DRAFT INTERNATIONAL STANDARD

ISO/DIS 5430

ISO/TC **61**/SC **14**

Secretariat: **DIN**

Voting begins on:
2022-05-20

Voting terminates on:
2022-08-12

Plastics — Marine ecotoxicity testing scheme for soluble decomposition intermediates from biodegradable plastic materials in products intentionally used in the marine environment – Test methods and requirements

ICS: 13.020.40; 83.080.01

This document is circulated as received from the committee secretariat.

Reference number
ISO/DIS 5430:2022(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

There is a growing interest in using biodegradable materials in products used in the marine environment (e.g. farming and fishing gears, floats, buoys). These products are subject to wear and tear and, therefore, tend to be sources of macro- and microplastics. Biodegradability is a factor that in principle mitigates the environmental impacts of fragmentation, thanks to persistence times in the environment that are shorter than that of non-biodegradable materials. Therefore, test methods to measure the level of biodegradation and disintegration of plastic materials in different marine habitats have been established by ISO/TC 61/SC 14 in recent years in order to better characterize the degradation of plastics in these very particular environments:

— The test standards like ISO 18830 (or ISO 19679), ISO 22404, ISO 23977-1 (or ISO 23977-2) are suited to investigate the biodegradation of plastic materials exposed to marine environmental samples (sediments and seawater).

— The ISO standard specification ISO 22403 specifies test methods and requirements to assess the intrinsic biodegradability of materials exposed to marine inocula under mesophilic aerobic laboratory conditions.

— The ISO standard ISO 22766 describes methods for the determination of the degree of disintegration of biodegradable plastic materials exposed to sublittoral and eulittoral habitats under real field conditions.

Beside data on the biodegradability of plastics materials, tests on ecotoxicological effects of potential soluble decomposition intermediates of the biodegradation process to marine organisms are necessary to enable developer and manufacturer of materials to evaluate and to exclude negative effects on marine organisms. In addition, in combination with data on biodegradability, data on ecotoxicological effects may be used for e.g., risk assessment purposes.

This document specifies test methods and requirements for assessing potential adverse effects on different marine organisms caused by soluble decomposition intermediates (degradation products) resulting from the decomposition of plastic materials that are intentionally disposed to marine areas.

Comprehensive ecotoxicity testing schemes and evaluation criteria are already part of ISO standard specifications like ISO 17088 and ISO 23517. The scheme and criteria given in ISO 23517 are equivalent to the requirements specified in the CEN-standard EN 17033. The CEN-document prEN 17427:2020 on carrier bags suitable for treatment in well-managed home composting installations includes an ecotoxicity testing scheme that follows the same basic principles as laid down in above mentioned ISO- and EN-standard specifications: adverse effects are assessed based on results from three tests covering organisms representing different trophic levels.

This document is aiming to provide a suitable ecotoxicity testing scheme for marine organisms.

Plastics — Marine ecotoxicity testing scheme for soluble decomposition intermediates from biodegradable plastic materials in products intentionally used in the marine environment – Test methods and requirements

1 Scope

This document specifies test methods and evaluation criteria by addressing potential ecotoxicological adverse effects on marine organisms. Adverse effects on marine species may be caused by soluble degradation products of plastic materials such as intermediates or remaining residues resulting from the biodegradation of plastic materials that are used in products for marine applications (e.g. nets for fish farming, dolly ropes, floats, buoys, etc.) and which are intentionally disposed to coastal areas, e.g. eulittoral, sublittoral or pelagic zones.

The ecotoxicity testing scheme covers marine organisms from four trophic levels, primary producer, primary and secondary consumer and decomposer:

— toxicity to marine algae,

— toxicity to marine invertebrates,

— toxicity to marine fish,

— toxicity to marine microorganisms.

This document is not suitable for the assessment of adverse effects caused by solid, non-biodegradable plastic materials of any size.

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 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 10210, *Plastics — Methods for the preparation of samples for biodegradation testing of plastic materials*

ISO 10253, *Water quality — Marine algal growth inhibition test with Skeletonema sp. and Phaeodactylum tricornutum*

ISO 11348-1, *Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) — Part 1: Method using freshly prepared bacteria*

ISO 11348-2, *Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) — Part 2: Method using liquid-dried bacteria*

ISO 11348-3, *Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) — Part 3: Method using freeze-dried bacteria*

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

ISO 18830, *Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sandy sediment interface — Method by measuring the oxygen demand in closed respirometer*

ISO 19679, *Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface — Method by analysis of evolved carbon dioxide*

ISO 22404, *Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment — Method by analysis of evolved carbon dioxide*

ISO 23977-1, *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1: Method by analysis of evolved carbon dioxide*

ISO 23977-2, *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 2: Method by measuring the oxygen demand in closed respirometer*

OECD (2019), Test No. 203 *Fish, Acute Toxicity Test,* OECD Guidelines for the Testing of Chemicals*, Section 2*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472:2013 and ISO 17088:2021 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 https://www.electropedia.org/

3.1
biodegradable plastics
plastics capable of being broken down or mineralized by microorganisms

4 General

The marine ecotoxicity testing scheme uses test methods with several marine organisms belonging to different taxonomic groups and trophic levels (see Table 1). The selected test methods, in combination with defined test acceptability criteria, allow the characterization of toxic properties of soluble degradation products of biodegradable plastic materials (i.e. intermediates or remaining residues) to marine organisms.

Table 1 — Ecotoxicity testing scheme for evaluation of adverse effects on marine organisms that represent different tropic levels in marine aquatic food webs.

Marine taxa – species	Ecological process	Test method
Algae — *Skeletonema costatum* — *Phaeodactylum tricornutum*	Primary producer	Marine algal growth inhibition test according to ISO 10253 with modifications specified in Annex A
Invertebrate (marine copepod) — *Acartia tonsa* — *Tisbe battagliai* — *Nitocra spinipes*	Primary consumer	Acute lethal toxicity test to marine copepods according to ISO 14669 with modifications specified in Annex B
Fish (optional) — *Cyprinodon variegatus* — *Dicentrarchus labrax* — *Pagrus major*	Secondary consumer	Fish acute toxicity test according to OECD 203 with modifications specified in Annex C
Micoorganisms — *Aliivibrio fischeri (old name: Vibrio fischeri)*	Decomposer	Determination of inhibitory effect on light emission of luminescent bacteria according to ISO 11348 (all parts) with modifications specified in Annex D

Ecotoxicological effects on marine organisms shall be determined by comparing marine matrices from preceding marine biodegradation tests produced with or without the addition of a test material.

5 Test methods

5.1 Preparation of marine matrices for ecotoxicity testing

Plastic materials preferably in the form of film or powder with dimension <500 µm shall be exposed to marine inocula under mesophilic laboratory test conditions following ISO standard test method ISO 18830 (or ISO 19679), ISO 22404 or ISO 23977-1 (or ISO 23977-2), respectively for up to two years. Powder can be prepared according to ISO 10210.

The minimum initial test item concentration in the marine biodegradation tests shall be the concentration recommended in the standard test method used for the production of the matrices in order to ensure proper biodegradation (e.g. at least 25 mg/100 g of sediment for ISO 22404, at least 100 mg/l of seawater plus sediment for ISO 19679).

Same applies to a reference material. Use microcrystalline cellulose or ashless cellulose filters as a reference material[1)]. If possible, the TOC, form, and size should be comparable to that of the test material.

In addition to marine matrices exposed to plastic materials (test sample) or to reference material (reference sample), marine matrices not exposed to plastic materials (control sample) are incubated in parallel.

The reactors for preparing the matrices for the test samples shall be run until significant biodegradation is clearly identifiable and degradation products might have released into the seawater. There are two options to test for ecotoxicity of soluble decomposition intermediates from biodegradable plastic materials:

1) Microcrystalline Cellulose "Avicel" produced by Merck or Laboratory filter paper Whatman n° 42 has been found satisfactory for this purpose and are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Option 1 – limit test (to be used for pure polymers): the marine test samples for ecotoxicity testing may be used for the indicated ecotoxicity tests when the mineralization level reaches 50 %.

Option 2 – time depending test (to be used for blends): the marine test samples for ecotoxicity testing may be used for the indicated ecotoxicity tests when mineralization reaches 20 %, 40 % and a plateau (equal or greater than 60 %).

For both options, the biodegradation should be monitored following existing ISO standard test method ISO 18830 (or ISO 19679), ISO 22404 or ISO 23977-1 (or ISO 23977-2).

The preparation of matrices for ecotoxicity tests derived from tests in marine sediment (ISO 22404) shall be done following ISO 5667-16. Conserve the samples at low temperature (approximately 4 °C) until processing. It is recommended that the samples are used for ecotoxicity testing within one week after sampling.

5.1.1 Enriched cultures

It is recommended to use pre-exposed inoculum to reduce the expected long exposure time due to the high initial test item concentration. To pre-adapt polymer degrading microbial communities, one gram of fresh marine sediment and 10 ml of fresh sea water can be mixed and shaken vigorously. After stopping the shaking and waiting for one minute that the sediment settles down, 1 ml of the supernatant is taken to inoculate 50 ml seawater amended with 53,5 mg/l NH_4Cl and 17,4 mg/l K_2HPO_4. In addition, 0,1 g/l to 1,0 g/l peptone is added as additional C- and N-source. Test material in the form of film, for example 5 cm^2 piece, is added to the cultures and incubated under conditions with constant shaking or stirring at mesophilic test conditions. After the polymer material is significantly disintegrated analyzed by means of visual inspection, a 1 ml aliquot is transferred into 50 ml fresh seawater amended with above mentioned inorganic and organic nutrients and new test material. After 4 to 5 transfers, a pre-adapted culture should be available able to degrade the test material in a more efficient way (complete biodegradation in a few weeks instead of months). The inoculum should be used to test according to ISO 23977.

5.2 Determination of ecotoxicological effects on marine algae (mandatory)

Basis for the determination of adverse effects on marine algae is ISO 10253. Marine unicellular algae are exposed to marine matrices and the increase in cell number or in biomass of the algae is determined after 72 h.

Both, the principles of the standard test method ISO 10253 and the modifications given in Annex A shall be followed to meet the special needs for testing marine matrices, exposed to biodegradable materials.

In Annex A a description of the ecotoxicity test is given including calculation of test results, validity criteria and evaluation criteria.

5.3 Determination of ecotoxicological effects to the marine invertebrates (marine copepods) (mandatory)

Basis for the determination of adverse effects on marine invertebrates (marine copepods) is ISO 14669. Marine adult copepods are exposed to marine matrices and the immobility or mortality of the copepods is determined after 48 h.

The principles of the standard test method ISO 14669 and the modifications given in Annex B, which are required to meet the special needs for testing marine matrices exposed to biodegradable materials, shall be followed.

In Annex B a description of the ecotoxicity test is given including calculation of test results, validity criteria and evaluation criteria.

5.4 Determination of ecotoxicological effects to the marine fish (optional)

Basis for the determination of adverse effects on marine fish is OECD 203. Marine fish are exposed to marine matrices and the mortality and visible abnormalities related to appearance and behaviour of the fish is determined after 96 h.

The principles of the standard test method OECD 203 and the modifications given in Annex C, which are required to meet the special needs for testing marine matrices exposed to biodegradable materials, shall be followed.

In Annex C a description of the ecotoxicity test is given including calculation of test results, validity criteria and evaluation criteria.

5.5 Determination of ecotoxicological effects on marine microorganisms (mandatory)

Basis for the determination of adverse effects on marine microorganisms is ISO 11348 (all parts). A cell suspension of a bioluminescent bacterium is exposed to marine matrixes and the inhibition of light emission (bioluminescence) is determined after 30 min.

The principles of the standard test method ISO 11348 (all parts) and the modifications given in Annex D, which are required to meet the special needs for testing marine matrices exposed to biodegradable materials, shall be followed.

6 Test report

The test report shall provide all relevant information, including:

a) all information necessary to identify the product or material tested;

b) a description of requirements of this document and a statement, for each requirement, as to whether or not the test result is in agreement with the requirement to

- 5.2 and Annex A regarding adverse effects to marine algae;
- 5.3 and Annex B regarding adverse effects to marine invertebrates (marine copepods);
- 5.4 and Annex C regarding adverse effects to marine fish;
- 5.5 and Annex D regarding adverse effects to marine microorganisms.

c) the documentation enabling the identification of any supplementary information (including externally sourced technical data) necessary to support the conclusions reached in the assessments.