
**Plastics — Soil biodegradable
materials for mulch films for use
in agriculture and horticulture —
Requirements and test methods
regarding biodegradation, ecotoxicity
and control of constituents**

Plastiques — Matériaux biodégradables dans le sol pour les films de paillage pour utilisation en agriculture et horticulture — Exigences et méthodes d'essai concernant la biodégradation, l'écotoxicité et le contrôle des constituants



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*.

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

Biodegradable mulch films are used worldwide in agriculture and horticulture for many years. They enhance growing conditions and contribute to increased yields and improved crop quality by, for example:

- inhibiting the development of weeds;
- reducing significantly the consumption of water and other resources;
- control of soil temperature;
- reduction in leaching of mineral elements and other fertilizer;
- reduction in soil compaction;
- protecting the crops from soil.

Biodegradable mulch films are not designed to be recovered from soil at the end of the intended service life. Therefore, it is no longer necessary for farmers to retrieve the biodegradable mulch film from the field for disposal or recycling after the harvest. Farmers can simply plow it under along with what remains from the plants so that it is incorporated into soil.

This document defines the standard specification to be met for biodegradable mulch films to be used in agriculture and horticulture. It is suited to characterize both the plastic materials which are used to manufacture mulch films and the mulch films itself with respect to characteristics such as biodegradation, adverse effects on terrestrial organisms and control of constituents.

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Plastics — Soil biodegradable materials for mulch films for use in agriculture and horticulture — Requirements and test methods regarding biodegradation, ecotoxicity and control of constituents

1 Scope

This document is applicable to biodegradable plastic materials used to produce mulch films or biodegradable mulch films ready to be used for mulch applications in agriculture and horticulture.

This document specifies test methods and evaluation criteria by addressing the following characteristics:

- a) control of constituents;
- b) biodegradation;
- c) negative effects on terrestrial organisms.

NOTE This document is construed in a way that it can be used to assess other soil biodegradable plastic products that do not qualify as mulch films. For example: drip tape, twine, clips, and plant pots.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. 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 11268-1, *Soil quality — Effects of pollutants on earthworms — Part 1: Determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei**

ISO 11268-2, *Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia andrei**

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants*

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

ISO 15685, *Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation*

ISO 17556, *Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved*

EN 14582, *Characterization of waste — Halogen and sulfur content — Oxygen combustion in closed systems and determination methods*

OECD. (2006), *Test No. 208, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2*

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 **mulch film**

film made from thermoplastic material intended to be used in agriculture and horticulture to cover the ground in order to improve growing conditions of crops and depending on the colour to control weeds

Note 1 to entry: It is assumed that a transparent mulch film does not allow to control weeds.

[SOURCE: EN 17033:2018, 3.1.1]

3.2 **material**

homogenous preparation of biodegradable polymer and additives, as necessary, such as carbon black and colour pigments

Note 1 to entry: Additives are usually introduced under the form of masterbatches using as carrier resin a biodegradable polymer.

[SOURCE: EN 17033:2018, 3.1.2]

3.3 **ultimate aerobic biodegradation**

breakdown of an organic compound by microorganisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) plus new biomass

[SOURCE: ISO 17088:2021, 3.9]

3.4 **mineralization**

decomposition of organic matter or organic substances into carbon dioxide, water and the hydrides, oxides or other mineral salts

[SOURCE: ISO 11074:2015, 3.3.19]

3.5 **disintegration**

physical breakdown of a material into fragments

[SOURCE: ISO 18606:2013, 3.3]

3.6 **degradation**

irreversible process leading to a significant change in the structure of a material, typically characterized by a change of properties (e.g. integrity, molecular mass or structure, mechanical strength) and/or by fragmentation, affected by environmental conditions, proceeding over a period of time and comprising one or more steps

[SOURCE: ISO 472:2013, 2.262]

3.7**total dry solid**

amount of solids obtained by taking a known volume of test material or compost and drying at about 105 °C to constant mass

[SOURCE: ISO 17088:2021, 3.8]

3.8**volatile solids**

amount of solids obtained by subtracting the residue of a known volume of test material or compost after incineration at about 550 °C from the *total dry solids* (3.7) of the same sample

Note 1 to entry: The volatile-solids content is an indication of the amount of organic matter present.

[SOURCE: ISO 17088:2021, 3.10]

3.9**organic constituent**

chemical constituent that contains carbon covalently linked to other carbon atoms and to other elements, most commonly hydrogen, oxygen or nitrogen

Note 1 to entry: Inorganic carbonates, carbides, cyanides and simple oxides such as carbon monoxide and carbon dioxide are not considered as organic constituent.

Note 2 to entry: Allotropes of carbon, such as diamond, graphite, carbon black, fullerenes, and carbon nanotubes are also not considered as organic constituent.

[SOURCE: ISO 17088:2021, 3.15]

3.10**natural soil**

soil collected from the surface layer of fields and/or forests

[SOURCE: EN 17033:2018, 3.3.1]

3.11**poly- and perfluoroalkyl substances****PFAS**

organofluorine compounds containing only carbon-fluorine bonds and carbon-carbon bonds but also other heteroatoms

4 Basic requirements**4.1 General**

In order to be identified as a biodegradable mulch film in accordance with this document, the mulch film or the material of the mulch film under investigation shall fulfil all requirements specified in [Clause 4](#) and [Clause 5](#), respectively.

If not all requirements are met, no reference to this document shall be made.

4.2 Control of constituents

The mulch film or the material of the mulch film shall be identified and characterized prior to testing including:

- determination of the presence of regulated metals and other elements;
- determination of the presence of organic [poly- and perfluoroalkyl substances (PFAS)] and inorganic fluorine (determined as fluorine);

— evaluation of the presence of other hazardous substance as specified in [Annex B](#);

taking legal compliance into consideration.

In addition, volatile solids shall be determined.

4.3 Ultimate aerobic biodegradation

The ultimate level of aerobic biodegradation shall be established by testing under controlled conditions as laid down in [5.2.1](#).

4.4 Negative effects on terrestrial organism

The mulch film or the material of the mulch film shall have no adverse effects on terrestrial organisms.

5 Detailed requirements

5.1 Control of constituents

5.1.1 Regulated metals and other elements

The concentrations of regulated metals and other elements in a mulch film or material of the mulch film shall be less than 50 % of those prescribed for sludges, fertilizers and composts in the country where the final product will be placed on the market or disposed of (see [Annex A](#) for examples).

Regulated metals and other elements shall be determined and reported.

5.1.2 Organic and inorganic fluorine

5.1.2.1 Organic fluorine (PFAS)

From a precautionary perspective, poly- and perfluoroalkyl substances (PFAS) shall not be intentionally added to the mulch film or to a material of the mulch film.

NOTE Most of poly- and perfluoroalkyl substances (PFAS) are extremely persistent in the environment and in addition, certain PFAS are suspected to have bioaccumulative properties and adverse effects for environment and human health.

5.1.2.2 Inorganic fluorine

The concentration of inorganic fluorine in the mulch film or in a material of the mulch film shall be less than 100 mg fluorine/kg material (dry matter) (see [Annex B](#)).

5.1.3 Other hazardous substances

From a precautionary perspective, hazardous substances as specified in [Annex B](#) shall not be intentionally added to a mulch film or material of the mulch film.

Information on the use of hazardous substances shall be recorded and can be based on a self-declaration.

5.1.4 Volatile solids

A mulch film or material of a mulch film shall contain a minimum of 60 % of volatile solids.

Volatile solids shall be determined and reported.

5.2 Ultimate aerobic biodegradation

5.2.1 Test method and evaluation criteria

The ultimate aerobic biodegradability shall be determined for the whole material or for each organic constituent.

Test samples shall not be subjected to conditions or procedures, such as a pre-treatment by heat and or an exposure to radiation exposure, designed to accelerate biodegradation prior to testing according to ISO 17556.

The material is considered to have demonstrated a satisfactory rate and level of biodegradation in soil if, when tested in accordance with ISO 17556, it achieves a minimum biodegradation percentage as specified hereunder:

- a) 90 % of the organic carbon shall have been converted to CO₂ by the end of the test period (relative to a reference material). Both the reference material and the test item shall be tested for the same length of time and the results compared at the same point in time;
- b) as an alternative, 90 % (in absolute terms) of the organic carbon shall have been converted to carbon dioxide by the end of the test period.

If the level of biodegradation exceeds 90 % (relative to a reference material or in absolute terms), then the biodegradation test can be terminated. However, the test period shall be no longer than 2 years.

The biodegradation test shall be performed at a temperature constant to within ± 2 °C in the range between 20 °C and 28 °C, preferably 25 °C.

Use, as reference material, a well-defined biodegradable polymer (microcrystalline-cellulose powder or ashless cellulose filters). If possible, the physical form and size of the reference material should be comparable to that of the test material.

The validity criteria as stated in ISO 17556 shall be fulfilled.

NOTE Biodegradability is assessed by measuring the mineralization level, i.e. the conversion of the organic carbon of a product or a material into CO₂ with the consumption of O₂ under aerobic conditions, or into CO₂ and CH₄ under anaerobic conditions. During biodegradation, part of the organic carbon is also assimilated as biomass. This biomass yield typically ranges from 10 % to 40 %, depending on the substrate. As a consequence, the mineralization level rarely reaches 100 % also when the biodegradation is 100 %, because of biomass formation. Standard test methods for the accurate determination of product's or material's carbon assimilated in biomass during biodegradation are not available yet.

5.2.2 Requirements regarding constituents

Organic constituents which are present at concentrations of less than 1 % (dry mass) do not need to demonstrate biodegradability. However, the sum of such constituents shall not exceed 3 % (dry mass).

For organic constituents which are present in the material at a concentration between 1 % and 15 % (by dry mass), the level of biodegradation shall be determined separately and meet the criteria specified in [5.2.1](#). Organic constituents at a concentration between 1 % and 15 % (by dry mass) that turned out to be readily biodegradable in a ready biodegradation test according to an OECD test guideline (OECD 301, Methods A to F^[30]; OECD 310^[31]) are considered as biodegradable in the context of ISO 23517.

As an alternative to testing the single organic constituent used between 1 % and 15 % (by dry mass), the level of biodegradation of that organic constituent can be determined using an artificial blend of the same material consisting of at least 15 % by total organic carbon (TOC) content. In case this artificial blend meets the criteria specified in [5.2.1](#), then the organic constituent in question is considered to be biodegradable in the context of ISO 23517 and can be used at the same or lower concentration in a material on the condition that the co-substrate is present as tested in the artificial blend.

Carbon black which is frequently used in mulch film is an inert solid. Therefore, it is not considered as an organic constituent and shall not be accounted in the calculation of the degree of biodegradation.

NOTE The objective of testing an artificial blend is to demonstrate that a constituent which does not meet the biodegradability requirements of ISO 23517 when tested alone, may become biodegradable in combination with another biodegradable constituents of a material. The concentration of the constituent in the artificial blend was set at a minimum of 15 % in order to avoid false-positive results, as theoretically a material with, for example, 10 % of a non or moderately biodegradable constituent may still reach the pass level for biodegradation specified in 5.2.1.

5.3 Negative effects on terrestrial organism

5.3.1 Ecotoxicity testing scheme

Ecotoxicity tests shall be performed in order to investigate possible adverse effects caused by degradation products resulting from the degradation of the material of a mulch film in soil at the end of the intended service life.

The test scheme takes into account:

- all relevant terrestrial organism groups as plants, earthworms (invertebrates) and microorganisms;
- important ecological processes critical due to their role in maintaining soil functions as breakdown of organic matter, formulation of soil structure and cycling of materials;
- all relevant exposure pathways as soil pore water, soil pore air and soil material.

The link between soil organism groups of major ecological importance covering all significant soil exposure pathways and suitable test methods for the evaluation of ecotoxicity of the materials of mulch films and their degradation products is shown in Table 1.

Table 1 — Test scheme for the assessment of ecotoxicity

Organism group	Ecological process	Soil exposure pathway	Test methods
plants: - higher plants	primary production	mainly soil pore water (by root uptake)	plant growth test according to OECD 208 or ISO 11269-2 with the modifications specified in Annex C
invertebrates: - earthworms	breakdown of organic matter, formation of soil structure	diverse and multiple up-take routes: - soil pore water; - ingestion of soil material; - soil air	acute earthworm test according to ISO 11268-1 with the modifications specified in Annex D or alternatively chronic earthworm toxicity test according to ISO 11268-2 with the modifications laid down in Annex E
microorganisms: - bacteria	recycling of nutrients	mainly soil pore water	nitrification inhibition test with soil microorganisms according to ISO 15685 with the modifications specified in Annex F

Ecotoxic effects on terrestrial organism shall be determined by comparing soils produced with or without the addition of a test material.

5.3.2 Preparation of soils for ecotoxicity tests

The ecotoxicity of degradation products shall be evaluated according to the test methods specified in 5.3.3, 5.3.4 and 5.3.5 using test soils prepared according to Annex G.

The initial test item concentration in the reactor with test material shall be 1 % dry weight.

NOTE Rationale for an initial test item concentration of 1 %: a 1 % loading is much higher than the expected application loading of a biodegradable mulch film on soil. For example: a typical mulch film is $1,5 \times 10^{-5}$ m thick and has a density of $1\,250\text{ kg m}^{-3}$. This means $1,875 \times 10^{-2}\text{ kg m}^{-2}$ for one application. The soil depth where a mulch film is typically used or remains after use is presumed to be 0,20 m, in agreement with the normal depth of soil tillage. Therefore, 1 m^2 of mulch film covering 1 m^2 of soil surface will typically be mixed with a volume of soil equal to $0,2\text{ m}^3$. This amount of soil weighs approximately 300 kg, considering a soil bulk density of $1\,500\text{ kg m}^{-3}$. Therefore, the typical loading of the mulch film in normal use will be approximately 0,006 3 % ($1,875 \times 10^{-2}\text{ kg}/300\text{ kg} \times 100$) which is far below the initial test item concentration of 1 % taking into account also various film thickness and repeated applications.

5.3.3 Acute toxicity plant growth test

The purpose of this test is to determine the possible toxic effects of mulch films incorporated in soil on the emergence, the early stages of growth and development of terrestrial plants. The basis for the determination is OECD 208 or, alternatively, ISO 11269-2. The principles of the standard test methods shall be followed with the modifications given in the [Annex C](#).

The germination rate and the plant biomass of the tested plant species in the soil exposed to the test material shall be more than 90 % of those from the corresponding blank soil not exposed to the test material.

If the germination rate and the plant biomass of the tested plant species grown on the soil exposed to the test material as well as on the soil exposed to the reference material (e.g. microcrystalline-cellulose) is less than 90 % of those from the corresponding blank soil not exposed to any material, then this could be considered as an indication that a transient phytotoxicity caused by the biodegradation of the high amount of biodegradable materials added to the soil is present and affecting the test. The test is to be considered as not valid and to be repeated after a further maturation of the soil.

The validity criteria as stated in OECD 208 or, if applicable, in ISO 11269-2 shall be fulfilled in blank soil samples (soil without the addition of test material).

Test items that have been already assessed for plant toxicity following EN 17033^[14], EN 13432^[11], EN 14995^[12], ISO 17088^[5], ISO 18606^[8], ASTM D6400^[17], ASTM D6868^[18], AS 4736^[15] or AS 5810^[16] or equivalent standard specifications and fulfilled the pass level for plant toxicity laid down in the respective standard specifications do not need to be retested.

5.3.4 Earthworm test

5.3.4.1 General

The effects of mulch films incorporated in soil shall be determined in accordance with the method described either in [5.3.4.2](#) (acute toxicity earthworm test) or in [5.3.4.3](#) (chronic toxicity earthworm test).

5.3.4.2 Acute toxicity earthworm test

The purpose of this test is to evaluate the possible acute toxic effects of mulch films incorporated in soil on the mortality and the biomass (mean weight) of *Eisenia fetida* or *Eisenia andrei*. The effects of mulch films incorporated in soil following the acute toxicity earthworm test shall be determined in accordance with ISO 11268-1 with the modifications specified in [Annex D](#). Alternatively, the chronic toxicity earthworm test given in [5.3.4.3](#) may be conducted.

The survival and the biomass of the surviving adult earthworms in the soil exposed to the test material shall be more than 90 % of those from the corresponding blank soil not exposed to the test material.

If the survival and the biomass of the surviving adult earthworms grown in the soil exposed to the test material as well as in the soil exposed to the reference material (e.g. microcrystalline-cellulose) is less than 90 % of those from the corresponding blank soil not exposed to any material, then this can

be considered as an indication that a transient phytotoxicity caused by the biodegradation of the high amount of biodegradable materials added to the soil is present and affecting the test. The test is to be considered as not valid and to be repeated after a further maturation of the soil.

The validity criteria as stated in ISO 11268-1 shall be fulfilled in blank soil samples.

Test items that have been already assessed for toxicity to earthworms following EN 17033^[14], AS 4736^[15] or AS 5810^[16] or equivalent standard specifications and fulfilled the pass level for toxicity to earthworm laid down in the respective standard specifications do not need to be retested.

5.3.4.3 Chronic toxicity earthworm test

The purpose of this test is to evaluate adverse effects of materials on the reproduction of earthworms. The effects of mulch films incorporated in soil following the chronic toxicity earthworm test shall be determined in accordance with ISO 11268-2 with the modifications specified in [Annex E](#). Alternatively, the acute toxicity earthworm test given in [5.3.4.2](#) may be conducted.

The effects on reproduction of adult earthworms (species *Eisenia fetida* or *Eisenia andrei*) exposed to mulch films samples incorporated in soil are compared to those observed for samples exposed to blank soil. In addition, observations on growth and survival of adult earthworms are determined.

The survival and the biomass of the surviving adult earthworms in the soil exposed to the test material shall be more than 90 % of those from the corresponding blank soil not exposed to the test material after an incubation period of 28 days.

The observed number of offspring in soil exposed to the test material shall be more than 90 % of those from the corresponding blank soil not exposed to the test material after an incubation time of 56 days.

If the survival and the biomass of the surviving adult earthworms after an incubation period of 28 days, or if the counted number of offspring after an incubation period of 56 days grown in the soil exposed to the test material as well as in the soil exposed to the reference material (e.g. microcrystalline-cellulose) is less than 90 % of those from the corresponding blank soil not exposed to any material, then this could be considered as an indication that a transient phytotoxicity caused by the biodegradation of the high amount of biodegradable materials added to the soil is present and affecting the test. The test is to be considered as not valid and to be repeated after a further maturation of the soil.

The validity criteria as stated in ISO 11268-2 shall be fulfilled in blank soil samples.

Test items that have been already assessed for chronic toxicity to earthworms following EN 17033^[14] or equivalent standard specifications and fulfilled the pass level for long-term toxicity to earthworm laid down in the respective standard specification do not need to be retested.

5.3.5 Nitrification inhibition test with soil microorganisms

The effects of materials on the microbial nitrification activity in soil shall be determined following ISO 15685 with the modifications specified in [Annex F](#).

The nitrite formation in soil exposed to the test material shall be more than 80 % of those from the corresponding blank soil not exposed to the test material.

If the nitrite formation in soil exposed to the test material and the reference material (e.g. microcrystalline-cellulose) is less than 80 % of those from the corresponding blank soil not exposed to any material, then this could be considered as an indication that a transient toxicity caused by the biodegradation of the high amount of biodegradable materials added to the soil is present and affecting the test. The test is to be considered as not valid and to be repeated after a further maturation of the soil.

The results are considered to be valid, if the variation between replicate blank soil samples and test samples is less than ± 20 %.

Test items that have been already assessed for toxicity to soil microorganisms following EN 17033^[14] or equivalent standard specifications and fulfilled the pass level for toxicity to soil microorganisms laid down in the respective standard specification do not need to be retested.

6 Test report

The test report shall provide all pertinent information, including:

- a) all information necessary to identify the mulch film tested;
- b) a description of requirements of this document and a statement, for each requirement ([Clauses 4](#) and [5](#)), as to whether the test result was in agreement with the requirement or not;
- c) the documentation enabling the identification of any supplementary information (including externally sourced technical data) necessary to support the conclusions reached in the assessments.

Annex A (informative)

Examples of maximum concentrations of regulated metals and other elements

A.1 Regulated metals

Table A.1 — Examples of maximum concentrations of regulated metals and elements and analytical test methods

Values given in mg/kg of dry material

Element ^f	US ^a	Canada ^b	EU + EFTA countries ^c	Japan ^d	China ^e	Test method
Zn	1 400	463	150	180	150	ISO 17294-2
Cu	750	189	50	60	50	ISO 17294-2
Ni	210	45	25	30	25	ISO 17294-2
Cd	17	5	0,5	0,5	0,5	ISO 17294-2
Pb	150	125	50	10	50	ISO 17294-2
Hg	8,5	1	0,5	0,2	0,5	ISO 12846
Cr	—	265	50	50	50	ISO 17294-2
Mo	—	5	—	—	1	ISO 17294-2
Se	50	4	—	—	0,75	ISO 17294-2
As	20,5	19	—	5	5	ISO 17294-2
Co	—	38	—	—	38	ISO 17294-2
F	—	—	—	—	100	EN 15408

^a The maximum metal concentrations given here for the US are 50 % of those prescribed by 40 CFR 503.13, Table 3 (as per ASTM D6400 requirements).

^b The maximum metal concentrations for Canada are those prescribed in 6.1 of BNQ 9011-911-1/2007.

^c The maximum metal concentrations are 50 % of those prescribed in ecological criteria for the award of the Community eco-label to soil improvers [COMMISSION DECISION (EU) 2015/2099 of 18 November 2015 establishing the ecological criteria for the award of the EU Ecolabel for growing media, soil improvers and mulch (notified under document C(2015) 7891].

^d The maximum metal concentrations for Japan are 10 % of those prescribed in the Fertilizer Control Law (Ministry of Agriculture, Forestry and Fisheries) and Guidelines for Quality of Composts (Central Union of Agricultural Co-operatives).

^e GB/T 35795-2017. Biodegradable Mulching film for Agricultural Uses. GB Standard of the People's Republic of China.

^f Countries not listed in the table shall use one of the available maximum concentrations.

The test methods given in [Table A.1](#) or other appropriate recognized international or national standards shall be used to determine the regulated metals. The applied test methods shall be reported in the test report.

Annex B (normative)

Maximum concentrations of organic and inorganic fluorine and other hazardous substances

B.1 Organic and inorganic fluorine

B.1.1 Organic fluorine (PFAS)

From a precautionary perspective, per- and polyfluoroalkyl substances (PFAS) shall not be intentionally added to the mulch film or a material of the mulch film.

B.1.2 Inorganic fluorine

The concentration of inorganic fluorine in the mulch film or a material of the mulch film shall be less than 100 mg fluorine/kg material (dry matter). The inorganic fluorine concentration shall be analysed according to EN 14582.

If a testing on a material containing, for example, talcum results in a value of more than 100 mg F/kg material (dry matter), then, in addition, the total fluorine content of a material without talcum shall be determined.

NOTE Talcum or talc is an inert mineral composed of hydrated magnesium silicate with the chemical formula $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$. The mineral is used in many applications including baby care products, cosmetics and packaging and packaging materials. Depending on the geographical origin of the inorganic natural product it can contain appreciable amounts of fluorine. As fluorine is almost entirely fixed in the matrix of talcum, just a marginal proportion of it is water-soluble and hence bioavailable. Talcum is not classified as hazardous to the environment.

B.2 Other hazardous substances

From a precautionary perspective a mulch film or a material of a mulch film shall not

- a) be classified as hazardous according to the UN Globally Harmonized System for Classification and Labelling of Chemicals (GHS)^[21]

and

- b) be intentionally produced with a hazardous substance
 - meeting criteria of classification according to the UN Globally Harmonized System for Classification and Labelling of Chemicals (GHS)^[21] as
 - 1) carcinogenic (category 1A or 1B) or
 - 2) mutagenic (category 1A or 1B) or
 - 3) toxic for reproduction (category 1A or 1B), or
 - having endocrine disrupting properties^[22], or
 - having persistent, bioaccumulative and toxic properties, or
 - having very persistent and very bioaccumulative properties, and

- exceeding a concentration limit of 0,1 % (by weight) in the mulch film or in the material of a mulch film.

NOTE 1 Safety Data Sheets or other reliable sources such as the website of the European Chemicals Agency (ECHA)^[32] which provide comprehensive information about a substance or a mixture, can be used for the identification of hazardous substances fulfilling the above-mentioned criteria.

NOTE 2 In the European Union, hazardous substances meeting the above outlined criteria are categorized as substances of very high concern (SVHC). SVHCs are those which appear on the Candidate List of substances of very high concern for Authorization^[23].

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Annex C (normative)

Determination of acute effects of materials on the emergence and growth of higher plants

C.1 General

The purpose of this test is to determine the possible toxic effects of mulch films incorporated in soil on the emergence, the early stages of growth and development of terrestrial plants. The basis for the determination is the OECD 208 or alternatively ISO 11269-2. The principles of the standard test methods shall be followed with the modifications given in this annex.

C.2 Procedure

C.2.1 Selection of plant species

Use at least one plant species from each family:

- monocotyledonae (e.g. summer barley: *Hordeum vulgare*; wheat: *Triticum aestivum*; perennial ryegrass: *Lolium perenne*);
- dicotyledonae (e.g. white mustard: *Sinapis alba*; garden cress: *Lepidium sativum*; radish: *Raphanus sativus*; mung bean: *Phaseolus aureus*).

C.2.2 Performing the tests

Fill pots with a minimum of 200 g (dry weight) of either test soil or blank soil prepared according to the description given in [Annex G](#).

Soil nutrients may be added to the soil samples in order to compensate nutrient depletion caused by increase of microbial biomass during the biodegradation of the test item and in order to maintain good growth vigor and plant vitality. For example, $\text{NO}_3\text{-N}$ is an essential macro-element for plant growth. In test soil samples that are prepared according to [Annex G](#), the $\text{NO}_3\text{-N}$ content can be significantly lower when compared to the blank soil, due to the microbial mineralization of the test item. Therefore, it is recommended to measure the nitrate content in test soil as well as in blank soil shortly before the start of the plant growth test. If necessary, soil nutrients may be added to the test soil till a similar level as in the blank soil. Recommendations for nutrient solutions are also given in ISO 11269-2:2013, Annex D. Applied measures shall be documented in the test report.

Add 50 seeds of the selected plant species (see [C.2.1](#)) on the top of each pot. Cover the seeds with a thin layer of inert material, such as siliceous sand or perlite.

Perform the tests in four replicates for each soil type.

Add water until 70 % to 100 % of the water holding capacity is reached. Supply evaporated water periodically during the whole test duration as needed.

The pots are kept under conditions that allow a satisfactory development of the selected species, i.e. 14 d to 21 d after 50 % of the seedlings have emerged in the control pots.

NOTE It is an advantage to keep the pots at a dark place or to cover them during the germination period.

C.3 Evaluation of the results

The number of germinated plants and the plant biomass of a test soil and the blank soil are compared. Germination rate and biomass are both calculated as per cent of the corresponding values obtained with the blank soil. If applicable, the same evaluation procedure applies to soil samples exposed to the reference material (e.g. microcrystalline cellulose).

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Annex D (normative)

Determination of acute effects of materials on earthworms

D.1 General

The purpose of this test is to evaluate the possible acute toxic effects of mulch films incorporated in soil on the mortality and the biomass (mean weight) of *Eisenia fetida* or *Eisenia andrei*. The basis for the determination is ISO 11268-1.

D.2 Procedure

Perform the test according to ISO 11268-1.

Fill each test container with 500 g (dry weight) of samples prepared according to [Annex G](#) and place 10 randomly selected worms to each test container. Perform the tests in four replicates. Add water until 40 % to 60 % of the water holding capacity is reached. Supply evaporated water periodically during the whole test duration as needed.

The number of dead earthworms is determined at day 7 and day 14 in each test container and the body weight of each earthworm is recorded at the beginning (day 0) and at day 14.

The biomass change of earthworms in each test container (mean weight of surviving worms) is calculated as percent biomass of earthworms at the beginning (day 0).

D.3 Evaluation of the results

The mortality and the biomass (mean weight of surviving worms) of earthworms added to the test soil and the blank soil are compared. Both parameters are calculated as per cent of the corresponding values obtained with the blank soil. If applicable, the same evaluation procedure applies to soil samples exposed to the reference material (e.g. microcrystalline cellulose).

Annex E (normative)

Determination of effects on reproduction of earthworms

E.1 General

The effects on reproduction of adult earthworms (species *Eisenia fetida* or *Eisenia andrei*) exposed to mulch films samples incorporated in soil are compared to those observed for samples exposed to blank soil. In addition, observations on growth and survival of adult earthworms are determined.

The basis for the determination is ISO 11268-2.

E.2 Procedure

Perform the test according to ISO 11268-2.

Fill each test container with 500 g to 600 g (dry weight) of samples prepared according to [Annex G](#) and place 10 randomly selected adult worms to each test container. Perform the tests in four replicates. Add water until 40 % to 60 % of the water holding capacity is reached. Supply evaporated water periodically during the whole test duration as needed. The earthworms are fed once a week during the test period. At the beginning the mass of living worms is measured in each container.

Remove the adult earthworms after four weeks (28 days) and record the total number and mass of living adult worms in each container. The biomass change of earthworms in each test container (mean weight of surviving worms) is calculated as percent biomass of earthworms at the beginning (day 0).

Incubate the test containers for another period of four weeks to allow offspring to develop. At the beginning of this period, juveniles are fed once. After 56 days, the number of offspring per test container hatched from the cocoons is counted.

E.3 Evaluation of the results

After 28 days, compare the effect of mulch films on the mortality and the biomass (mean weight of surviving worms) of adult earthworms added to the test soil and the blank soil. Both parameters are calculated as per cent of the corresponding values obtained with the blank soil. If applicable, the same evaluation procedure applies to soil samples exposed to the reference material (e.g. microcrystalline cellulose).

After 56 days, the effect of mulch films on the reproduction of earthworms is determined by comparing the number of offspring counted in test soil and the blank soil. The effect on reproduction is calculated as per cent of the corresponding value obtained with the blank soil. If applicable, the same evaluation procedure applies to soil samples exposed to the reference material (e.g. microcrystalline cellulose).

Annex F (normative)

Determination of nitrification of soil microorganisms

F.1 General

The purpose of this test is to assess possible adverse effects of mulch films incorporated in soil on nitrification activity of soil living microorganisms. The basis for the determination is the ISO 15685.

According to ISO 15685, ammonium oxidation, the first step in autotrophic nitrification in soil, is used to assess the potential activity of microbial nitrifying populations. Autotrophic ammonium-oxidizing bacteria are exposed to ammonium sulfate in a soil slurry buffered at pH 7.2. Oxidation of the nitrite performed by nitrite-oxidizing bacteria in the slurry is inhibited by the addition of sodium chlorate. The subsequent accumulation of nitrite is measured over a 6 h incubation period and is taken as an estimate of the potential activity of ammonium-oxidizing bacteria. As the generation time of ammonia-oxidizing bacteria is long (>10 h), the method provides a measure of the potential activity of the nitrifying population at the time of sampling. It does not measure growth of the nitrifying population.

F.2 Procedure

Perform the test according to ISO 15685 in three replicates for each soil sample, prepared according to [Annex G](#).

The ammonium oxidation is measured by means of analysing the nitrite formation [mg NO₂/g soil (dry weight)] in each sample after 2 h and 6 h of incubation.

N-containing plastic materials may affect the nitrite concentration in test soil samples because of possibly occurring nitrification during the preceding preparation of soils for ecotoxicity testing according to [Annex G](#). Therefore, if nitrification in test soil samples is anticipated, the nitrite content in test soil as well as in blank soil samples should also be measured at the beginning of the test. Corrections should be made if the nitrite concentration in test soil samples is 15 % higher compared to values obtained with blank soil. To do this, the measured nitrite concentrations in test soil after 2 h and 6 h should be corrected by the mean difference value observed at the beginning of the test (0 h).

F.3 Evaluation of the results

The nitrite formation in the test soil is compared with that in blank soil.

The nitrite formation in the test soil is calculated as a percentage of the corresponding value obtained with the blank soil. If applicable, the same evaluation procedure applies to soil samples exposed to the reference material (e.g. microcrystalline cellulose).

Annex G **(normative)**

Preparation of soils for ecotoxicity testing

G.1 General

The purpose of this annex is to give the instructions to prepare soils for ecotoxicity testing.

Natural soil shall be used in order to prepare soil for ecotoxicity testing.

G.2 Preparation of the soil

G.2.1 Collection and sieving of natural soil

Use natural soil collected from the surface layer of fields and/or forests. Sieve the soil to give particles of less than 5 mm, preferably less than 2 mm, in size and remove obvious plant material, stones and other inert materials.

Record the sampling site, its location, the presence of plants or previous crops, the sampling date, the sampling depth, the soil analysis, if available, and, if possible, the soil history, such as details of fertilizer and pesticide application.

G.2.2 Adjustment of the water content and the pH of the soil

Adjust the water content of the soil to a suitable value for the test material by adding an appropriate amount of water to the soil, or by drying the soil in the air in a shaded place followed by addition of an appropriate amount of water.

The optimum water content of the soil should be between 40 % and 60 % of the total water-holding capacity. The water content shall be determined according to ISO 11274.

Adjust the pH of the soil to between 6,0 and 8,0 in accordance with ISO 10390 if it is not already within this range.

It is recommended that the ratio of organic carbon in the test material to nitrogen in the soil (C:N ratio) be adjusted to at least 40:1, if required, by adding nitrogen, for example, aqueous solution of ammonium chloride as specified in ISO 17556.

G.3 Reactors

Prepare a sufficient number of reactors, each consisting of a box made of polypropylene or other suitable material, with the following dimensions: L = 30 cm; w = 20 cm; h = 10 cm. The box shall be covered with a lid assuring a tight seal to avoid excessive evaporation. In the middles of the two 20 cm wide sides, a hole of 2,5 mm diameter shall be made approximately 6,5 cm from the bottom of the box. These two holes provide gas exchange between the inner atmosphere and the outside environment and shall not be blocked.

At least a minimum of 4 000 g (dry weight) blank soil and a minimum of 4 000 g (dry weight) test soil is required to perform ecotoxicity tests outlined in [5.3.3](#), [5.3.4](#) and [5.3.5](#).

G.4 Procedure

The initial test item concentration shall be as specified in [5.3.2](#).

Fill a reactor with the soil/material mixture so that the soil layer is between 5 cm and 10 cm thick.

Prepare at the same moment a reactor with blank soil (i.e. soil without test material).

Record the mass of each reactor before and after addition of the mixture soil/test material.

If transient toxicity effects due to the rapid biodegradation of the test material in soil are likely to occur, prepare in parallel a polypropylene reactor with the reference material (e.g. microcrystalline cellulose) added at the same concentration applied for the test item.

Incubate each reactor in an environment with a temperature range between 20 °C and 28 °C, preferably 25 °C. Report the temperature of environment effectively applied during testing.

Weigh and mix the reactors weekly and restore the original mass with deionized water. Report these operations.

Additional technical measures may be indicated if transient toxicity effects cannot be excluded due to the rapid biodegradation of high amount of biodegradable plastic materials^[20]. As the temporary toxic effects can interfere with results from ecotoxicity tests ([5.3](#)), mix soils at regular intervals at least once a week in order to limit transient effects. Control pH at regular intervals and if necessary, re-adjust to pH between 6,0 and 8,0. In addition, extend incubation time until suspected transient toxicity can be expected to be over. Report these operations.

From a practical point of view, it is recommended to use a powder of the test item in the test rather than the original mulch film in order to avoid effect of sticking together of pieces of the mulch film which may have a negative impact on the biodegradation of the film. It is recommended that the preparation of powder is performed according to ISO 10210^[2].

G.5 Test duration

The reactor for preparing the blank soil as well as for the soil exposed to the test item shall be run until significant biodegradation is clearly identifiable and intermediates might have been released into the soil.

The test for preparing samples for ecotoxicity testing may be started simultaneously with the requested biodegradation test, for example, according to ISO 17556. If the biodegradation degree exceeds at least 50 % in the biodegradation test where the sample is tested in the same physical size, the soil samples for ecotoxicity testing may be used for the indicated ecotoxicity tests.

Optionally, the progress of the biodegradation may be assessed qualitatively by means of a slide frame test, as follows:

- a) use a film of the thickness 15 µm;
- b) fix pieces of the mulch film in approximately three to five slide frames;
- c) place the slide frames randomly in the soil for preparing ecotoxicity samples;
- d) the progress of the disintegration of the mulch film should be visually monitored at regular intervals;
- e) if the mulch film clearly started to disintegrate, then the soil is ready for ecotoxicity testing. An example for qualitative evaluation of the disintegration in a slide frame test is given in [Annex H](#).