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## Principles for the analysis of microplastics present in the environment

*Principes d'analyse des microplastiques présents dans  
l'environnement*

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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](http://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](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 249, *Plastics*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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](http://www.iso.org/members.html).

## Introduction

The analysis of plastics and microplastics is a new field in relation to other areas of environmental analysis. A large number of scientific publications exist, but they do not apply a uniform analysis, which makes it difficult to compare the results.

This document sets out key principles for the investigation of microplastics in the environment, which should be taken into account in the subsequent development of specific procedures for sampling, sample preparation and detection. A large number of the principles described in this document can be applied, analogously, to other matrices and products, including foodstuffs and drinking water. The objective is to present a pool of methods and notes that is as harmonized as possible and to make it available for use in science, businesses and administrations.

What is true for analytics is also true for definitions in the same way. On the one hand, the terms used in this document are based on existing definitions in the subject area, but on the other hand, analytical requirements are also taken into account. This applies, for example, to the term “large microplastics”. The particle size to be investigated is closely related to the detection method to be selected. In the course of future specific work, it can be necessary to modify existing definitions slightly and adapt them to new knowledge and requirements.

With regard to the definitions, including the ideas of size classes, it is pointed out, that the discussion is ongoing in various technical committees in ISO and other standardization bodies. The definitions in this document show the status in ISO TC 61/SC 14. The definitions chosen in this document are adapted from the ISO report on plastics. The basis of the classification is based on the metric sizes and the associated designations. Microplastics is thus derived from micrometres.

NOTE Microplastics can also stem from different sources not specifically mentioned in this document, such as textiles, paints and tyres.

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# Principles for the analysis of microplastics present in the environment

## 1 Scope

This document describes the principles to be followed in the analysis of microplastics in various environmental matrices. This includes the unique particle size classification of plastics, the use of certain apparatus with regard to sampling, sample preparation, and the determination of representative sample quantities.

The purpose of this document is to specify minimum requirements until specific standards for the different case situations are available. This is important to ensure that the development of the specific standards is done on a consistent basis to ensure that comparison or correlation of results is possible.

This document does not include requirements for monitoring actions.

## 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 472, *Plastics — Vocabulary*

## 3 Terms and definitions

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For the purposes of this document, the terms and definitions given in ISO 472 and the following 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

#### large microplastic

any solid plastic particle insoluble in water with any dimension between 1 mm and 5 mm

Note 1 to entry: Microplastics may show various shapes.

Note 2 to entry: Typically, a large microplastics object represents an item consisting of plastics or a part of an end-user product or a fragment of the respective item.

[SOURCE: ISO/TR 21960:2020, 3.10, modified — term number in Note 1 to entry was removed.]

### 3.2

#### microplastic

any solid plastic particle insoluble in water with dimension between 1 µm and 1 000 µm (= 1 mm)

Note 1 to entry: Primary microplastics object represents a particle intentionally added to end-user products for example cosmetic means, coatings, paints etc. Secondary microplastics object can also result as a fragment of the respective item.

Note 2 to entry: Microplastics have regular and irregular shapes (see ISO 9276-6:2008).

Note 3 to entry: The defined dimension is related to the longest length of the particle.

[SOURCE: ISO/TR 21960:2020, 3.9, modified — Note 1 to entry was removed, all other Notes to entry were changed.]

**3.3 additives**

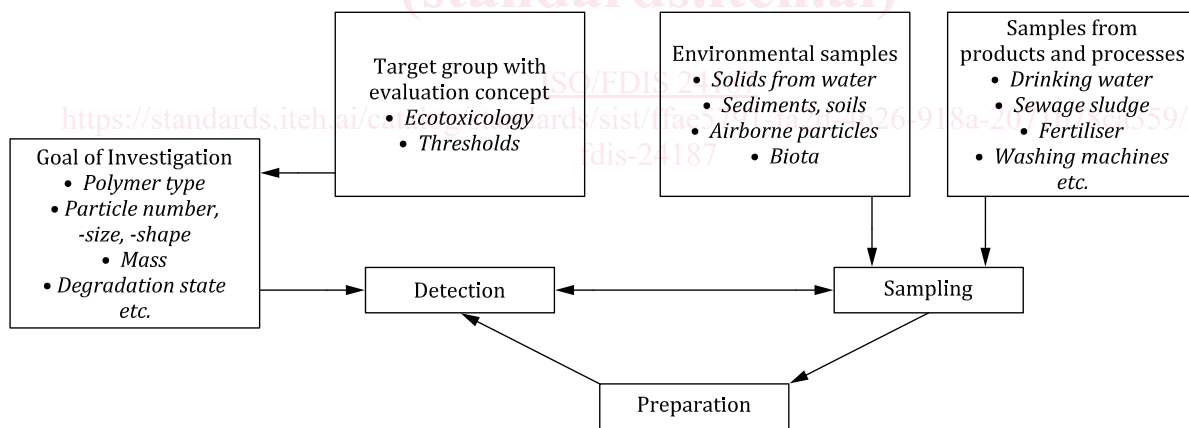
substances which are used to process plastics or to modify end use properties of plastics

Note 1 to entry: Important additives such as fillers/reinforced materials, softeners and flame retardants are referenced according to ISO 1043-2 to ISO 1043-4.

**4 General aspects**

Microplastics is a term that comes along with different physical and chemical properties, such as shape, size (range), type of polymer(s), presence of additives, presence of fillers, state of degradation and so on. The amount of microplastics in a given matrix can be measured in different ways, i.e. as number (of particles) or mass content/fraction in relation to the sample’s quantity, which itself can be based on various units (volume, weight, etc.). Hence, before selecting a suitable (set of) method(s), the question(s) to be answered and properties to be measured need to be specified carefully. This applies not only to detection methods but also to the sampling and processing/preparation methods associated with them, right up to the statistical evaluation of results.

A schematic representation of the interdependencies of microplastics analysis is shown in Figure 1. As a rule, the objective or objectives of a measurement or a measurement program is/ are based on a clear question/task or on an evaluation concept involving necessary assessment parameters, respectively (for example integration into an overall ecological context, thresholds for monitoring). A suitable detection method is then selected, which generates the desired result parameters (such as polymer type, mass content, number, shape, size, degradation status).



**Figure 1 — Schematic representation of interdependencies during microplastics analysis in environmental and related matrices**

**5 General requirements for all analytical steps**

All analytical steps (sampling, sample preparation, detection) shall be undertaken in plastics-free or low-plastics working conditions. These include the avoidance of standard plastics products (for example tubes, vessels). Contamination, especially cross-contamination shall be avoided, the user should avoid using plastics equipment wherever possible. Instead, alternatives made of metal, glass or ceramics should be used. As an exception and after it was proved by experiments (for example by characterizing the container), types of plastics that are not to be detected or evaluated can be used as well. Care should be taken that personal protective equipment (e.g. lab coats, gloves) are also made of non-synthetic material or material that does not interfere with the analyses. Recovery tests should be performed for each analytical step.



If feasible, samples should be handled in laminar flow boxes in the laboratory or clean rooms (class 3 according to ISO 14644-1, especially during the preparation process of samples and during the determination of particle numbers).

It shall be determined beforehand whether hygienization of samples is necessary. Sterilization is a standard recommendation for the analysis of dry samples from wastewater, sewage sludge and organic wastes. Various methods can be applied, but each of them has specific impact on the integrity of microplastics particles in the sample.

- a) Steam sterilization: risk of melting microplastics (for example PE, PP).
- b) Radiation sterilization (gamma, beta radiation, UV radiation): risk that the polymer structure is degraded (cleavage of polymer chains and oxidation).
- c) Chemical sterilization: risk that polymer structure or the particles' surface is chemically modified.

Relevant information about the measurement conditions and control processes (quality assessment and quality control/QAQC) shall be recorded, including all analytical steps. For general quality control measures in laboratories, see ISO/IEC 17025:2017. For intercomparison tests, see ISO 13528.

Blank value determination for the applied detection methods is essential, since contamination (for example by airborne particles) during sampling, preparation and detection can easily occur. Determination of blank values is essential; the number of blanks depends on the concrete method to be applied. More specific requirements have to be given in upcoming standards.

A classification of microplastics into size classes according to [Table 1](#) is recommended. Small particles that occur in higher quantities are grouped into narrower classification classes than the larger particles, which are more relevant in terms of mass and classified into wider classes. This also enables a higher methodological feasibility of processes (including feasibility of filtration, detection limits in analytics) and a better integration of particle quantities/masses in impact analyses (i.e. for environmental assessments). The proposed size classes are given in [Table 1](#). The maximum dimension/diameter/length of a particle defines the size class.

**Table 1 — Particle size classification**

Classification		Microplastics						Large microplastics
particle size classes	µm	1 to < 5	5 to < 10	10 to < 50	50 to < 100	100 to < 500	500 to < 1 000	1 000 to 5 000
average particle size	µm	3	7,5	30	75	300	750	3 000
mass <sup>a</sup>	mg	$1,4 \times 10^{-8}$	$2,2 \times 10^{-7}$	$1,4 \times 10^{-5}$	$2,2 \times 10^{-4}$	0,014	0,22	14
number of particles in 14,13 mg	number	$1,0 \times 10^9$	$6,4 \times 10^7$	$1,0 \times 10^6$	$6,4 \times 10^4$	1 000	64	1

<sup>a</sup> Mass here is estimated from the average particle size (3 000 µm) assuming spherical particle with a density of 1.

## 6 Identification of appropriate detection methods

### 6.1 General

The selection of one or more quantitative or qualitative detection method(s) depends specifically on the objectives and tasks of a project or an existing requirement. The various detection methods differ regarding the generated result per measurement. These include identification of the polymer (type of polymer) and other qualitative properties (i.e. presence of additives, chemical composition, molecular weight and morphology of particle surface, particle size and shape) and quantitative properties (particle number, particle mass fraction).

Depending on the objective of the analysis, it can be sufficient to apply a (pre-)screening method that may give limited information, but does not require sophisticated instrumentation. For (pre-) screening purposes relatively simple and inexpensive techniques could be used. Like this, cost-effective routine analyses can be carried out with a higher throughput than more performance but highly time consuming and costly techniques.

## 6.2 Detection techniques

Different detection methods based on various measurement principles are available for microplastics analysis.

Spectroscopic methods can capture and assign the characteristics of specific chemical structures of polymers using reference spectra. Used methods are based on vibrational spectroscopy techniques (including on microscopic level) including different measurement setups:

- Fourier transform infrared spectroscopy (FTIR);
- attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR);
- focal plan array detector Fourier transform infrared spectroscopy (FPA-FTIR);
- quantum cascade laser induced infrared spectroscopy (QCL-IR);
- near or short-wave infrared spectroscopy (NIR, SWIR);
- Raman spectroscopy.

In thermo-analytical methods, the sample is pyrolysed under inert conditions and specific decomposition products of the individual polymers are detected. Currently well-established are gas chromatography-mass spectrometry (GC-MS) methods. They differ regarding the heating procedure (filament based, micro furnace, Curie point), the sample amounts or sample preparation of individual selected or concentrated particles (pyrolysis - Py-GC-MS) as well as pyrolysis of complete filter residues (thermal extraction desorption - TED-GC-MS). Further methods are suitable, an alternative is the use of methods, which detect the specific melting process of semi-crystalline polymer materials (differential scanning calorimetry, DSC).

Chemical methods are used to decompose the samples and detect specific fragments of polymers or elements. Examples are inductively coupled plasma mass spectrometry (ICP-MS) for tyre and road wear particles or liquid chromatography (LC) for PET, PC or PA, respectively.

Further methods are suitable, such as visual sorting of larger items using microscopy or hot needle test. Such visual sorting is subjective and depends on the expertise of the experimenter. An alternative is also the detection of dyed particles by fluorescence microscopy and spectroscopy. These methods are (partly) restricted regarding the analytical accuracy of polymeric particles but represent fast screening solutions.

All the tools differ regarding the preparation of the samples, the maximum number and sizes of measurable particles or sample mass, the measurement time and the lower detection level regarding the lateral resolution or limit.

## 6.3 Identification of objective to be addressed

Mass content is a monitoring parameter used to estimate the occurrence of microplastics. They are suitable when it comes to the regular, repeated determination of microplastics in the context of monitoring and the control of the effectiveness of measures against plastics inputs. The nominal range of particle size for which these detection analyses are to be made shall be defined in advance. This grouping into size classes ([Table 1](#)) makes it possible to assign the total contents to a specific particle size range. The contents of the different plastics can be measured in a consistent way, regardless of particle shape, number and size. In principle, it should be taken into account that a few large particles are more significant in terms of mass balance than many small particles.

Determining the exact number, size and shape of particles provides a very comprehensive, detailed picture of the occurrence of microplastics in environmental samples. This is important for toxicological studies and assessment. The suitability of the measurement technique for the nominal particle size range to be investigated shall be ensured in advance. For spectroscopic results it is possible to evaluate the particle size during or after measurement. The particles of the different plastics can thereby be measured in a consistent way according to particle shape, number and size. Classification into size classes (see [Table 1](#)) allows for comparing the total contents for a specific particle size range. The analysis of very small particles (<5 µm) is complex and partly limited for real samples. The evaluation methods shall guarantee homogeneity of the analysed environmental sample aliquots, as often only a fraction of the sample can be analysed.

The individual characterization of specific properties of identified plastics particles, for example the state of degradation, the surface structure or condition, and the analysis of additives can be relevant for evaluating the interaction with the environment, but also for assessing their sources, entry paths, and fate. Such analyses may require prior, and in some cases very complex, isolation of individual particles.

## 7 Sampling of water

### 7.1 General

Determination of microplastics in the various environmental matrices are a relatively new field of research. In the following, reference is made to existing standards, some of which, however, were not developed for microplastics sampling. They give a first indication of the procedure. Microplastics is similar in shape, size and density to natural particles. However, a 1:1 transfer of the previous procedure has not yet been realized.

In principle, there are a large number of references in the ISO 5667 series (ISO 5667-1, ISO 5667-4, ISO 5667-6, ISO 5667-8, ISO 5667-9 and ISO 5667-17) of standards for the sampling of water. This includes the sampling of fresh waters (for example lakes, rivers and ground waters) and marine waters. However, these International Standards have not been developed specifically for the sampling of microplastics. These International Standards are a good basis but shall be examined in detail for their suitability in relation to the issues at hand and, where appropriate, adapted as necessary. Refrigerating (max. 4 °C) of samples is recommended in order to avoid microbiological growth, to slow degradation of samples by bacteria and to extend storage time.

For macroplastics, other sample strategies shall be applied, further developed and validated.

### 7.2 Sampling volume

The sample volume depends on the detection and/or quantification limit of the selected analysis technique, the expected particle number or mass content of the microplastics under investigation as well as the size range of the microplastics under investigation: it is assumed that the smaller the diameter of the particle, the more of them that are present in the environmental medium under investigation (for example in water). In this respect, a smaller sample volume may be sufficient if many small particles are present and particles are counted in the detection method. For detection methods determining mass contents, the mass of particle must be sufficient to reach limit of detection or limit of quantification, respectively.

The lower the particle content, the more sample is required in order to examine both sufficient mass and a sufficient number of particles.

The sample volume in the lower µm range (this means approximately <10 µm) can be smaller (in the millilitre or litre range) because the statistical probability of obtaining a representative cross-section of small particles expected is greater with a high number of particles. However, the present particles in such a sample must reach the limit of detection/limit of quantification for detection. If the entire size range down to the upper µm range (this means approximately > 100 µm) is to be covered during sampling, significantly larger volumes of water shall be filtered (several litres to over several cubic meters). Very large representative sample volumes are necessary to be taken in the almost solids-free