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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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Contents

Page

Foreword

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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.

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 are 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 idea of size classes, it is pointed out that 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 ISO/TR 21960:2020. 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.

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

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

ISO and IEC maintain terminology 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).

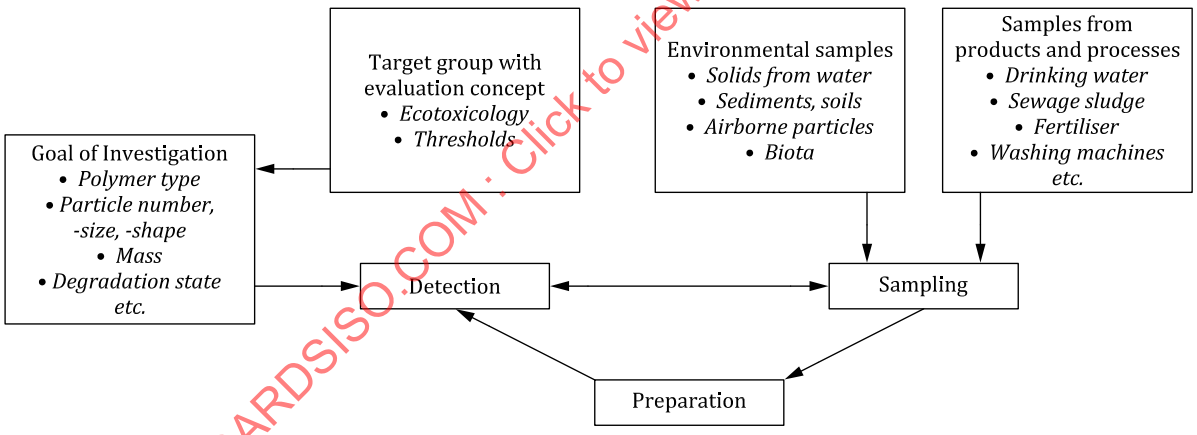

Figure 1 — Schematic representation of interdependencies during microplastics analysis in environmental and related matrices

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. 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. 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[a]	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

[a] 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 plane 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. It is 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 is 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 are 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 Internationals 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 Sample 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 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 volume 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

water body. Depending on the detection method, however, the sample volumes may differ. Especially in the small and very small particle range (this means approximately < 10 µm), microscopic-spectroscopic methods manage with smaller amounts of water. For macroplastics, other sample volumes shall be determined or otherwise specified in such a way that a representative sample can be taken.

7.3 Mesh sizes

For all water filtration processes, it is recommended to use the particle size classes shown in Table 1, so that results can be evaluated according to the size classes and for comparison of different investigations. In the case of high concentrations of solids in water sample and large sample volumes, a fractionated filtration contributes to reducing filter cake formation and subsequent blockage or partial blockage of filters.

In the case of filter cartridges or sieve cascades, a verification of the defined pore size or the nominal mesh size shall be carried out and recorded. Filterability shall be ensured over the entire sampling period as well as the complete removal of filter residues from previous measurements in case of repeated use of the materials. For quality assurance regarding the filtration process, recovery tests are recommended. They are recommended regarding the filter materials (pore sizes) using standardized procedures (ISO 2942). For sampling particles smaller than 10 µm, pressure or vacuum filtration is necessary (approximately 20 Pa to 60 Pa) due to the low water permeability of the filters.

The immersion depth and orientation of the sampling container/sampling device opening with respect to the flow direction (angle to the incident flow) during the sampling process is to be documented. Ideally, the hydrodynamic conditions should be documented as well (possibility of isokinetic sampling). When using neuston or plankton nets or cascades (especially for marine water), the same particle classification described above (see Table 1) should also be used.

Other commonly used sampling methods can be applied, such as sediment traps, membrane filter systems and flow centrifuges, but have not yet been sufficiently characterized for microplastics measurements. Therefore, no recommendations are made to date. Furthermore, there are no recommendations for preferred sampling by means of random samples or aggregate samples. When using collection containers for continuous sampling, care shall be taken to homogenize the sample during further processing (biological growth, sedimentation or flotation effects).

In the documentation of sampling methods, the depth from surface during sampling, the sampled water volume and the effectively filtered water volume shall always be reported.

7.4 Filter materials

The selection of the filter material can be decisive with regard to the research question and the planned analysis techniques. The filter materials should also be plastics-free. Non-polymer filter materials (e.g. stainless steel, silica, alumina) have proven to be very helpful.

Check the filter for inorganic or organic contaminants or residues before use.

8 Sampling of terrestrial, semiterrestrial and subhydric soils

8.1 General

All soil sampling standards refer to nutrients or contaminants that are either adsorbed to soil particles or are probably finely dispersed. The sampling and pre-treatment of terrestrial, semiterrestrial and subhydric soils as well as solid materials fundamentally require validation tests.

8.2 Sampling of terrestrial soils

In this subclause, first hints for the sampling of terrestrial soils are given. So far, there is not much experience with microplastics analysis of soils. As with the taking of water samples, the determination of a representative sample is also the decisive factor here. This depends on the number of particles and

particle size. As a first step, ISO 18400-203, ISO 18400-205, ISO 23611-2, ISO 23611-3, ISO 23611-4 and ISO 23611-5 should be followed.

8.3 Sampling of semiterrestrial soils

Semi-terrestrial soils are under the influence of (ground)water and may also be subject to occasional flooding. However, the flooding periods are irregular and short. This may also include river banks and beaches by the sea. In principle, the same extraction methods can be used as for terrestrial soils.

8.4 Sampling of subhydric soils (sediments)

Subhydric soils are permanently under water. Depending on the discipline, they are also referred to as lake sediment or marine sediment. ISO 5667-12 provides guidance on the sampling of unconsolidated sediments for the determination of their geological, physical and chemical properties, as well as the determination of biological, microbiological and chemical properties at the water and sediment interface. Guidance on obtaining sediment cores is given specifically for the measurement of rates of deposition and detailed strata delineation. The environments considered are:

— limnic (rivers, streams and lakes, natural and man-made);

— estuarine, including harbours;

— marine sediments.

9 Sampling of air

9.1 Indoor air

ISO 16000-34 specifies the general strategies for determining the concentration of airborne particles indoors and covers the size range from approximately 1 nm to 100 µm. In addition, this document describes methods for identifying typical indoor particle sources and gives general recommendations for obtaining a representative sample. The main sources of indoor particulate matter are described in this document, together with indoor particle dynamics. Various measurement methods are described, along with their advantages, disadvantages and areas of application, as well as some general sampling recommendations. Measurement strategies for determining airborne particles indoors are discussed, including reference case studies with more specific sampling recommendations.

Additional documents in the ISO 16000 series will focus on each fraction of airborne particulate matter and give specific recommendations for these measurements.

9.2 Outdoor air

The ambient air quality at a particular location or region is generally variable with time, this variation being caused by a number of factors, especially meteorological conditions, topography and patterns of emissions. Such circumstances may require that a large number of measurements be made over a long interval of time to ensure that a sufficiently wide range of conditions is covered. Stratified sampling is one method which reduces the number of measurements needed to assess certain aspects of ambient air quality. This technique has been applied for example in ambient air quality surveys and in noise surveys [38].

ISO 9359 specifies a method for the assessment of certain aspects of ambient air quality in terms of percentiles and means using the principle of stratified sampling.

10 Sampling of sludges and other similar materials

Similar to the water and soil, the preliminary work in the relevant ISO committees shall also be taken into account when investigating sewage sludge. However, as already mentioned above, previous

experience with sampling cannot be easily transferred to the field of plastics. The size and number of plastics particles, in particular, play an important role in representative sampling. However, ISO 5667-13 should be mentioned here in any case.

In addition to the standards from ISO/TC 147 “Water quality”, standards from ISO/TC 134 “Fertilizers, soil conditioners and beneficial substances” or ISO/TC 275 “Sludge recovery, recycling, treatment and disposal” can also contain helpful information.

Fertilizers are commercial products and are available in various types of packaging. Sampling is often carried out on heaps. ISO/TR 5307 gives first indications. From ISO 7410, ISO/TR 7553, ISO 7742 and ISO 3963, further information about what has to be considered for such sampling can be obtained.

11 Sampling of mineral and other inorganic materials

ISO 14488 specifies methods for obtaining a test sample from a bulk of particulate material (powder, paste, suspension or dust) that can be considered to be representative of that bulk with a defined confidence level. It is particularly relevant to the measurement of particle size, size distribution and surface area.

12 Sampling of biota

When sampling biota, local laws and regulations governing such actions can exist. In some cases, there are specifications with regard to animal protection and specifications under which circumstances animals may be taken from nature and euthanised for experiments.

Initial information can be taken from the following standards, for example:

— ISO 10870;

— ISO 23611-1;

— ISO 23611-2;

— ISO 23611-3;

— ISO 23611-4;

— ISO 23611-5;

— ISO 23611-6.

13 Sample preparation

13.1 General aspects

The selection and sequence of sample preparation steps depends on various factors, such as (see also Figure 1):

— sample composition, which depends on the investigated environmental matrix determining the amount and type of (potentially) interfering matter present;

— sample size;

— concentration of microplastics in the sample;

— the chosen detection method.

Various steps can be needed, such as reduction of size of matrix constituents (for example milling, grinding), removal of interfering matter, homogenization, and/or concentration of the sample (for example filtration, drying). Filtration is included in Clause 7.

For any such step, the impact on the microplastics to be measured needs to be investigated via preliminary tests with the matrix under investigation, doped with microplastics material. If available, reference materials shall be used for this purpose.

13.2 Drying

The usual sample preparation of aqueous or wet samples include a drying step, which shall be carried out at temperatures not higher than 40 °C in order to prevent the plastics to be tested from being affected. For the analysis of certain polymers, temperatures up to 40 °C can be more appropriate to prevent affecting the plastics particle. Freeze-drying is therefore a favourable alternative.

13.3 Milling and grinding

When grinding samples, care shall also be taken to work below the glass transition temperature. It is not suitable for spectroscopic methods as the size and number of the particles can be affected. The use of a cryo mill is recommended. Milling and grinding as sample preparation steps should be avoided or handled with extreme care in order to prevent the plastics to be tested from being affected. However, if particle size and number is not of interest, grinding and milling could be utilized below the glass transition temperature.

13.4 Removal of inorganic matter

For the preparation of most water samples (filter residues) removal of the inorganic matrix is proposed for all spectroscopic detection methods. For solid samples (for example soil, sediment) a density separation for removal of the inorganic matrix is always recommended.

Methods for density separation using saturated salt solutions (for example NaCl, $ZnCl_2$, Wolframates, NaI, $CaCl_2$, KBr, potassium formate) are generally proposed. These salt solutions represent different densities and enable the separation of particles based on the effect that less dense material floats and separates from the denser sinking material. It should be noted that the viscosity of the solution and the wettability of the particles can be critical, as well as the particles' shape, size and density. Suitable separation times have to be chosen to achieve appropriate separation, taking into account the expected floatation speed of the particles, which depends on their size and density as well as on the density and viscosity of the selected liquid. In addition, the size of the separation container and the duration of the separation can influence the detection result, which should therefore always be constant and reported. The pH value of the solutions shall be checked (risk of precipitation of carbonates or degradation of plastics particles).

It should be noted that certain agents can interact with and modify polymers (affecting the detection result) and that experimental conditions (for example elevated temperature) can alter the particles, thus impacting the identification of plastic materials.

13.5 Removal of organic matter

For samples from water (filter residues) and for samples from solids (for example soil, sediment) treatment processes for removing the natural organic matrix are proposed for all spectroscopic detection methods and in specific cases also for thermoanalytical methods.

The parameters relevant to the processes (type of chemicals or enzymes, concentration, enzyme activity, exposure time, temperature, pH value) shall be comprehensively represented, also with regard to sampling and subsequent detection. The treatment of the samples with oxidizing solutions (for example, hydrogen peroxide or Fenton reagent) is proposed most frequently. A temperature of 25 °C in the laboratory should not be exceeded with respect to lab safety aspects and protection of the analyst. Handling of samples with diluted or concentrated acids or bases is also common. An alternative is

enzymatic processing. It should be noted that certain agents and experimental conditions (for example temperature) can affect the plastics particle or the particle surface, which can affect the detection result.

14 Data processing

14.1 General aspects

Considering the objectives and analytical instruments presented in Clause 6 (identification of appropriate detection methods) the detection of values such as mass of microplastics, chemical structure, particle number, size and shape from measurement data are still a non-trivial task, for which further standardization is required. Numerous analytical approaches are applied for microplastics quantification (see Clause 6) and the way data are processed will depend on the detection method.

Any detection method generates output data, for example counting results, microscope images, spectra for IR and Raman or chromatograms for the chromatography-based methods. These shall be interpreted to identify the microplastics content of a set of analysed particles. At the most fundamental level, this is done by scrutinizing a spectrum or chromatogram and applying chemical specialist knowledge for its interpretation. In the case of spectroscopic techniques, identifying chemical bonds in an FTIR or Raman spectrum and from these bonds allow to deduce the chemical structure of a particle. This process is time-consuming and can be sped up by the use of reference spectra databases, where the shape and peaks of a spectrum are compared to many references. In its first instance, this process is manual, but it can be automated for the various detection methods. Whenever the detection method generates a very large number of spectra, a further layer of automation can be necessary as the sheer number of spectra or potential particles to be evaluated can be overwhelming.

14.2 Single spectra/chromatogram interpretation

Interpretation of a spectrum or chromatogram requires a person with chemical expertise in the respective area. Automated references library searches cannot substitute the specialist but will allow a faster and less biased identification.

The algorithms and reference data applied should be targeted to the detection method and the microplastics as they actually are in the environment to be studied. For example, applying a database made by ATR-FTIR of virgin materials on data sets made by transmission-FTIR of environmental microplastics will yield less accuracy in the prediction than applying a database made by transmission-FTIR of such microplastics.

14.3 Interpretation of large spectra/chromatogram data sets

Interpretation of spectra or chromatograms requires that the personnel have chemical expertise in the respective area. However, the specialist cannot perform the analysis without automation of the interpretation process as data sets are too large (e.g., when applying µFTIR imaging microscopy). A number of algorithms and approaches for semi- or fully-automatic data analysis have been proposed to overcome this issue. These can be divided in algorithms for:

— Supervised machine learning, which creates a function which maps an input to an output based on examples of input-output pairs.

— Unsupervised machine learning, which does not rely on input-output pairs but looks for previously unseen patterns in the data without human supervision.

In Annex A, further guidance towards advanced data processing, considerations for the application, validation of databases and algorithms as well as related approaches are given.

15 Aspects of analytical quality assurance

15.1 Reference materials

Reference materials (RM) play a fundamental role for analytical measurements (e.g. for calibration, method validation, assessment of trueness and precision, interlaboratory comparison or comparison test). According to ISO Guide 30:2015, RM are materials that are "sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process. Two types of RM can be distinguished:

a) neat materials in which the target analyte (here: microplastics/microplastic particles) is the main component that is only associated with potential impurities and/or additives and may be dissolved or suspended in a pure solvent;

b) 'matrix RM' where the target analyte is a minor component and embedded in a matrix (e.g. soil, water, air, biota) in order to mimic real samples.

The level of complexity for microplastics analysis is far more pronounced than other contaminants due to numerous factors. These include the chemical composition of the particles (large variety of polymers are present in the environment, their degree of aging varying with time and locations and a large scale of additives, colorants or other modifiers), their size and shape, and the size distribution. The polymer types most frequently detected in the environment are PE, PP, PS, PET, PA and PVC, all of them being thermoplastics. Such polymers are rarely present in the environment in their original composition or morphology. Rather, with time, their surface is modified by various weathering conditions occurring in the environment (e.g. humidity, UV radiation, temperature, chemical and/or mechanical stress) or they can be degraded to smaller particles by such processes. Often, weathered microplastic particles are oxidized at their surface, and thus, can be more hydrophilic than pristine material. They frequently show irregular or fibrous shapes.

When selecting microplastic RM for the validation of analysis methods for environmental samples, special attention shall be paid to use materials with relevant composition and properties for the particular analysis method. This applies to the microplastic particles as well as, for matrix RM, to the selected matrix (water, soils, airborne dust, foodstuff, etc). Thus, considering the diversity of microplastic materials to cover and of the (research or regulatory) questions to answer, a very high number and variety of RM would be needed to satisfy the various needs. ISO Guide 33 provides general recommendations on the use of RM, including their selection.

Microplastics RM should be made available for as many different polymers relevant for environmental samples as possible. They should cover the whole size range of the microplastics or a specific size class within that range (see for example Table 1) and, if possible, include particles of different shape (including sphere-like particles, fibres, films, fragments). This huge demand makes a prioritisation of the most needed RM necessary. Depending on the (research or regulatory) question to answer and the analytical method applied, different quantities of RM are needed.

For individual polymer types (mainly PS), particles with a narrow size distribution are commercially available. However, such particles are designed for specific purposes (e.g. calibration of particle sizing instruments) that do not necessarily make them suitable as RM for microplastics analysis in environmental samples, because their properties do normally not correspond to the characteristics of microplastics found in environmental samples. In addition, such commercial products may contain additives (e.g. detergents) and therefore may require clean-up before use.

Production of microplastic particles for use in RM can generally be done in two ways, i.e. by de-novo synthesis or by size reduction of larger plastic particles/items (such as ultrasonic treatment and cryo-milling). Various production methods are described in the scientific literature, but most often a milling approach is applied. With cryo-milling, the resulting particles often cover a wide range of sizes. Additional sieving can therefore be needed to extract the required size class. ISO/TS 4807 specifies requirements for particle size reference materials.

Production of particle sizes significantly smaller than 10 µm by cryo-milling is even more difficult. On the one hand, only a small proportion of the grinded material reaches these small particle sizes, on the

other hand, losses of the small particles can occur during processing, as these particles are very easily carried into the air. For these smaller particles, using ultrasonic treatment may be more reasonable. Particles smaller than 10 µm can also be produced by a controlled synthesis from polymeric basic components, as is already common for PS particles in the nanometre range.

Matrix RM could be prepared from a clean matrix spiked with an analyte (or a group of analytes) followed by homogenisation or from a matrix already containing the analyte(s), sampled in a large quantity and homogenized or a combination of both. In case of matrix RM with microplastics, the used approach to date is spiking with pristine, well-defined, and well-characterized plastic particles.

Matrix RM must fulfil all requirements posed for a material to become a RM – they must be sufficiently homogeneous and stable with respect to one or more specified qualitative or/and quantitative property(ies) (see ISO Guide 35), e.g. the mass and/or the number of microplastic particles.

For all microplastics, neat RM and matrix RM, a chemical characterization of the composition, a homogeneity test as well as a stability test shall be carried out. The ISO standards which set the requirements for both of them (as well as the general rules for RM production) are ISO 17034 and ISO Guide 35. For certified RM (CRM), the result of this test shall be traceable to a recognized international reference (preferably SI based) and shall also include a measurement uncertainty. However, with the lack of standardized methods for microplastics, it is necessary to use a stepwise approach, letting both the reference materials and the analytical methods develop in parallel.

Until a sufficient variety of RM are available, laboratories could prepare their own in-house materials in the form of quality control materials (QCM) as outlined in ISO Guide 80. QCM can be prepared according to the needs of a laboratory. The requirements for QCM are less strict than for CRM (see ISO Guide 80). For example, stability for transport does not have to be assessed and a traceability statement is not required. However, these materials cannot be used to assess the trueness of an analytical method or for calibration. Their main field of application is for an instrument quality chart and/or method precision.

Preparing QCM, even though the requirements are less strict than for CRM, is also challenging. They should resemble, as much as possible the materials to be analysed. Often, such in-house materials are produced with particles of pristine plastic. Pristine plastic microparticles however have different properties than microplastics found in environmental samples. The latter, having been exposed to environmental conditions, are more hydrophilic, contrary to pristine ones. While preparing QCM suspensions/spiked matrices, their different behaviour should be taken into account, as it can lead to inhomogeneity, lack of stability and erroneous results. Depending on the type of polymer (density, polarity), the surface properties (oxidation, coating with agglomerated natural particles or biofilm), the size and the shape of the particles, microplastics are differently distributed in matrices.

15.2 Performance of interlaboratory comparison tests

An interlaboratory comparison can be carried out with different objectives. They are for example used for the validation of analytical methods, for testing the suitability of laboratories or for the characterization of samples (for example RM). This objective is a central element of external quality assurance for testing laboratories and as such is explicitly recommended in the standard ISO/IEC 17025. Also, ISO/IEC 17043 deals with this aspect. In the following the specific aspects regarding microplastics analysis is addressed.

Interlaboratory comparisons are basically performed by simultaneous measurements of several laboratories on identical (homogeneous) subsets of the sample or material using identical or different procedures (depending on the objective of the test). An appropriate number of repeat measurements and the determination of blank values shall be considered. The statistical evaluation of interlaboratory comparisons allows statements to be made about the accuracy (correctness and precision) of analytical methods, characteristic values of samples/RM and the measuring capacity of the participating laboratories. Interlaboratory comparisons for validation of analytical methods require in general a uniform homogeneous matrix RM.

Microplastics are not present in the environment as a pure powder, they are integrated into the environmental matrix (for example water, soil, sediment, biota) or matrix of a product (for example compost, sewage sludge). Interlaboratory comparisons for validation of analytical methods require

such a uniform homogeneous matrix RM. Depending on the question of the interlaboratory comparison, matrix RM shall be specifically selected, their microplastics contents or particle size distribution to be investigated shall be adjusted regarding the performance of the analytical method and the test scenario.

The addition of compatibility agents for microplastics suspensions or microplastics mixtures with natural particle quantities with comparable properties can lead to a better distribution in the medium, but such agents or particles can lead to additional processing steps or complications in detection.

The difficulty of producing homogeneous suspensions or mixtures means that matrix RM with very low microplastics contents or particle numbers can only be produced to a limited extent without additional agents or particles and according to the criteria of a RM (homogeneity of the microplastics contents with specified minimum sample quantity and stability of the microplastics distribution, i.e. no segregation after production). The conception of an interlaboratory comparison can therefore also be a compromise between the addressed problem, the production of homogeneous, realistic matrix RM and the methodical boundary conditions of the procedure to be evaluated.

For the validation of the detection methods within the framework of an interlaboratory comparison, ideally only pure microplastics particles are added to a matrix without significant contribution to the signal. It shall be taken into consideration that ultimately the transfer of the test sample into the analytical instrument (for example preparation on filters or in crucibles) already represents a separate preparation step which cannot be assigned to the pure methodological performance of the detection procedure.

The validation of the different sample preparation steps within the framework of an interlaboratory comparison shall be oriented to the assessment of the reduction of the environmental matrix (organic/inorganic fraction), as well as the integrity of the particle (i.e. particle size, particle mass). The selection of the matrix depends on the research question. Since the interlaboratory comparison for sample preparation shall be followed by a detection method, a defined number of particles / or a defined content of microplastics RM shall be added, which can be determined within the scope of the possibilities/performance of the various detection methods. It shall be considered here that the microplastics particle stability is determined by the type, the size and the aging condition. Therefore, it is particularly important to work with realistic particles.

The validation of the sampling in an interlaboratory test depends on the variety and the composition of the environmental medium. A defined number of particles or mass of microplastics RM shall be added to the different environmental media, which can be recorded within the scope of the possibilities of the different detection methods. It shall be considered here that in some cases very high microplastics particle numbers or microplastics contents shall be added, which can be critical in terms of costs.

Annex A
(informative)

Advanced Data Processing

A.1 Introduction to advanced data processing

There are two key issues which should be addressed when developing or applying semi-automatic or fully automatic analysis processes in the form of algorithms for the processing of this data.

— Manual repetitive tasks are a possible source of human bias. Ideally, the algorithms (or software) should require no human intervention regarding the data analysis process. While an expert audit of the analysis results may be necessary, the data should already be in a state, where the possibility of subjective interpretation is as low as possible. This also applies to parameter settings of the algorithm (or software) which should be kept constant across experiments in order to ensure comparability.

— It is often the case that an algorithm is designed to be applied for data originating from a certain measuring instrument. This then implies that when the same algorithm shall be applied for data originating from a different measuring instrument its applicability should be assessed to prevent a possible algorithmic bias.

Algorithms which are already in regular use for the task of analysing microplastics can be categorized into supervised and unsupervised learning approaches. Supervised learning is the machine learning task of creating a function which maps an input to an output based on examples of input-output pairs. With respect to the type of output, a regression task is referred to, if the output variable is continuous, and a classification task, if the output variable is categorical. On a mathematical level supervised learning can be further divided into model-based learning and instance-based learning. Instance-based learning directly applies the collection of input-output pairs to compute an output based on the input by using similarity measures (or distance metrics respectively). In model-based learning a statistical model is inferred from the collection of input-output pairs which is then applied for computing the output based on the input.

Unsupervised learning is a form of machine learning which does not rely on input-output pairs. It looks for previously unseen patterns in the data without any human supervision. However, the lack of human supervision does not imply absence of human bias or algorithmic bias.

It should be considered, that individual reference spectra which are bundled as reference libraries often have been measured using different measuring instruments and parameter settings. The measurement system (FTIR, Quantum Cascade Laser-QCL) geometry (for example ATR, transmission, reflection) as well as parameters such as number of scans as well as the detector type result in the generation of different data. Therefore, a spectral reference library where for example a PE spectrum measured with FTIR in reflection mode is replaced by a PE spectrum measured with QCL can behave differently when applied to the task of microplastics identification. Likewise, a library, which is based on QCL spectra, will perform differently when applied to data originating from FTIR measurements. From the viewpoint of machine learning changing the entries of a spectral reference library or adding additional spectra is the process of creating a new instance-based model, which may behave very differently than the original model. Without re-evaluating this new model, it should not be assumed that the statistical performance is the same or better. Further, if the spectral reference library has been enhanced by spectra originating from a different measuring instrument to enhance the applicability to other kinds of data, this does not make a proper re-evaluation dispensable.

NOTE 1 Typical examples for model-based machine learning approaches include multilinear regression, partial least squares, artificial neural networks, random forests and support vector machines.

NOTE 2 Typical examples for instance-based machine learning approaches include the k-nearest-neighbour algorithm and the commonly used spectral library searches, which are often based on the Pearson correlation coefficient.

NOTE 3 Typical examples for unsupervised learning approaches include principal component analysis and cluster analysis.

A.2 Methods for determining particle number and sizes (Spectroscopic procedures)

A.2.1 Chemical structure determination

Spectroscopic methods allow the determination of chemical structure by assessing the characteristic vibrational bands. This process can be automated by machine learning approaches, which are based on spectroscopic reference data. Table A.1 compares general aspects of both model-based and instance-based approaches for large data sets, which commonly arise in hyperspectral imaging applications.

Table A.1 — Comparison of machine learning approaches

Characteristics	Model-based	Instance-based
Mathematical basis	Statistical model	Similarity measure
Computational time (10^6 spectra)	minutes	hours
Time dependence on number of reference spectra	No	Yes
Experience required for creation	high	low

The objective of assigning an unknown spectrum to a known predefined class (or category) (for example a certain polymer type) is a classification task. If for example the degradation state or the determination of the concentration of additives in an already identified spectrum is the objective and the output variable is continuous, then this is a regression task. This distinction should be considered when selecting a machine learning approach, as suitability of different learning approaches varies with respect to classification and regression tasks.

NOTE If linear behaviour of the problem can be assumed (for example Lambert-Beer's law can be applied) then linear approaches such as partial least squares regression can be favourable over spectral reference libraries or other nonlinear approaches.

A.2.2 Spatial analysis of particle contours

Depending on the used technology spectroscopic instruments either create point-wise measurements, or a collective measurement in the form of a hyperspectral image by means of line array or focal plane array detectors. Due to time constraints point-wise measurement approaches often do not allow for a mapping of the whole filter surface. Nonetheless, point-wise measurements can be applied effectively if the particles are located before a spectroscopic measurement is applied. This can be done by capturing an image of the filter in visible light by means of a microscope or by measuring an intensity map at a certain wavelength. The particle contours can then be detected by means of binarization or a watershed algorithm. The point-wise measurements are then conducted at the centre of each particle. Hyperspectral imaging, on the other hand, captures the whole filter surface including all particles. While this comes with certain advantages the amount of data is considerably larger and therefore may require high-throughput approaches for data processing. Table A.2 aims at a basic comparison of the two approaches.

Table A.2 — Comparison of spatial analysis approaches

Characteristics	Point-wise	Hyperspectral imaging
Time dependence on number of particles	Yes	No
Applicable to agglomerated particles	No	Yes
Data set size	small	large

A.3 Methods for determining mass content (thermoanalytical and chemical methods)

A.3.1 Chemical structure determination

Thermoanalytical methods (TED-GC-MS, Py-GC-MS) allow the determination of chemical structure by assessing the characteristic decomposition products. The generated chromatograms are evaluated regarding the formation of characteristic mass fragments (*m/z*-values) at specific retention times. This process can be automated in principle by machine learning approaches, which are based on chromatographic reference data.

Chemical methods extract the structural microplastics information by chemical treatment reaction, which is not related to a data processing.

A.3.2 Quantification of mass content

Different quantification procedures (matrix dependent or independent response factor, external or internal calibration) can be chosen for the determination of mass content. The decision is made by the human evaluator.

The evaluation of data, for example peaks that arise at certain intervals, are integrated and multiplied with a response factor to arrive at a mass value. These response factors can be derived by means of linear regression or other kinds of statistical modelling, if cross-dependencies due to matrix effects require a more advanced approach.

The implementation of signal from an internal standard shall be considered.

This general approach is also valid for signals, which are obtained using chemical methods for microplastics identification.

A.4 General recommendations for evaluating databases and data processing approaches

There is a very rich set of mathematical approaches which can be applied for the purpose of microplastics detection. This applies to all mentioned spectroscopic, thermoanalytical and chemical techniques. While some algorithms are limited to very specific kinds of data there are others which are applicable for different kinds of data to a certain extent.

In order to test the applicability of a machine learning algorithm with respect to a certain kind of data, a test data set or a collection of test data sets has to be established. These data sets consist of instances (input) and their respective output variables, which may either be continuous or categorial depending on regression or classification tasks. A.4.1 and A.4.2 describe typical performance measures for evaluating or comparing algorithms. Note that these performance measures can only be compared if they originate from the same test data set.

NOTE 1 An error which can sometimes be found in scientific literature is that different microplastics related detection algorithms are compared based on published error rates. This is an invalid comparison as these performance measures were not computed from the same test data set.