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Textiles and textile products — Microplastics from textile sources —

Part 2: Qualitative and quantitative analysis of microplastics

Textiles et produits textiles — Microplastiques d'origines textiles —

Partie 2: Analyse qualitative et quantitative des microplastiques

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Foreword

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This document was prepared by Technical Committee ISO/TC 38, *Textiles*.

A list of all parts in the ISO 4484 series can be found on the ISO website.

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 significant evidence that the textile sector releases microplastics (MPs) into the environment.

These particles, when present in the environment, can affect the biota, and so, their number, shape and size can be relevant parameters for the assessment of their potential impact and, consequently, the development of a counting technique can be a helpful approach.

Moreover, many of the microparticles analysed are not of synthetic origin and therefore it is necessary to identify and distinguish them from microplastics (MPs).

This document is designed to provide the nature, numerical concentration, surface area and (estimated) mass of the microplastics produced or released by the textile sector and collected in a solid, aqueous or aeriform matrices.

Depending on the matrice, pre-treatment of the sample is necessary to concentrate the microplastics and eliminate inorganic and organic (for example biological) components that can interfere with their identification. This document involves a preliminary observation of the sample by an optical microscope (OM) and then identification of the microplastics (MPs) by molecular spectroscopy. This document provides the possibility of using two different techniques of molecular spectroscopy, Micro-FTIR and Micro-Raman to identify and count plastic particles down to submicron size.

This document is designed to allow the re-evaluation of microplastic counting data when toxicological and environmental impact indications become available.

This document describes the method of analysis for a single filter. However, errors in the qualitative and quantitative determination of microplastics that can result from the variability between different filters imply that replicates should be performed to establish precision.

This document provides useful information (e.g. dimensional classes, shape, composition, etc.) that can be taken into account for a possible eco-toxicological assessment of health and environmental impacts. It is well known that some microplastics (MPs) are lipophile and can be vehicles for toxic compounds (e.g. PCBs, PAHs, dioxins) or vehicles of pathogenic microorganisms adhered to their surface and can be assimilated (with their dose of toxicity) and permeate into organisms and cells.

The sources of microplastics are numerous. Their shapes and sizes are also variable. In the case of those released by textiles, the typical (but not the only) morphology is fibrous and their diameter and length can vary depending on the construction parameters of yarns and fabrics or cleaning conditions.

Textiles and textile products — Microplastics from textile sources —

Part 2: Qualitative and quantitative analysis of microplastics

1 Scope

This document establishes a qualitative-quantitative analytical evaluation (i.e. determination) of microplastics to be able to define their:

— particle number;

— morphology (morphological characteristics);

— dimensional distribution;

— the type, chemical origin or nature of polymers and their colour, if present.

This document is applicable to the determination of microplastics (from the textile sector) collected in various matrices (for example textile process wastewater, clothes washing water, textile process air emissions, textile process solid waste).

This document specifies expression of results in terms of estimated surface area and mass of microplastics (MPs) per unit sample. It enables the expression of the results of the quantification of microplastics (MPs) from various sources, including samples related to the production, processing, treatment and use of textiles (raw material, manufacturing process, sample like wastewater from washing clothes, air, and industrial process water).

This document applies to textile sector samples of matrices of different physical states (solid, liquid or aeriform), for example:

— solid samples from textile production processes;

— water samples from the textile production process and/or from the washing of clothing (e.g. garments or other textiles, ISO 4484-1 or ISO 4484-3 can be applied in order to prepare a liquid to be tested);

— air samples to test the air quality in the workplace of textile companies.

This document, being able to provide information such as size, shape, surface and mass (estimated), enables the transfer of useful information for ecotoxicological assessments to specialists.

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 1833-4, *Textiles — Quantitative chemical analysis — Part 4: Mixtures of certain protein fibres with certain other fibres (method using hypochlorite)*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

EN 481, *Workplace atmospheres — Size fraction definitions for measurement of airborne particles*

EN 13284-1, *Stationary source emission — Determination of low range mass concentration of dust- Part 1: Manual gravimetric method*

EN 13284-2, *Stationary source emissions — Determination of low range mass concentration of dust — Part 2: Quality assurance of automated measuring systems*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
microplastic
MP
material consisting of a solid polymer containing particles, to which additives or other substances may have been added, and where a weight fraction of ≥1% particles have:

a) all sizes 100 nm ≤ × ≤ 5 mm,

b) for fibres, a length of 300 nm ≤ × ≤ 15 mm and a length/diameter ratio >3

Note 1 to entry: Polymers that occur in nature that have not been chemically modified (other than by hydrolysis) are excluded, as are polymers that are (bio) degradable.

[SOURCE: ECHA, ANNEX XV Restriction Report - Microplastics, 22 August 2019, par 1.2.2.1, modified on lower size recommended dimensions, by Commission Recommendation C/2022/3689 of 10 June 2022 on the definition of nanomaterial (OJ C 229, 14.6.2022, p. 1), modified — "≥1% w/w" was changed to "a weight fraction of ≥1 %"; additional information has been given as a note to entry.]

3.2
significant sample volume
amount of filtered volume to be analysed considering the source of the sample and the values of: total suspended solid (TSS) and chemical oxygen demand (COD)

Note 1 to entry: See Table 1 and Table 2.

3.3
subsample
aliquot (fraction) of the primary sample diluted (as known) with water

3.4
washing solution
solution used to wash equipment to recover any MP which can be left on the equipment

3.5
image analysis
identification and classification of particles/fibres according to their morphology (shape) and size, providing additional sample information.

Note 1 to entry: The sample information are, for example, distribution percentage, number and size of microparticles and microparticles with fibre shape.

3.6
molecular micro-spectroscopy
analysis (FTIR or Raman) coupled to optical microscope (OM)

Note 1 to entry: The equipment is used to identify the polymer that composes the particle and to classify the particles recognized by image analysis, providing information such as distribution, percentage, number and size of MPs identified.

4 Principle

In order to be able to determine the MPs in a matrice, it is first necessary to transfer a significant portion of them to a suitable filter (6.3) to allow subsequent microscopic analysis with regards to quantity (molecular spectroscopy).

Different approaches shall be followed depending on the physical state of the starting matrice:

— powdery solid, or a mixture of solid materials;

— aqueous (liquid) suspension;

— aeriform.

In the case of solid powdery samples, the preliminary dispersion of a representative aliquot of the samples is carried out in a known volume of water (5.1), or in a dispersing solution consisting of a non-ionic surfactant (5.4.2) in filtered water (5.1). If the sample is solid (non-powdery) an appropriate disintegration treatment (e.g. ultrasonic treatment) shall be carried out.

For the analysis of liquid suspensions, the sample shall be filtered through a filter (6.3) of suitable material, pore size and shape (depending on the spectroscopic technique used). Analysis of the material on the filter (6.3) according to the following description:

a) identification of the composition of microparticles present by Micro-FTIR (6.1) /Micro-Raman (6.2) spectroscopy in order to identify any plastic microparticles and related measurement of their dimensions by image analysis;

b) counting of the number and identification of the size class of particles and fibres observed, and possible calculation of the total masses of MP present on the filter (6.3) in accordance with Formula (6).

Aeriform samples shall be considered in accordance with EN 13284-1, and for dust collection in the air (air emissions, air working environment) shall be considered in accordance with EN 13284-2.

All MP assessments, regardless of the different matrices analysed, shall be compared. The data of the produced waste, the discharged water, the air of the workplace or the emissions into the atmosphere can be analysed and compared to obtain a balance of the MPs of:

— a specific textile production process;

— textile products during their life cycle as garments;

— any other textile processes/semi-finished/finished products.

According to the analytical method used, the different sampling, preparation and purification procedures shall be considered. They shall be chosen according to the characteristics of the sample to be tested.

The analytical method shall be adequately applicable to all samples prepared in advance.

Preliminary analysis should be performed in accordance with the different sample preparation and purification procedures and the analytical techniques subsequently applied.

In particular, in the case of textile sector source samples with potential presence of salts and organic substances, investigative analyses shall first be carried out, for example in the case of liquid samples of aqueous matrices:

— determination of conductivity;

— determination of COD;

— determination of TSS, with membrane filtration technique;

— pre-screening with OM for estimating microscopic image quality vision.

5 Reagents

All reagents [analytical reagent grade (AR)] or their solutions and the demineralised water (5.1), shall be filtered through filters (6.3) with a pore size of at least 0,45 µm.

5.1 Demineralised water, Grade 3 quality as specified in ISO 3696.

5.2 Hydrogen peroxide, with a volume fraction of 15 % in demineralised water (5.1).

For the oxidation of organic matter prior to filtration, hydrogen peroxide AR may be used.

5.3 Sodium hypochlorite (NaClO), 1 M or 1 mol/l.

Freshly prepared sodium hypochlorite solution containing (35 ± 2) g/l active chlorine (±1) mol/l, in accordance with ISO 1833-4 is used for the dissolution of wool fibre during standards preparation.

5.4 Washing solutions

5.4.1 Sodium chloride, with a mass per volume of 1 %.

Dilute 10 g of pure NaCl AR in 1 000 ml with demineralised water (5.1)

The solution shall be prepared by diluting pure NaCl AR salt in demineralised water (5.1).

A sample of this solution shall be analysed to determine the MPs content of the salt to be taken into account in the results of the analyses.

5.4.2 Non-ionic surfactant, with a mass per volume of 1 %.

Dilute 10 g of pure non-ionic surfactant in 1 000 ml of demineralised water (5.1).

The solution shall be prepared by diluting the surfactant, preferably, not ionic, for example Triton X (whose composition and/or IR/Raman spectrum shall be known in order to subtract it during analysis), in demineralised water (5.1).

A sample of this solution shall also be analysed in order to determine the MP content to be taken into account in the results of the analyses.

5.5 Ethanol solution

Ethanol with a volume fraction of 95 % mixed 1:1 with demineralised water (5.1)

It is possible to use the ethanol solution previously filtered with a filter (6.3) made of mixed esters of cellulose or cellulose nitrate.

5.6 Acetic acid solution 0,1 mol/l, with a volume fraction of 60 %.

Remove salt or organic material by dosing a solution obtained by diluting 5,7 ml of acetic acid (a volume fraction of 60 %) in 1 000 ml of demineralised water (5.1).

If necessary, the molarity of the solution can be increased to make the removal of salt or organic material more efficient.

A sample of this solution shall also be analysed in order to determine the MP content so that it can be taken into account in the results of the analyses.

6 Apparatus

6.1 Micro-FTIR, for particles greater than 10 µm with the following requirements:

a) transmission/transflectance or reflectance;

b) spectral resolution: minimum 4 cm^{-1};

c) Spectra format: absorbance;

d) Depending on the system an aperture of 150 µm × 150 µm can be used;

e) Detector spectra range 4 000 cm^{-1} to 675 cm^{-1};

f) Collection time and scans depend on the system and used sources.

6.2 Micro-Raman, for particles greater then 0,2 µm to 0,5 µm with the following requirements:

a) lasers can have a wavelength of 457 nm, 532 nm, 633 nm, and 785 nm;

b) several objectives with different magnifications and numerical apertures;

c) spectroscopic systems;

d) Ultra-High-Throughput spectrometer (UHTS300) in the VIS range Grating: minimum 600 g/mm;

e) Ultra-High-Throughput spectrometer (UHTS400) in the NIR range Grating: minimum 300 g/mm.

6.2.1 CCD camera with one of the following specifications:

a) black illuminated camera for operation in VIS range;

b) low dark current CCD camera in NIR range;

c) dark field microscopy; particle identification and measurement software

6.3 Filters, according to the type of spectroscopy chosen and the size of the particles to be determined.

The pore size that can usually be used are: 0,45 µm, 0,8 µm, 1 µm, 5 µm.

All the filters shall have a suitable shape and fit the instrument of molecular micro-spectroscopy. Circular or square shapes are preferred.

6.3.1 Micro-FTIR Filters

6.3.1.1 In the case of Micro-FTIR (reflection and transmission) analysis, filters (6.3) with a pore size area of at least 5 µm shall be used.

The filters shall be one of the following.

a) Aluminium oxide filter, spectral range between 4 000 cm^{-1} and 1 250 cm^{-1}, in transmission mode (pore diameter 0,02 µm to 0,2 µm).

b) Silicon filter, spectral range between 4 000 cm^{-1} to 600 cm^{-1} in transmission or reflection mode. Pore diameter 5 µm, pitch 12 µm, thickness 500 µm.

c) Gold polycarbonate filter, spectral range 4 000 cm^{-1} to 400 cm^{-1} in reflection mode (1 µm, 25 mm)

d) Cellulose acetate nitrate filter, spectral range between 4 000 cm^{-1} to 400 cm^{-1} in reflection mode or micro-ATR.

6.3.1.2 In the case of attenuated total reflectance (ATR) analysis, the possible filters (6.3) that can be used have a pore size area of at least 5 µm, in detail:

a) cellulose acetate nitrate;

b) pore diameter 0,45 µm and/or 0,8 µm; filter diameter 47 mm and/or 25 mm;

c) PVDF (polyvinylidene fluoride);

d) pore diameter 5 µm filter diameter 47 mm.

6.3.2 Micro-Raman filters

In the case of Micro-Raman (6.2), the possible filters (6.3) that may be used are:

a) cellulose acetate nitrate, gold coated polycarbonate membrane, silicon filter;

b) 13 mm diameter, 25 mm diameter, 47 mm diameter, 10 mm side.

All materials used shall be washed with demineralised water (5.1) and subsequently with ethanol (5.5) beforehand to remove any residual MP, and immediately after washing shall be left to dry in the air covered with aluminium foil or watch glass.

6.3.3 Reading the filter

To reduce bend upon drying after the filtration process, mount them as flat as possible for measurement.

Silicon filters have two faces (one mirror face and the other darker). Use the filter with the mirror face facing upwards (face to be used for filtering), in this way the micropores facilitate the adhesion of the MPs and their maintenance, even when the filter is dry, during the following counting and identification steps with Micro-FTIR (6.1) or Micro-Raman (6.2).

Silicon filters show strong Raman peaks but no fluorescence. Silicon peaks may be ignored in the particle identification.

6.4 Light microscope, suitable for fibre identification, involves the use of projection microscopes and visual microscopic image analysers. Transmitted-light microscopes with direct graduated scale equipped with an optical lens are also applicable.

6.5 Filtration system, made of steel or glass (see Figure 1), with funnel of 100 ml, 500 ml, 1 000 ml, 2 000 ml complete with sintered septum, clamp and coded Erlenmeyer flask.

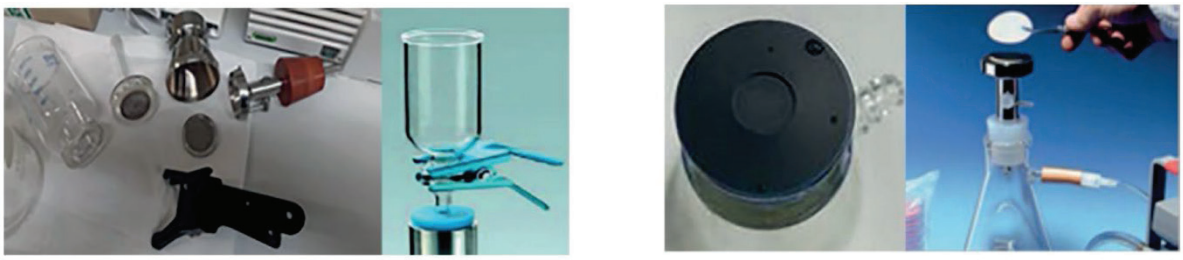

Figure 1 — Examples of filtration system, filter holder and filters

6.6 Flasks and bottles, made of glass, with glass cap.

6.7 Tweezers, made of steel.

6.8 Petri dishes, made of glass.

6.9 Microslides, made of glass.

6.10 Filter holder, (see Figure 1).

6.11 Mechanical stirrer.

6.12 Conductivity meter, accurate to a minimum of 5 microSiemens/cm (µS/cm).

6.13 Equipment for COD determination, rapid kits are also allowed.

6.14 Analytical balance, accurate to a minimum of 0,1 mg.

6.15 Ultrasonic bath, (see 7.3).

6.16 Vacuum filtration system.

6.17 Software, for automatic image analysis, morphological identification, dimensional classification, mapping.

7 Cleaning procedure

7.1 Cleaning of the materials and the test environment

Airborne fibre contamination is possible and using procedures to reduce it is recommended.

The following is a list of mandatory actions to be taken to reduce contamination and keep equipment clean:

a) All glassware shall be previously washed with demineralised water (5.1) filtered through filters (6.3) with a pore size of at least 0,45 µm (nitrate, acetate, mixed cellulose esters) and with washing solution, then rinsed before each use; then washed with ethanol solution (5.5) filtered through filters (6.3) with a pore size of 0,45 µm (nitrate, acetate, mixed cellulose esters) and with washing