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Soil quality — Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials

Qualité du sol — Lignes directrices relatives aux choix et à l'évaluation des essais appliqués pour la caractérisation écotoxicologique des sols et des matériaux de type sol

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Foreword

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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).

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

This second edition cancels and replaces the first edition (ISO 17616:2008), which has been technically revised. The main changes compared to the previous edition are as follows:

— definitions for “soil use” and “ecosystem service” [10] have been added to Clause 3;

— end points of ecotoxicity tests (e.g. mortality, reproduction, growth, genotoxicity, and other functional activities), as well as the overall principles and application of test batteries have been clarified in Clause 4;

— sub-chronic toxicity tests have been added;

— Figure 1 was revised;

— Tables 1 and 2 (test batteries for retention and habitat function assessment, respectively) have been revised (test categories, test organisms added/deleted, references updated).

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.

Introduction

The characterization of contaminated soils can be based on strategies considering chemical analyses and/or bioassays. ISO 15799 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 or re-use, and possible adverse effects on aquatic and soil dwelling organisms.

An assessment strategy giving instructions for the choice and evaluation of test results is hence proposed. The evaluation of the bioassays outcome is based on empirically-derived critical dilution levels that take into account the sensitivity of the test system and the intended use/re-use of the site under investigation. This approach intends to contribute to an effective and comparable assessment within the ecotoxicological characterization of contaminated soil or soil materials[1]. The test systems included in this approach are not mandatory and may be replaced or accomplished by other test methods. Nevertheless, the selected test systems have proved to appropriately characterize contaminated soils and soil materials with respect to their ecotoxic properties[2],[3], both towards aquatic and terrestrial organisms, the latter being responsible for maintaining essential soil functions.

Soil quality — Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials

1 Scope

This document is one of the family of standards (ISO 15799, ISO 19204) providing guidance on the characterization of soils and soil materials in relation to their retention and habitat functions and uses. It is appropriate to use it in conjunction with the two other standards in this family. It provides guidance on the choice and evaluation of tests applied for ecotoxicological characterization of soils and soil materials. Recommendations for test strategies with respect to the protection of ground and surface waters and the maintenance of the habitat function of soil are included. The tests recommended represent a minimum test battery that can be complemented by additional tests, or even be replaced by others, according to the intended uses or protection goals envisaged. The effect values indicated in this document do not refer to regulation but represent the lowest level at which an adverse effect is considered likely to occur.

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 http://www.electropedia.org/

3.1 Assessment

3.1.1
soil-related assessment
assessment of the ecotoxic potential of *soils* (3.2.1), soil substrates and *soil materials* (3.2.2) based on chemical analyses, biological tests and field inventories (monitoring) such as that mentioned in the TRIAD approach[4]

Note 1 to entry: TRIAD means an assessment approach based on a combination of chemical (i.e. residue analysis), ecotoxicological (i.e. laboratory ecotoxic tests) and ecological (i.e. monitoring) data.

3.1.2
risk
expression of the probability that an adverse effect on *soil* (3.2.1) functions will occur under defined conditions and the magnitude of the consequences of the effect occurring

3.1.3
LID-value
lowest-ineffective-dilution value
lowest value of the dilution factor above which the test gives an ecotoxicological relevant reduction (e.g. 20 % inhibition of luminescence)

EXAMPLE A LID 8 corresponds to a dilution of soil extract of 1 : 8 (i.e 1 part of soil extract: 7 parts of dilution water).

Note 1 to entry: The exact definitions are given in the standard of the respective bioassay. According to ISO 13829[5] for the umu-test, it is the "DLi value" (explanation in Annex D). According to ISO 16240[6] 3.4, it is the "decisive D_{min} value".

Note 2 to entry: For further information on results expression and interpretation based on LID values see Annex A of this document.

3.2 Types of soil and other soil materials

3.2.1
soil
upper layer of the Earth's crust composed of mineral particles, organic matter, water, air and organisms

[SOURCE: ISO 15799:2019, 3.1.1]

3.2.2
soil material
material coming from *soil* (3.2.1) and displaced and/or modified by human activity, including excavated soil, dredged materials, manufactured soils, remediated treated soils or fill materials

[SOURCE: ISO 17402:2008, 3.16]

3.3 Terms relating to Soil characteristics

3.3.1
habitat function
ability of *soils* (3.2.1)/ *soil materials* (3.2.2) to serve as a habitat for microorganisms, plants, soil-living animals and their interactions (i.e. biocenose)

[SOURCE: ISO 15799:2019, 3.2.1]

3.3.2
retention function
ability of *soils* (3.2.1)/ *soil materials* (3.2.2) to adsorb pollutants in such a way that they cannot be mobilised via the water pathway and translocated into the food chain

Note 1 to entry: The habitat and retention functions include the following soil functions according to ISO 11074:2015[7], 3.3.31:

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

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

— carrier of genetic reservoir;

— basis for the production of agricultural products;

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

[SOURCE: ISO 15799:2019, 3.2.2]

3.3.3
contaminant
substance or agent present in the *soil* (3.2.1) as a result of human activity

Note 1 to entry: There is no assumption in this definition that harm results from the presence of the contaminant.

Note 2 to entry: See also pollutant (3.2.3) and potentially harmful substance (3.2.5) definitions in ISO 15799:2019

[SOURCE: ISO 15176:2002[9], 3.2.6, modified — Note 2 to entry added.]

3.3.4
pollutant
substances, which due to their properties, amount or concentration cause negative impacts on the *soil* (3.2.1) function or soil use

Note 1 to entry: Adapted from ISO 15176:2002[9] 3.2.7.

3.4 Land and sites

3.4.1
re-use
useful and harmless utilisation of *soil materials* (3.2.2)

Note 1 to entry: In the context of this document re-use means the transfer of soil materials to another location for use in agriculture, horticulture, forestry, gardens, recreational areas and construction sites.

[SOURCE: ISO 15176:2002[9], 3.4.1; ISO 15799:2019, 3.3.1]

3.4.2
soil use
use of the *ecosystem services* (3.4.3) that *soil* (3.2.1) provides

3.4.3
ecosystem service
service that is (directly or indirectly) provided by an ecosystem to benefit people

[SOURCE: Based on Millennium Ecosystem Assessment]

4 Principles and applications of test batteries

The sensitivity of organisms (e.g. bacteria, plants, animals) to toxicants may vary significantly from one species to another. Thereby, it is admitted that only the results of several ecotoxicity tests can give a clear indication of the toxic effects of soil or soil materials. As such, the combination of ecotoxicity tests, defined as a battery, shall include organisms belonging to various trophic levels, several biological responses or end points (e.g. mortality, reproduction, growth, genotoxicity, as well as other functional activities), in order to take into account the variability of species sensitivity within the studied compartment.

The ecotoxicity tests included in batteries should at least have the following characteristics:

— sensitivity;

— practicability;

— compliance with standardized methods;

— high cost efficiency;

— representativeness of the soil ecosystem and/or of the selected application scenario (i.e. habitat or retention functions).

Depending on the use of the soil or soil material (e.g. the agricultural use of waste) or the protection goal (i.e. habitat or retention functions), the applied test battery can differ (see Clause 5 in ISO 15799 for guidance on test selection criteria)[1]. Nevertheless, the selected tests should allow the identification of the most sensitive trophic level(s) and give information on the toxic effects induced by solid samples.

The evaluation of results from the ecotoxicological tests should take into consideration the application purposes (further details on the field of application in ISO 15799, Clause 4), which can broadly be for:

— monitoring and control of the success of soil treatment (off-site, on-site, in situ) (see 5.1),

— assessment of soil / soil material quality or contamination effects according to its use or re-use (see 5.2).

Irrespective of the application purpose, the ecotoxicological characterization of soils and soil materials depends on the soil use/re-use and soil functions requiring protection[1], as aforementioned. Overall, it can essentially rely on the:

— assessment of mobile and bioavailable potentially harmful substances, in cases where the soil/ soil material (see 3.2.1 and 3.2.2) can affect the ground and/or surface water, as well as in cases where pollutants are added to soils (e.g. agricultural use of wastes like sludge, composts, etc.) (i.e. **retention function**), and/or

— assessment of the ecotoxic potential of soils and soil materials (see 3.2.1 and 3.2.2) and possible adverse effects on soil-dwelling organisms (i.e. **habitat function**).

5 Testing strategy and interpretation of test results according to the use and re-use of soils / soil materials and soil functions

5.1 Monitoring of soil treatment success

For evaluating the efficiency of soil treatments, the procedure below may be followed at different stages:

a) before the treatment – perform the ecotoxicological assessment of the soil sample(s) using a test battery (5.2);

b) monitoring during the soil treatment process – perform a simple test selected from the battery above [e.g. the most sensitive and practical test used among the battery applied in 5.1 a)];

c) at the end of the treatment – perform a new assessment with the same test battery used in 5.1 a), in order to judge the success of the treatment.

5.2 Assessment of the ecotoxic potential of soils / soil materials

5.2.1 General

If soils or soil materials are assessed with respect to their intended use or re-use, the tests (see ISO 15799) appropriate to evaluate their quality regarding the retention (see Table 1) and/or habitat functions (see Table 2) should be applied. A strategy for the assessment of the ecotoxicological characterization of soils and soil materials is proposed in Figure 1. The chemical characterization of soils should always be conducted to increase the reliability of interpretations of the ecotoxicological results obtained upon the selected test battery.

The test battery usually includes a set of acute and chronic/sub-chronic toxicity tests. If acute toxicity is detected, it is not necessary to perform other tests. On the other hand, if no acute effect is detected, chronic/sub-chronic toxicity and genotoxicity test(s) shall be conducted.

The assessment of soil and soil materials may be influenced by the collection, handling and storage methods followed. Thereby, standardized procedures should be conducted in accordance with ISO 18400-206[11].

5.2.2 Choice of test battery and evaluation of test results for assessing retention function

5.2.2.1 Acute and chronic/sub-chronic assays for assessing the ecotoxic potential of soil/soil materials

In a first approach, acute and sub-chronic tests using luminescent bacteria, algae and daphnids (see Table 1) are recommended to assess the retention function of soils through the testing of soil/soil material eluates. Depending on the legal requirements, other standardized bioassays may be selected (see Table 1 or Annex A of ISO 15799 for choosing additional assays).

For the assessment of effects, toxicity criteria are also given in Table 1. The toxicity criteria are provided as LID-values, percentages of inhibition or mortality. If these values are exceeded, then an ecotoxicological potential is highlighted, thereby indicating that soil pollutants are soluble in water, bio-available, and can be transported via the water path. If at least one positive test result is obtained, the use of the soil or soil material is limited, or the requirements of remediation are not fulfilled (see Figure 1).

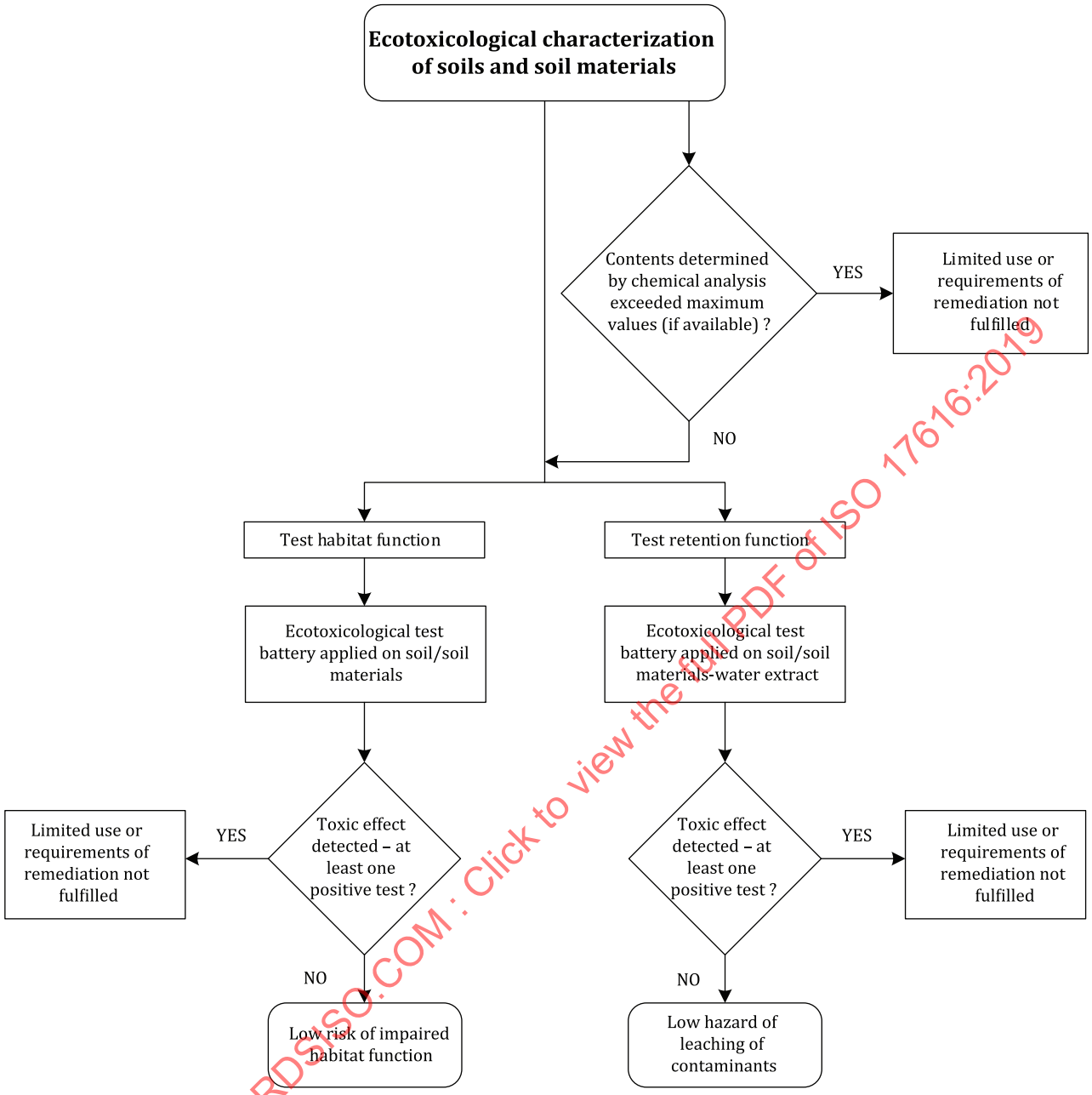

Figure 1 — Flowchart describing a strategy for assessing the ecotoxicological potential of soils and soil materials

Table 1 — Biological tests for the assessment of ecotoxic and genotoxic potential of liquid-extractable compounds possibly present in soils or soil materials (retention function)

Test category	Trophic level	Test species	Reference	End point	LID value[a,b]	Significant biological effect[b]
Acute ecotoxic tests	Decomposer	***Vibrio fischeri***[c]	ISO 11348[12]	Inhibition of light emission	LID > 8	20 % Inhibition
	Primary consumer	***Daphnia magna***	ISO 6341[13]	Immobilization	LID > 4	20 % Inhibition
Chronic/ sub-chronic ecotoxic tests	Primary producer	*Lemna minor*	ISO 20079[14]	Growth inhibition	—	25 % Inhibition
		Raphidocelis subcapitata	ISO 8692[15]	Growth inhibition	LID > 4	25 % Inhibition
	Primary consumer	*Ceriodaphnia dubia*	ISO 20665[16]	Mortality and reproduction	—	20 % Mortality 30 % Inhibition
		Brachionus calicy-florus	ISO 20666[17]	Mortality and reproduction	—	20 % Mortality 30 % Inhibition
Genotoxicity tests	Decomposer	***Salmonella chol-eraesuis subsp. choleraesuis***	ISO 13829[5]	Induction rate of umuC gene	D_{Li} W 3	—
		Salmonella chol-eraesuis subsp. choleraesuis	ISO 13829[5]	Induction rate of umuC gene in concentrated water extract 1 : 15 (water : soil)	D_{Li} W 3	—
		Salmonella typh-imurium TA 98 and TA 100	ISO 16240[6]	Induction rate of mutant colonies	Deci-sive D_{min} value W 2	—
	Primary producer	*Vicia faba*	ISO 29200[18]	Micronucleus frequency increase	—	Statistically sig-nificant increase on micronuleus frequency in the soil / soil material relatively to the negative control.

NOTE Tests given in **bold letters** are part of a minimum test battery.

[a] LID-values are relevant for a liquid/soil (L/S) ratio of 2 : 1. Further guidance on the preparation of soil eluates can be obtained in EN ISO/TS 21268-1[19], EN ISO TS 21268-2[20] or EN 14735[21].

[b] Toxicity criteria established on the basis of testing a wide variety of contaminated and uncontaminated soils. It should be guaranteed that no false positive assessment is obtained[22],[23].

[c] Reclassified as *Aliivibrio fischeri* (Beijerinck 1889) Urbanczyk et al.[24].

Nutrients extracted from soils and certain soil materials can interfere with the effects of microalgae growth inhibitors, since a surplus of essential macronutrients, like nitrates and phosphates may stimulate microalgae metabolism and physiological performance. Therefore, it shall be taken into account that the toxicity of pollutants can be masked by substances favouring algal growth, and that soil composition shall be considered when analysing results. The inclusion of a reference soil/soil material with similar nutrient and organic matter contents similar of the contaminated soil/soil material, could allow identification of the influence of those interfering factors on the algae response.

5.2.2.2 Assessment of the genotoxic and mutagenic potential of soil/soil materials

The assessment of soil(s) and soil material(s) genotoxic potential should be also evaluated, since genotoxic effects can occur far below the toxic threshold levels for acute and (sub-)chronic test systems[25] (see Table 1). A stepwise procedure, including tests of 2:1 water:soil extracts and 15-fold-concentrated water extract with the Umu-test[5], is recommended. The concentrated water extract shall be tested additionally if no genotoxic effects were determined in the soil eluate.

If neither the soil/soil material eluate nor the concentrated eluate extract present genotoxicity, but due to chemical analysis or former use of the site it is suspected that water-extractable mutagenic substances are present, the assessment should be completed by the Ames-test[6]. It is recommended to use the strains *Salmonella typhimurium* TA 98 and TA 100 with and without metabolic activation.

5.2.3 Choice of test battery and evaluation of test results for assessing habitat function

The habitat function of soils can be characterized by the recommended tests in Annex A of ISO 15799. Notwithstanding, a list of selected standardized tests, which proved to be valid for soil quality testing and provide relevant expressions of test parameters appropriate to be used as toxicity criteria, is presented in Table 2. Other test methods not yet harmonized or sufficiently validated can be appropriate as well, and should be added as supplementary methods to the proposed test battery, or can replace others for economic or ecological reasons.

If there is one positive test result among the minimum test battery, a detailed assessment should be undertaken, considering the analytical data (e.g. metal concentrations) and physical-chemical soil properties, particularly the soil texture and pH value. If more than one of the terrestrial tests has positive effects, the habitat function of the soil is significantly restricted.

Table 2 — Overview on relevant soil organisms and respective test systems considered in the test battery for the assessment of the habitat function of soils

Test category	Trophic level	Test organism(s)	Reference	End point	Toxicity criteria
Acute/short-term ecotoxic tests	Primary producer	**Plant species**	ISO 11269-2[26]	**Early growth inhibition**	Growth reduction >30 %, or $G_m + SD < 0{,}9 \times G_{calc}$ where G_m is the determined growth in the mixture SD is the standard deviation of Gm G_{calc} is the calculated mean growth of the test and control soil $(G_{soil} + G_{control}) \times 2^{-1b}$
	Autotrophic microbes	**Soil microflora -Ammonium-oxidizer microbes**	ISO 15685[27]	**Ammonium oxidation**	Soils are assessed as being positively affecting the nitrification process if the activity in the mixture with control soil deviates more than 10 % of the average activities of both individual soils: $A_m + SD < 0{,}9 \times A_{calc}$ where A_m is the determined activity in the mixture A_{calc} is the calculated mean activity of the test and control soil $(A_{soil} + A_{control}) \times 2^{-1a}$ In case of adding a material (e.g. sewage sludge) to soil it is recommended to use an effect criterion of 25 % inhibition

NOTE Tests given in **bold letters** are part of a minimum test battery.

[a] Toxicity criteria according to the respective standards.

[b] Toxicity criteria established on the basis of testing a wide variety of contaminated and uncontaminated soils, and a round robin test[39],[22],[40],[41]. Even if the physicochemical soil properties of the test soil or soil material differ significantly from the control soil or substrate, it should be ensured that no false positive assessment is obtained.

Table 2 *(continued)*

Test category	Trophic level	Test organism(s)	Reference	End point	Toxicity criteria
	Decomposer and other microbes	**Soil microflora**	ISO 17155[28]	**Inhibition of respiration**	Respiratory activation quotient (Q_R) >0,3 Or $t_{peak\ max}$ >50 h at Q_R 0,2 to 0,3[a] In case of adding a material (e.g. sewage sludge) to soil it is recommended to use an effect criterion of 25 % inhibition of basal respiration
		Arthrobacter globiformis	ISO 18187[29]	**Dehydroge-nase activity**	>30 % inhibition of enzyme activity compared to the negative control[a,b]
	Primary consumer	*Eisenia fetida/andrei*	ISO 11268-1[30]	Mortality	>20 % mortality compared to the control[a,b]
		Eisenia fetida/andrei	ISO 17512-1[31]	**Impact on avoidance behaviour**	Avoidance behaviour >80 % indicates limited habitat function[a,b]
		Folsomia candida	ISO 17512-2[32]		Avoidance behaviour >70 % indicates limited habitat function
Chronic ecotoxic tests	Primary producer	*Brassica rapa* *Avena sativa*	ISO 22030[33]	Growth and reproductive capacity	Growth reduction >30 %, or Reproductive capacity reduced by >50 % compared to the control[a,b]
		Vicia faba	ISO 29200[18]	Micronucleus frequency increase	Statistically significant increase of micronucleus frequency in the soil/soil material relatively to the negative control.
	Primary consumer	*Caenorhabditis elegans*	ISO 10872[34]	Reproduction	Reproduction rate reduced by >50 % compared to the control[b]
		Eisenia fetida/andrei	ISO 11268-2[35]	**Reproduction inhibition**	Reproduction rate reduced by >50 % compared to the control[b]
		Folsomia candida	ISO 11267[36]	**Reproduction inhibition**	Reproduction rate reduced by >50 % compared to the control[b]
		Enchytraeus sp.	ISO 16387[37]	Reproduction inhibition	Reproduction rate reduced by >50 % compared to the control[b]
		Helix aspersa	ISO 15952[38]	Growth inhibition	Biomass reduced by >40 % compared to the control[b]

NOTE Tests given in **bold letters** are part of a minimum test battery.

[a] Toxicity criteria according to the respective standards.

[b] Toxicity criteria established on the basis of testing a wide variety of contaminated and uncontaminated soils, and a round robin test[39],[22],[40],[41]. Even if the physicochemical soil properties of the test soil or soil material differ significantly from the control soil or substrate, it should be ensured that no false positive assessment is obtained.

Annex A
(informative)

Tests with soil eluate — Expression of results

In order to conform with assessment purposes, LID values were defined for each ecotoxicological test, which, if exceeded, indicate an ecotoxicological potential (i.e. a sample is classified as “toxic” in regard of a specific organism and biological response).

For example, in the case of the bacteria luminescence and algae growth tests, the LID-value (i.e. >8 and >4, respectively) is defined as the dilution at which the effect on the respective end point is below 20 %, while in the test with daphnids the LID-value (i.e. >4) is 10 %. For the three test types is assumed the use of a dilution series based on a spacing factor of 2. If the respective LID-value is not reached, an ecotoxic potential of the soil via water path is not detected. This situation is described as “non-toxic” and indicates a low risk of contaminants entry into adjacent waterbodies (i.e. soil/soil material retention function is preventing the dispersal of contaminants).

The LID values-based toxicity criteria presented in Table 1 (i.e. LID > 8 for *Vibrio fischeri* luminescence inhibition, LID > 4 for the Daphnia *magna* survival test and the algal growth inhibition test, D_{Li} W 3 for the umuC-test, and decisive D_{min} value W 2 for the *Salmonella* microsome test) were discussed and set after testing large numbers of uncontaminated and contaminated soil samples, and after ring testing[22]. One important aspect considered is that on the basis of these toxicity criteria, soil samples/soil materials that are definitely uncontaminated are not classified as “toxic”. For example, the German standard soil “Lufa 2.3” has a LID value of 8 in the *Vibrio fischeri* luminescence inhibition test[42].

NOTE Explanations are extracted from Reference [43]. Additional information is given in References [22] and [44].