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ISO 11268-3

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Soil quality — Effects of pollutants on earthworms —

Part 3: Guidance on the determination of effects in field situations

Qualité du sol — Effets des polluants vis-à-vis des vers de terre —

Partie 3: Lignes directrices relatives à la détermination des effets sur site

Reference number
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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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 11268-3:1999), which has been technically revised.

ISO 11268 consists of the following parts, under the general title *Soil quality — Effects of pollutants on earthworms*:

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

— *Part 2: Determination of effects on reproduction to Eisenia fetida/Eisenia andrei*

— *Part 3: Guidance on the determination of effects in field situations*

Introduction

The earthworm field test is based on a method being developed by the German Federal Biological Research Centre for Agriculture and Forestry for the testing of pesticides.[6] Later, it was internationally standardized by the International Organization for Standardization (ISO), taking into account results and recommendations of an international workshop in 1991 in Sheffield, United Kingdom, [7] "Ecotoxicology of Earthworms", as a tool for characterizing soil quality. Growing experience has shown that the practical performance of the test can be improved. In two meetings organized by the Federal Biological Research Centre for Agriculture and Forestry (Braunschweig, 2002) and by the German Federal Agency for Consumer Protection and Food Safety (Lille, 2005), an ad-hoc working group of experts from various countries and institutions proposed recommendations that should be taken into account if revision has been approved by voting in the periodical review. A report of the discussions, comments, and recommendations has been published.[8]

In cases where earthworms and other organisms are used as bioindicators to assess the soil quality of a site as a habitat for soil organisms, guidance for extraction procedures and advice for planning a survey is given in ISO 23611-1 to ISO 23611-6.

Soil quality — Effects of pollutants on earthworms —

Part 3:
Guidance on the determination of effects in field situations

1 Scope

This part of ISO 11268 specifies techniques for determining the effects of substances on earthworms in the field and provides a basis for determining the effects of chemicals applied to or incorporated into soil, including soil injections or drilled pelleted formulations.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

ISO 23611-1, *Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and formalin extraction of earthworms*

3 Units

Rates of application of test substances are expressed in kilograms per hectare (kg/ha) or litres per hectare (l/ha) of the substance applied. When this is a formulated material, the application rate is expressed in terms of the amount of active ingredient applied.

The concentrations of test substances incorporated in the soil are given in mg active ingredient (a.i.)/ kg soil dry mass, d_m. The same units are used when comparing the results of this field test with those gained in laboratory studies.

4 Principle

Species, numbers, and biomass of earthworms collected by sampling plots treated with a test substance are compared with those collected from treated control and reference plots. Sampling is performed as specified in ISO 23611-1. The duration of the study depends on the characteristics of the test substance but is usually of one year's duration. Sampling dates are chosen to lie within the periods of activity of the earthworms.

The test is of a randomized complete block design with four replicates per treatment. Statistical analysis of numbers of each species collected at each sampling occasion is used to determine the effects of treatments by comparing abundance, biomass, and diversity between control and treated plots.

NOTE The test also generates samples of earthworms from treated plots for residue analysis where such information is appropriate.

5 Reagents and material

5.1 Formalin [formaldehyde solution, 4 % (volume fraction)].

5.2 Formalin [formaldehyde solution, 37 % (volume fraction)].

5.3 Ethanol, 70 % (volume fraction).

5.4 Carbendazim, e.g. applied as Derosal®[1)] formulation (360 g a.i./l) as reference substance.

6 Apparatus

Use standard laboratory equipment and the following.

6.1 Plastic vessel, (250 ml and 500 ml) for storing the worms.

6.2 Plastic hand gloves.

6.3 Forceps.

6.4 Piece of thick plastic (1 m^2 to 2 m^2).

6.5 Spade or shovel.

6.6 Dissecting microscope, with low magnification (10× to 40×).

6.7 Balance (0,01 g to 200 g).

6.8 Water-can, (preferably 20 l) with water (20 l per sampling plot).

6.9 Pencil, note book, water resistant marker, labels.

6.10 Thermometer, e.g. for measuring air temperature.

6.11 Drying cabinet, for soil water content determination.

7 Procedure

7.1 Sampling of earthworm populations

Sampling of earthworms is done by a combination of two different methods: hand-sorting and formalin extraction. Based on several comparative studies, this combination is clearly recommended in the various reviews on earthworm ecology (e.g. [9], [10], [13]). For details of extraction procedures, see ISO 23611-1.

Sampling should be done at times of the year where the animals are not forced by the environmental conditions (i.e. low soil water content and/or extremely high or low temperatures) into diapause (i.e. are not reacting to formalin).

Due to the individual size of the worms, a large plot shall be identified: a square of 50 cm × 50 cm is often sufficient in the Holarctic where most adult earthworms have a length approximately between

1) Derosal is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

1 cm and 20 cm. However, at places with a low density of earthworms [e.g. soils with low pH (<4,5) or which are anthropogenically used like crop sites], larger plots (i.e. 1 m^2) are recommended. On the other hand, at sites with a high earthworm density (e.g. many meadows in temperate regions), a smaller plot of 0,125 m^2 is sufficient.[12] The individual samples are taken randomly over the test plot.

NOTE 1 If in special situations, such as in Southern Europe, anecic species do not occur, sampling by hand-sorting up to a soil depth of 30 cm is sufficient. When sampling conditions are not optimal, e.g. at extremely low soil water content or dense root layer, hand-sorting can even be the only effective extraction method.

In case in a behavioural sampling method formalin alone is used, an additional check by hand-sorting is necessary and it should be performed as described in this International Standard. Hand-sorting combined with the formalin method usually yields satisfying results. If a combination of hand-sorting and an extraction method is used, no additional efficiency check is necessary.

7.2 Preservation

Preservation shall be carried out according to ISO 23611-1.

7.3 Determination of biomass

Determination of biomass shall be carried out according to ISO 23611-1.

8 Preparation for the test

8.1 Test site

8.1.1 Selection and description

In general, the test site should be as homogenous as possible to improve the statistical power of the test. Gradients in environmental conditions should be avoided (e.g. adjacent ditches), canopy influences as woodland borders, or compacted tractor tracks on the site. The site should be on level ground and should have the same cropping and soil characteristics throughout.

Grassland is the preferred study site for testing effects of substances on earthworms. In grassland, earthworm density and diversity are generally higher and more stable than on arable land, which makes it easier to detect significant effects on earthworm populations. If effects on earthworms are observed on a grassland site, a refined risk assessment should include specific scenarios (crops and regions) covering the intended use patterns of the test substance (e.g. pesticide). Orchards are not recommended for testing because of the heterogeneity of the site due to tree rows and strips without trees. If an orchard is used, it shall be ensured that the higher variability is compensated by taking more samples or restricting sampling to specific areas. A suitable grassland test area should have an earthworm density of at least 100 individuals per square metre. With lower population densities, more samples should be taken than recommended in 8.1.3.

If information on effects on bare soils is required, then arable plots may be used, provided that there are at least 60 earthworms per square metre present at the start of the test according to the results of the pre-sampling.

The experimental plots should support a mixed population of earthworms[11] which are generally representative of the type of environment selected. In agricultural areas, for example, important anecic and endogeic species should be present at a sufficiently high density (at least 10 % of the population for each group) that plots can be taken as representative. Care should be taken not to select plots where uncharacteristic species predominate.

NOTE Due to natural reasons, a certain ecological group possibly does not occur in some regions (e.g. anecic worms in parts of the Mediterranean). In such cases, expert knowledge is required in order to identify the ecologically most important species of that region.

In order to satisfy these requirements, samples should be taken from prospective plots before the start of the study for preliminary investigation of species distribution.

Extreme soil types, e.g. very sandy, clay, or moory soils, should be avoided when selecting the test site.

A description of the test site should contain the following physicochemical and biological information:

— particle-size distribution (as specified in ISO 11277);

— organic-carbon content (as specified in ISO 10694);

— pH-value (as specified in ISO 10390);

— water-holding capacity, $WHC_{\max}$ (in the A-horizon, as specified in ISO 11274);

— description of vegetation.

Determination of these characteristics should be made using standard methods.

Microclimate measurements (soil and air temperature, soil water content, rainfall quantity, sunshine duration) are particularly important for the period of chemical application and temperature, and rainfall quantity should be recorded over the year.

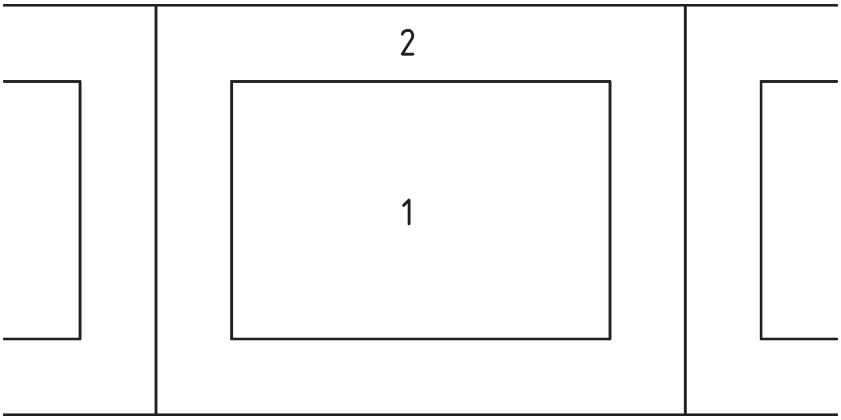
The history of the test site should be known (e.g. applications of pesticides, mineral fertilizers, sewage sludge, tillage).

8.1.2 Design of experiment

The experimental design depends on the objectives of the study and the amount and quality of information available from the study site. Usually (i.e. in the case of testing a chemical substance), a negative control (i.e. plots sprayed just with water) and a positive control (i.e. plots sprayed with a reference substance, e.g. a substance known to be toxic to earthworms) are sprayed. In general, it should be taken into consideration that a dose-response design clearly facilitates environmental risk assessment as compared to single-dose studies. In any case, the reasons for the selected test design shall be explained in the study report.

The test should be designed as a randomized complete block. The number of the treatment groups and planned sampling dates determine the number of plots and, therefore, the surface area of the field site.

However, the size of the individual study plots should be at least 100 m^2 (10 m × 10 m). The samples are taken exclusively from the central area of the plots so that around the sampling area, there is a 1 m to 2 m wide edge strip which is also treated (see Figure 1). The sampling area of the samples taken per treatment, plot (replicate), and date depends on the earthworm density and distribution of the selected experimental field (see 7.1) and can range between 1 m^2 and 0,125 m^2, but most often, 0,25 m^2 is appropriate.



Key

1 sampling area

2 edge strip

Figure 1 — Schematic view of a test plot

Samples taken on the same date should be at least 2 m apart and sampled areas should not be used for sampling at subsequent sampling dates.

The required number of random samples depends, among other things, on the density and distribution of the earthworm population over the test area.[14]

For each test variant (control, reference substance, test substance), at least four replicates should be used and four random samples taken per replicate (i.e. 16 individual samples per test variant).

On grassland, a sampling area of 0,25 m^2 per individual sample is sufficient. Use of a metal or plastic enclosure with a size of 50 cm × 50 cm (square) or a diameter of 56 cm and a height of 10 cm to 15 cm is recommended. On arable land, the sample area shall usually be increased to 1 m^2 due to low population density or non-homogeneous distribution of the worms.

On grassland, the vegetation at the sampling area should be cut carefully before sampling so that all earthworms appearing on the surface can be seen and collected.

Care should be taken that the entries of earthworm holes are not blocked and, therefore, operators should avoid walking on sampling areas.

NOTE Guidance concerning application rates of pesticides is given in Annex A.

8.1.3 Maintenance of test fields

Grassland fields should be mulched regularly (two times to six times per year) using grass clippings of a mulching lawn mower in order to keep the grass cover short. Mulching should be carried out one week to two weeks before the application of the test substance to ensure that the grass on the surface, which acts as a food source for some earthworms, has been in contact with the test material. The last mulch before application of the test substance may only remain on the field, provided that it does not create a coherent grass mat. In the case of mulching over the course of the year, the mulch should remain on the field as it serves as food for some earthworm species.

If a test is carried out on arable land, usual agricultural practice should be used. However, ploughing and other soil treatment measures should be avoided as much as possible during the experiment.

Pesticides should not be used on the test area, but if an application is unavoidable, then the chemical chosen should be non-toxic to earthworms (the selection should be based on the risk assessment for the respective compound as published in EFSA dossiers). For herbicides to be tested, arable land or grassland should be used which is treated with another herbicide (which is not the test substance),

cultivated, and re-sown on the whole site before starting the test. The same substance should also be applied to the control plots. With respect to the interpretation of the test results, it shall be kept in mind that even if this chemical does not affect earthworms, interactions between residues of the non-toxic chemical and the test substance can occur. Therefore, a time gap of at least one week should be kept between the spraying of the non-test herbicide and the herbicide to be tested.

In individual cases, artificial overhead irrigation of the test field can be useful as earthworms only become active and rise to the surface when sufficient soil water content is present. Irrigation one week to two weeks before sampling can facilitate or even be necessary to allow sampling since this depends on the worms' activity. If little or no rainfall occurs within three days after each application, irrigation of the site is considered necessary to achieve optimal conditions for exposure. Irrigation can be necessary to ensure exposure of earthworms. The amount used for irrigation should be realistic according to regional and climatic conditions. An even distribution over the experimental area shall be assured at any irrigation treatment. A total of 10 mm of precipitation (rainfall plus irrigation) within three days after each spray application is desirable.

9 Procedure

9.1 Application of test substance

The test substance and reference substance should be fully identified in the test report and information on physical and chemical properties should be included if this is helpful in the interpretation of the test results.

When chemicals are designed for soil application (e.g. agricultural chemicals), application rates, formulations, and modes of application are specified by suppliers. In such cases, they should be followed. Ideally, in such cases, application in the test should be carried out using application equipment similar to that used in practice (e.g. when testing pesticides, application should be carried out using appropriate agricultural spraying equipment designed to deliver equivalent volumes in the same manner). All equipment should be adjusted prior to use to deliver the chemical at a rate equal to the maximum which would reasonably be used in practice.

With pesticides which are applied in water, a water application rate of 200 l/ha to 300 l/ha should be used on arable land. On grassland, 400 l/ha to 800 l/ha should be applied to ensure penetration. If several applications are planned, they should be carried out at intervals corresponding to usual application procedures.

Immediately after application, the concentration of the test substance in soil shall be determined once by residue analysis to verify the actual exposure concentration in soil. Collection of soil samples for residue analyses should be performed according to standard protocols (e.g. Reference[15]). Since the residue analysis in the earthworm field study is only needed to confirm the application rate, a soil sample of 10 cm depth (including the "biological active" zone) is sufficient also in grassland studies. In light of the wide variability in field studies, a range of 50 % to 150 % of the nominal concentration in soil should be achieved for quality assurance measures.

When the effects of accidental spillages or leaching of chemicals are under study, then application should aim to mimic realistic circumstances as closely as possible but with regard to sampling constraints (e.g. even distribution over plots). As earthworms in temperate countries are most active in spring and autumn, it is recommended that the test should begin in spring.

9.2 Sampling dates

A pre-treatment sampling should be conducted to gain information about the species composition, density, and homogeneity of the site. A full sampling is recommended in order to use the information on variability for the statistical evaluation of the other sampling dates. Statisticians should be consulted to check the necessity of pre-treatment data for the statistical analysis in general.

One to two days after each application of the test substance and/or after irrigation, the soil surface shall be searched for alive and/or dead earthworms.

After application of the test substance, at least three sampling dates should be planned, which shall fall in periods of earthworm activity:

— First sampling: about one month to three months after application;

— Second sampling: about four months to six months after application;

— Third sampling: about 12 months after application.

The test duration depends on the properties of the test chemical. Any further sampling that can be necessary should be carried out at half-year intervals during periods of earthworm activity.

9.3 Reference substance

The simultaneous testing of a reference substance (toxic standard) is necessary to obtain information on the effect of a test substance under the specific experimental site conditions.

The active ingredient carbendazim, which is toxic to earthworms, is suitable for this purpose.[16],[17],[18] A field application rate of 6 kg to 10 kg of active ingredient per hectare is considered suitable to obtain the desired effect (reduction of population by 40 % to 80 %). One application of the reference substance during the test is considered to be sufficient (independently from the application pattern of the test substance).

For studies conducted so far, the reference substance should usually yield a statistically significant difference of at least 40 % (4 kg to 8 kg a.i. carbendazim/ha) on overall abundance and/or biomass compared to the control at least at one sampling date. For future studies, the reference substance should yield a statistically significant difference of at least 50 % (6 kg to 10 kg a.i. carbendazim/ha) on overall abundance and/or biomass compared to the control at least at one sampling date. Experience shows that studies without a clear effect of the reference substance can hardly be used in the risk assessment.

NOTE 1 The third sampling of the plots treated with the reference substance 12 months after the application can be skipped if significant effects have been observed at one or both of the first two sampling dates. Experience has shown that the effects of carbendazim on earthworm populations can already be demonstrated four weeks after application.

NOTE 2 The effects of carbendazim significantly decrease in times when earthworms are less active or in diapause (in Central Europe, often the time between beginning of May and mid-September). This fact should be kept in mind when planning an earthworm field test.

10 Data assessment

10.1 End points

The evaluation of the earthworm community at each sampling date shall include the following:

— total abundance and biomass of earthworms per square metre;

— abundance and biomass of the dominant species (species with >10 % dominance and/or at least 10 individuals/m^2 to 15 individuals/m^2). If at a sampling date no species is dominant in one of the endogeic or anecic groups, earthworms belonging to the same ecological group (e.g. endogeics) or belonging to the same morphological groups (e.g. *Tanylobous* and *Epilobous*) can be combined. Evaluation shall be performed at the species level or for each of the respective ecological or morphological groups;

— abundance and biomass of juvenile and adult earthworms belonging to non-dominant species as described above. Since juvenile earthworms of the same genus can often not be identified to the species level, the evaluation can be restricted to morphological groups (*Tanylobous* and *Epilobous*).

NOTE 1 Despite the fact that the selection of the test site was based on the occurrence of at least one dominant species from the two most important ecological groups (anecics and endogeics), it cannot be ensured that during the course of the study, these species are always dominant.

NOTE 2 Certain species can be over-represented or under-represented when activity-related extraction methods are used. For example, adult worms of the species *Lumbricus terrestris* which are living mainly deep in the soil are not covered representatively when electrical extraction methods are used.[19] Similarly, smaller endogeic species of worms in the soil can be killed as a result of formalin extraction and can thus be under-represented.[20]

10.2 Identification of earthworm species

Identification down to species level is made by means of the relevant identification literature, [21], [22] and the nomenclature used should be in accordance with References [23], [24], and [25]

The earthworms collected may be fixed in 5 % formaldehyde solution and kept there until identification. Ethanol (70 %) may also be used as a fixation and preservation liquid. However, ethanol has the disadvantage of bleaching the worms, which makes identification more difficult. Identification of living earthworms in the field is also possible but requires that operators have the skills necessary to identify species.

Adults and young worms of a species are counted separately. With young worms which are difficult to identify, a distinction between *Tanylobous* and *Epilobous* should at least be made.

NOTE To facilitate the taxonomic identification of juvenile earthworms, it is helpful and sufficient to differentiate between *Epilobous* and *Tanylobous* species. The first body segment, the peristomium, surrounds the mouth and dorsally carries a forwardly directed fleshy lobe, the prostomium. When the worm is at rest, the prostomium acts as a flap and seals the entrance to the mouth or buccal cavity; otherwise, it is employed as a tactile and chemo-sensory probe. In *Lumbricus* spp., it is additionally prehensile and used to draw grasses and leaves into the burrow. The prostomium can be continuous posteriorly with the peristomium (*Zygolobous*), have a simple demarcation (*Prolobous*), have a short posterior tongue-like projection (*Epilobous*), or have the tongue-like projection extend back to the first furrow and divide the peristomium dorsally (*Tanylobous*).

10.3 Determination of biomass with gut content

Before weighing, the fixed worms are put onto filter paper to remove any adhering liquid. The mass of the worms is recorded on the basis of species and age (for details, see ISO 23611-1).

11 Calculation and expression of results

For each species collected on each sampling occasion, determine the number of adults and juveniles and their mass. Comparisons between treatments and controls are made using suitable statistical methods. Statistical testing and inference depend on whether the replicate values are normally distributed and are homogeneous with regard to their variance. The reference substance should not be included in the statistical comparison of treatment and control.

In order to test for normality and variance homogeneity, use Shapiro-Wilks and Levene's test procedure, respectively. With normally distributed and homogeneous data, multiple comparisons for randomized complete block design (RCB) such as Dunnett's or William's test ($\alpha = 0{,}05$, one-sided) should be performed. If data do not fulfil the criterion of normality, they can be transformed (logarithmic, square-root) or evaluated using generalized linear models or non-parametric tests, e.g. the Bonferroni U-test in accordance with Reference [13] or the Jonckheere-Terpstra Step-down-test can be applied. If only one treatment has been performed and the prerequisites (normality, homogeneity) of parametric test procedures are fulfilled, use the pairwise Student's t-test, or otherwise the Mann-Whitney U-test procedure. In addition to uni-variate methods, multi-variate statistical tools, such as PRC (Principal response curves), can be helpful.

12 Validity of the test

Minimum earthworm density required for testing substances in the field determined by a pre-treatment sampling are as follows:

— grassland: 100 individuals/m^2;

— arable land sites: 60 individuals/ m^2.

13 Test report

The test report shall include the following information:

a) reference to this part of ISO 11268 (i.e. ISO 11268-3);

b) results, expressed as in Clause 11;

c) detailed description of the test substance and information on physical and chemical properties, if helpful for the interpretation of the test results;

d) characteristics of the test site (see 8.1.1);

e) weather conditions during the test period;

f) detailed description of the test design and the management of the test site (size of test plots, number of replicates, number of samples);

g) extraction method used for sampling;

h) overall abundance and mass of the earthworms collected for all sampling dates together;

i) tables showing the percentage change per test plot, treatment, and date compared to the control;

j) overall abundance and mass of each species for all sampling dates together;

k) tables showing the numbers and mass per sample and date for each species;

l) graphical representation of the abundance and mass change for each individual species during the test period;

m) results obtained with the reference substance;

n) any operational details not specified in this part of ISO 11268 and any incidents liable to have affected the results.

Annex A
(informative)

Additional requirements of pesticide testing

A.1 Aspects of the test design to be considered (8.1.3)

For non-persistent compounds (i.e. $DT_{90\ field}$ < 365 days), it is recommended to test the full single application rate and, if applicable according to the intended use pattern, further additional application rates separately. These additional applications should take into account possible interception factors by the crop.[26] For persistent compounds (i.e. $DT_{90\ field}$ > 365 days), it is also recommended to test the full single application rate and, if applicable according to the intended use pattern, further additional application rates separately. However, for persistent compounds, the first application should also contain a dose which corresponds to the long-term plateau concentration calculated for a soil depth of 10 cm. [27] All application rates except the first one should take into account possible interception rates by the crop.[26]

NOTE $DT_{90\ field}$ is time required for 90 % dissipation (DT_{90}) of the initial substance under field conditions.

Annex B
(informative)

Information on specific earthworm species or communities in different climatic or geographic regions

A guideline which is relevant for (at least) Europe should acknowledge that earthworm communities can differ considerably in different regions, depending e.g. on soil properties or climatic conditions. This is true for the species level as well as for ecological groups. Therefore, as an example, the average community structure and abundance of Central-European sites is given in Table B.1. The numbers are based on an evaluation of sampling results from of 86 crop sites and 48 grasslands in Germany.[28] This information can be helpful when selecting a new test site. It can be relevant not only for Germany but also for wide regions of Northern Europe, i.e. those dominated by a small number (about 25) mainly peregrine (i.e. widely distributed, often by human activities; sometimes considered to be invasive) lumbricids. Comparable information for other parts of the world is missing so far.

The occurrence of identified species (in percent of the total number of the sampled sites belonging to the two specified land use types), their number, and mean abundance is shown in Table B.1. Please note that juveniles (only determined to the genus level) are not included in this compilation.[28]

Table B.1 — Occurrence of identified species

Species	**Crop sites** (n = 86)		**Grassland** (n = 48)	
	Occurrence (%)	**Individuals/m²**	**Occurrence** (%)	**Individuals/m²**
Allolobophora chlorotica	31,4	6,4	35,4	4,8
Aporrectodea caliginosa	**84,9**	23,1	**91,7**	28,1
Aporrectodea longa	19,8	2,6	10,4	0,6
Aporrectodea rosea	**55,8**	7,1	**56,3**	6,6
Dendrobaena octaedra	2,3	0,0	12,5	1,3
Dendrodrilus rubidus	0,0	0,0	8,3	0,7
Lumbricus castaneus	9,3	0,8	31,3	2,4
Lumbricus rubellus	24,0	1,3	**62,5**	10,3
Lumbricus terrestris	**55,8**	5,2	**75,0**	8,6
Octolasion tyrtaeum	17,4	0,9	41,7	3,6
Σ (Individuals/m²)	49,3		75,6	
Number of species	3,3		5,0	

NOTE 1 Only adult individuals determined to the species were considered here.

NOTE 2 Typical species for each of the two land use types (i.e. those occurring at more than 50 % of all sites belonging to the respective land use type are shown in bold).

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