulfoxide or 20 % glycerol in 2 ml ampoules at a temperature not above – 80 °C.
Feeding	TGA-culture medium, consisting of tryptone, glucose and ampillicin
7. Test substrate	Water and waste water samples
Sample preparation	—
Storage temperature	4 °C or – 18 °C
pH	7,0 ± 0,2
8. Test conditions	
Incubation	Microplate incubator with shaker
Temperature	37 °C ± 1 °C (28 °C ± 1 °C for measuring induction of the umuC-gene)
9. No. container, no. replicates	96 well microplates, 3 replicates/sample
10. Test duration	6 h to 7 h
11. Neg. control.	Dilution water + inoculum + TGA-culture medium
Blank	Dilution water + TGA
12. Positive control/reference toxicant	4-nitroquinoline-N-oxide (4-NQO: 50 ng/ml), aminoanthracene (2-AA: 200 ng/ml)
13. Validity criteria	The test is considered valid if the positive controls reach an induction ratio of at least 2 under the test conditions.
14. Statistics	Means, standard deviation
15. Test parameter(s)	Smallest dilution level at which the induction ratio $I_R < 1,5$
16. End points	Bacterial growth and induction of the umuC-gene. Growth factors, A_{600} and A_{420} values, β-galactosidase units [(A_{420}/A_{600})-values]
17. Limitations/comments	
Undissolved substances can falsify the test result and/or affect reproducibility.	
In heavily coloured and/or turbid samples, light loss due to absorption can occur during photometric measurement. In this case, the uninoculated sample should be taken as the blank.	
If a sample contains high levels of cytotoxic materials, these could impede cell division and can even lead to cell death.	

A.3.3 Salmonella/microsome test

See Table A.23.

Table A.23

1. Title of test	Water quality — Evaluation of genotoxicity by measurement of the induction of micronuclei
2. Harmonization	International
3. Reference	ISO/WD 21427 (To be published.)
4. Principle	The possible mutagenic activity of the test sample is detectable by comparing for the respective bacterial strain and the respective activation condition the number of mutant colonies on plates treated with the negative control and on plates treated with undiluted and diluted test samples, respectively.
5. Test type	Genotoxicity to procaryotes, static
6. Test organism	*Salmonella typhimurium*
Stock culture	*Salmonella typhimurium* TA 100 and TA 98
Feeding	Nutrient broth
7. Test substrate	Water and waste water samples
Sample preparation	Samples containing solids should be centrifuged to separate solids. Sterile filtration of water and waste water prior to the test
Storage temperature	0 °C (1 to 2 days), $< -$ 18 °C (up to 2 months)
pH	No adjustment to neutral (except with excessive low or high pH values)
8. Test conditions	
Incubation	Incubation in the dark
Temperature	37 °C $\pm$ 1 °C
9. No. container, no. replicates	2 to 3 replicates
10. Test duration	48 h to 72 h
11. Neg. control.	Dilution water without test sample
12. Positive control/reference toxicant	Nitrofurantoin (NF) TA 100: + 100 colonies), 4-nitro-1,2-phenylenediamine (4-NPDA) TA 98: + 50 colonies, 2-aminoanthracene (2-AA) TA 100: + 800 colonies, TA 98: + 800 colonies
13. Blank	Dilution water
14. Validity criteria	The means of negative controls have to be within the defined range. The means of positive controls have to show at least the defined induction rates. Titer determinations must demonstrate sufficient bacterial density per millilitre ($>$ 10:8).
15. Statistics	Means, standard deviation
16. Test parameter(s)	Lowest dilution level (D-value) at which no genotoxic effects are found for plates treated with the test sample or dilutions thereof.
17. End points	Increase in mutant colonies per plate above the defined induction rate defined per strain in correlation to dose.
18. Limitations/comments A strong bacteriotoxic effect of the test sample may lead to a reduction of viable bacteria and to a reduction of mutant colonies as compared with the corresponding negative control counts. In extreme cases of bacteriotoxicity, the number of surviving bacteria can be reduced to such an extent (several hundred) that the traces of histidine in the minimal softagar are sufficient to allow these bacteria to grow up to visible colonies mimicking the growth of mutant colonies. This can lead to false positive results.	

Bibliography

[1] ISO 6341, *Water quality — Determination of the inhibition of the mobility of* Daphnia magna *Straus* (Cladocera, Crustacea) — *Acute toxicity test*

[2] ISO 7346 (all parts), *Water quality — Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio *Hamilton-Buchanan (*Teleostei, Cyprinidae*)]*

[3] ISO 8692, *Water Quality — Fresh water algal growth inhibition test with* Scenedesmus subspicatus *and* Selenastrum capricornutum

[4] ISO 10253, *Water quality — Marine algal growth inhibition test with* Skeletonema costatum *and* Phaeodactylum tricornutum

[5] 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*

[6] ISO 10706, *Water quality — Determination of long term toxicity of substances to* Daphnia magna *Straus* (Cladocera crustacea)

[7] ISO 11074-1:1996, *Soil quality — Vocabulary — Part 1: Terms and definitions relating to the protection and pollution of the soil*

[8] ISO 11074-4, *Soil quality — Vocabulary — Part 4: Terms and definitions related to the rehabilitation of soils and sites*

[9] ISO 11267, *Soil quality — Inhibition of reproduction of* Collembola (Folsomia candida) *by soil pollutants*

[10] ISO 11268-1, *Soil quality — Effects of pollutants on earthworms* (Eisenia fetida) — *Part 1: Determination of acute toxicity using artificial soil substrate*

[11] ISO 11268-2, *Soil quality — Effects of pollutants on earthworms* (Eisenia fetida) — *Part 2: Determination of effects on reproduction*

[12] ISO 11269-1, *Soil quality — Determination of the effects of pollutants on soil flora — Part 1: Method for the measurement of inhibition of root growth*

[13] 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*

[14] ISO 11348 (all parts), *Water quality — Determination of the inhibitory effect of water samples on the light emission of* Vibrio fischeri *(Luminescent bacteria test)*

[15] ISO 13829, *Water quality — Determination of the genotoxicity of water and waste water using the umu-test*

[16] ISO 14238, *Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes*

[17] ISO 14240-1, *Soil quality — Determination of soil microbial biomass — Part 1: Substrate-induced respiration method*

[18] ISO 14240-2, *Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method*

[19] ISO 14442, *Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water*

[20] ISO 14669, *Water quality — Determination of acute lethal toxicity to marine copepods* (Copepoda, Crustacea)

[21] ISO 15176:2002, *Soil quality — Characterization of excavated soil and other soil materials intended for re-use*

[22] ISO 15685, *Soil quality — Determination of potential nitrification — Rapid test by ammonium oxidation*

[23] ISO 16072, *Soil quality — Laboratory methods for determination of microbial soil respiration*

[24] ISO 17155, *Soil quality — Determination of abundance and activity of soil microflora using respiration curves*

[25] ISO 20079[1)], *Water quality —Determination of toxic effect of water constituents and waste water to duckweed* (Lemna minor) *— Duckweed growth inhibition test*

[26] ISO 20963[1)], *Soil quality — Effects of pollutants on insect larvae* (Oxythyrea funesta) *— Determination of acute toxicity*

[27] AFNOR NF T90-325, *Water quality — Evaluation of genotoxicity with larvae of amphibians* (Xenopus laevis, Pleurodeles waltl)

[28] ASTM E1676-97, *Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests With Lumbricid Earthworm* Eisenia fetida

[29] ASTM E1598-94, *Standard Practice for Conducting Early Seedling Growth Tests*

[30] LEON, C.D. and VAN GESTEL, C.A.M. *Selection of a set of laboratory ecotoxicity tests for the effects assessment of chemicals in terrestrial ecosystems*, Discussion paper, VrijeUniversiteit, Amsterdam, Report D 94004, 1994

[31] TORSTENSSON, and L. & PETERSSON, I. *Draft Environmental hazard classification criteria for chemical substances: Terrestrial environment-fate in the soil and soil compartment effects*. Nordic Project Group for Criteria for Classification of Substances Dangerous for the Environment: Soil/Terrestrial Environment, 1996

[32] CARACAS. *Risk Assessment for Contaminated Sites in Europe*, Vol. 1 Scientific Basis, L & M Press Nottingham, 1998

[33] KREYSA G. and J. WIESNER Eds. Bioassays for Soils/Ad-Hoc-Committee *Methods for Toxicological/Ecotoxicological Assessment of Soil*s. DECHEMA Frankfurt/M, 1995

[34] KUHNT, G. and MUNTAU, H. Eds. *EUROSOILS — Identification, collection, treatment, characterization*, European Commission, special publication No. 1.94.60., Ispra (Italy),1994

[35] VAN STRAALEN, N. M. and LØKKE, H. *Ecological Risk Assessment of Contaminated Soils*, Chapmann & Hall, London, Weinheim, New York, Tokyo, Melbourne, Madras,1997

[36] OECD, Algae, Growth Inhibition Test. *Guidelines for Testing of Chemicals*. No. 201, 1984

[37] OECD, *Daphnia* sp. Acute Immobilization Test and Reproduction Test. *Guidelines for Testing of Chemical*s. No. 202, 1984

1) To be published.

[38] OECD, Earthworm, Acute Toxicity Tests. *Guidelines for Testing of Chemicals.* No. 207, 1984

[39] OECD, Daphnia magna Reproduction Test. *Guidelines for Testing of Chemicals*. No. 211, 1984

[40] RÖMBKE, J. and MOSER, T. *Organisation and performance of an international ring test for the validation of the enchytraeid reproduction test*. Volumes I and II, UBA-Texte 4/99, Umweltbundesamt, Berlin,1999

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