EasyMP™: Diverse and environmentally relevant microplastic reference materials encompassing fragments and fibers

O. Hagelskjær[1,2,a*], F. Hagelskjær[3,b], H. Margenat[2,c], J.E. Sonke[4,d], G. Le Roux[2,e]

[1]Microplastic Solution, 31320 Castanet-Tolosan, France.

[2]Centre de Recherche sur la Biodiversité et l'Environnement (CRBE), Université de Toulouse, CNRS UMR 5300, IRD, Toulouse INP, Université Toulouse III – Paul Sabatier (UT3), 31326 Toulouse, France

[3]SDU Robotics, Mærsk Mc-Kinney Møller Institute, University of Southern Denmark, 5230 Odense M, Denmark.

[4]Géosciences Environnement Toulouse, CNRS UMR5563 - IRD UR 234, Université Toulouse III - Paul Sabatier (UT3), 14 Avenue Edouard Belin, 31400 Toulouse, France.

[*]Corresponding author.

[a]oskar@microplasticsolution.com | https://orcid.org/0000-0001-8790-1992.

[b]frhag@mmmi.sdu.dk | https://orcid.org/0000-0002-0000-0348.

[c]henar.margenat-hervas@univ-tlse3.fr | https://orcid.org/0000-0002-3650-963X.

[d]jeroen.sonke@get.omp.eu | https://orcid.org/0000-0001-7146-3035.

[e]gael.le-roux@cnrs.fr | https://orcid.org/0000-0002-1579-0178.

This manuscript has been submitted for publication in Wiley: Nano Select (eISSN 2688-4011.) and is currently undergoing peer-review. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer reviewed Publication DOI' link on the right-hand side of this webpage. Please feel free to contact the corresponding author.

EasyMP™: Diverse and environmentally relevant microplastic reference materials encompassing fragments and fibers

O. Hagelskjær[1,2,a*], F. Hagelskjær[3,b], H. Margenat[2,c], J.E. Sonke[4,d], G. Le Roux[2,e]

[1]Microplastic Solution, 31320 Castanet-Tolosan, France.

[2]Centre de Recherche sur la Biodiversité et l'Environnement (CRBE), Université de Toulouse, CNRS UMR 5300, IRD, Toulouse INP, Université Toulouse III – Paul Sabatier (UT3), 31326 Toulouse, France

[3]SDU Robotics, Mærsk Mc-Kinney Møller Institute, University of Southern Denmark, 5230 Odense M, Denmark.

[4]Géosciences Environnement Toulouse, CNRS UMR5563 - IRD UR 234, Université Toulouse III - Paul Sabatier (UT3), 14 Avenue Edouard Belin, 31400 Toulouse, France.

[*]Corresponding author.

[a]oskar@microplasticsolution.com | https://orcid.org/0000-0001-8790-1992.

[b]frhag@mmmi.sdu.dk | https://orcid.org/0000-0002-0000-0348.

[c]henar.margenat-hervas@univ-tlse3.fr | https://orcid.org/0000-0002-3650-963X.

[d]jeroen.sonke@get.omp.eu | https://orcid.org/0000-0001-7146-3035.

[e]gael.le-roux@cnrs.fr | https://orcid.org/0000-0002-1579-0178.

Abstract: The field of microplastic (MP) research has expanded significantly since the terminology's inception in 2004. Despite the exponential increase in studies, the availability of environmentally relevant MP reference materials (RMs) remains limited, and no certified MP RMs exist. This study addresses the need for diverse RMs by presenting data on MP RMs of fragments (10-100 µm) and fibers (50-1000 µm), suspended in 95 vol.% ethanol solution at various concentrations. Five samples each of fragments and fibers, derived from four subsamples, were prepared and evaluated for repeatability, with relative standard deviation (RSD) determined at 10 and 9%, respectively. Novel size group-specific RSD evaluation was also conducted. The study confirms the homogeneity and distribution consistency of these RMs, demonstrating RSDs below 20% for fragments and within acceptable ranges for fibers. These RMs, branded as 'EasyMP™,' will be available for purchase, providing essential tools for accurate MP analysis and experiments, contributing to reproducible MP studies.

Keywords: true-to-nature, standards, plastic filament, microfibers 50 µm, micro fragments 10-100 µm, self-validation study

1. Introduction

The field of microplastic (MP) research has experienced considerable growth since the term was first introduced in 2004.[1] Between 2010 to 2021, the annual number of studies published on MPs increased exponentially by 40%, reaching thousands of publications per year.[2] Despite this, access to environmentally relevant MP reference materials (RMs) has been limited.[3–5] To this date, no certified MP RMs exist and the lack of commercially available RMs hinders the harmonization of analytical methods and the generation of comparable data.[6]

While most MP recovery experiments, toxicological studies and other method validation approaches have relied on surrogate microbeads,[7] the majority of environmental MPs are either fragments or fibers.[8–12] Depending on environmental setting (e.g., indoor vs. outdoor, anthropogenic vs. remote) and target MP size range, the proportion of fibers to fragments in environmental samples may diverge significantly.[13,14] Volume is also a concern; for example, dosing by weighing of dry MP powders is unfeasible in the size range below 100 µm,[15] and may contribute to laboratory experiment dosages orders of magnitude above environmental concentrations.[16]

For comprehensive validation of methods used in MP analysis and experiments, it is therefore important that RMs of both fragment and fiber-type morphology are made available in environmentally relevant concentrations. RMs of different polymer types should also be available due to variation in density, polarity and chemical resistance.[17] For ease of use and rapid detection without applying chemical identification methods, pigmented RMs may under specific conditions be advantageous.[18]

The current study presents experimental data to self-validate MP RMs of fragments in the 10-100 µm range and fibers from a length of 50 µm, suspended in ethanol solution in varying concentrations. For fragments and fibers respectively, five samples, each based on four subsamples, were prepared and evaluated for repeatability. Relative standard deviation (RSD) was determined for the total number of particles as well as mass. In addition, as a novel approach, RSD within size groups was also evaluated. The presented variants of RMs, along with many other polymer types, will be made available for purchase under the retail name 'EasyMP™' on www.microplastic.store and www.microplasticsolution.com.

2. Methods and materials

2.1. Fragment and fiber production

Fragments were manufactured through the process of cryomilling of larger plastic items, using liquid nitrogen, in concordance with best current practices.[19] By vacuum filtration, the incident fragments were sieved in succession through 500, 100 and 5 µm meshes. Note that although the cut-off value of evaluated

fragments is 10 µm, the utilization of a 5 µm mesh allows for the inclusion of elongated fragments with aspect ratios below 2, in the finest size fraction. Fiber filaments are cut in varying lengths from millimeters down to 50 µm. The resulting particles, either fragments or fibers, were transferred into 100-mL glass vials with built-in pipette screw caps and suspended in prefiltered (0.45 µm) 95 vol.% laboratory grade ethanol. Solutions were either diluted or concentrated to meet the required specifications.

2.2. Data acquisition and presentment

A total of ten 100 mL EasyMP™ samples were prepared; five fragments samples [10-100 µm] and five fiber samples [50-1000 µm] of different polymer composition. For each individual sample, four subsamples between 0.5 to 1 mL (depending on particle concentration in the relevant sample) were pipetted directly from the sample vial using the built-in pipette screw cap, onto four individual 5 µm, 25 mm membranes. Particle count and morphological features, including size, were registered using static image analysis dark-field microscopy (ColSpec® MK2, LightForm® inc.), capable of visually eliminating the filter background while avoiding particle glare. Multiple micrographs were stitched together to form high-resolution mosaics on the order of 2 µm/pixel (Fig. 1).

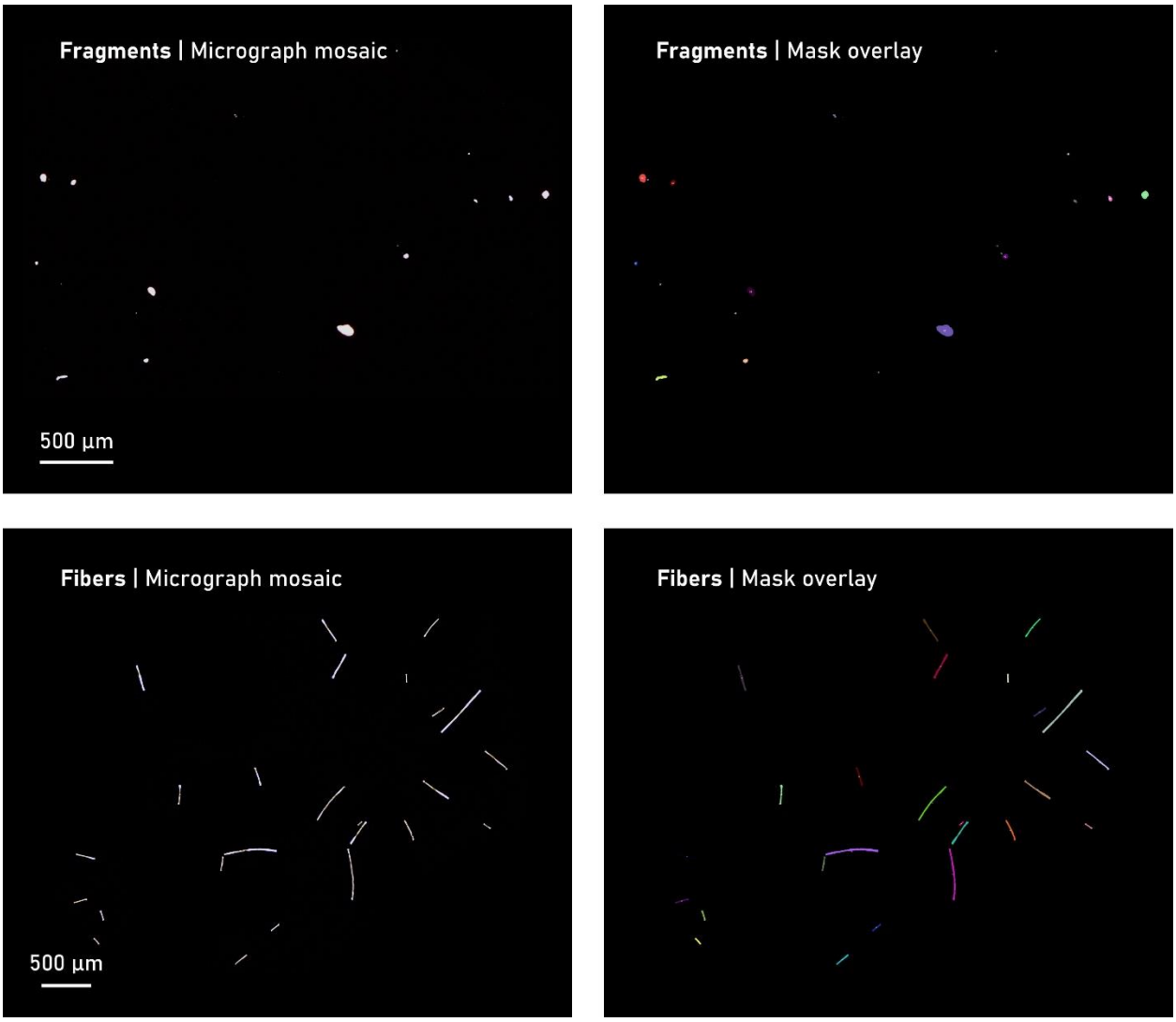

Figure 1 - Excerpt of micrographs captured under darkfield illumination of fragments (top) and fibers (bottom). 'Micrograph mosaic' refers to composites of the original images, while 'Mask overlay' uses distinct colors to highlight the defined individual particles.

The technique effectively eliminates the filter background, allowing for automated identification and extraction of morphological data of particles as small as 10 µm on their largest dimension, including both fragments and fibers. The method ensures a sufficiently homogeneous distribution, resulting in good particle spread with minimal overlap, if particle area coverage (A%) does not surpass 5%.[18] Additionally, each mosaic was manually reviewed and adjusted to address any visual particle partitioning or agglomeration.

For each particle, the data extracted included: area, Feret diameter, minimum Feret diameter, circularity and aspect ratio. The volume of a fragment ($V_{fragment}$) is calculated as a function of area (A) and height (h), where h is assumed to be half of the minor axis ($\text{Feret}_{\text{minimum}}$) of the fragment in question (Eq. 1).

$$V_{fragment} = A \cdot h = A \cdot (0.5 \cdot \text{Feret}_{minimum}) \quad (1)$$

The volume of a fiber (V_{fiber}) is approximated based on a cylindrical model (Eq. 2).

$$V_{fiber} = \pi \cdot r^2 \cdot L \quad (2)$$

Where r is mean radius (n = 10) and L is the length of the fiber filament in question. For both fragments and fibers, mass is calculated by multiplying volume by the specific gravity of the relevant polymer type. Volumetric MP concentration, particle size distribution and mass distribution are based on the mean of the four investigated subsamples, and is unique to each individual sample. Each certificate of analysis (COA) and the relevant safety data sheet (SDS) is available in dedicated Google drives, only accessible by weblink or by scanning the QR-code on the sample label (Fig. 2).

Figure 2 - 100 mL EasyMP™ '*Fiber sample #1*', containing cotton (cellulose) fibers from 50-1000 µm in length. The label provides basic information such as particle concentration in both counts (n/mL) and mass (µg/mL). The QR code grants access to a dedicated cloud folder containing the COA and SDS.

3. Results and discussion

3.1. Repeatability

To determine repeatability of EasyMP™ RMs, RSD was evaluated on the basis of five individual samples of fragments and fibers, respectively. MP concentrations and standard deviation (SD) was calculated on the basis of four subsamples. Samples were labeled using the prefix "*fragment*" or "*fiber*" followed by "*sample #1*", "*sample #2*", and so forth.

To provide an example of the data provided with EasyMP™, the partial COA's of '*fragment sample #1*' and '*fiber sample #1*' are presented in Table 1. The samples demonstrate 7 and 9% RSD in number of MPs and 10 and 8% RSD in terms of calculated mass for '*fragment sample #1*' and '*fiber sample #1*', respectively. The table includes mean MP particle count, mass- and particle size distribution, as well SD within the respective size groups. The data is also illustrated in Fig. 3.

EasyMP™ | Certificate of analysis (COA) | Fragment sample #1

	SS_1		SS_2		SS_3		SS_4		Number of particles (n/mL)			Mass of particles (µg/mL)			Particle size distribution (PSD)		
Diameter (µm)	n/mL	µg/mL	n/mL	µg/mL	n/mL	µg/mL	n/mL	µg/mL	Mean	SD (n)	RSD (%)	Mean	SD (µg)	RSD (%)	Mean diameter (µm)	PSD (%)	Cum. PSD (%)
10-20	1524	0.83	1340	0.75	1984	1.03	1508	0.87	1589	239	15	0.87	0.10	12	10	N/A	1
20-30	892	2.09	978	2.44	1022	2.23	724	1.79	904	114	13	2.14	0.24	11	15	37.9	37.9
30-40	632	3.97	564	3.38	578	3.31	522	3.35	574	39	7	3.50	0.27	8	25	21.6	59.5
40-50	318	4.18	314	4.01	310	3.57	342	4.42	321	12	4	4.04	0.31	8	35	13.7	73.1
50-60	220	4.96	208	4.76	186	3.88	194	4.39	202	13	6	4.50	0.41	9	45	7.7	80.8
60-70	188	6.56	140	4.70	120	3.59	156	5.38	151	25	16	5.06	1.08	21	55	4.8	85.6
70-80	132	6.60	102	5.06	100	5.44	100	5.14	109	14	13	5.56	0.62	11	65	3.6	89.2
80-90	68	5.54	82	6.08	78	5.46	92	7.86	80	9	11	6.23	0.97	16	75	2.6	91.8
90-100	60	5.83	54	6.38	52	5.00	68	8.36	59	6	11	6.39	1.24	19	85	1.9	93.7
100-110	44	5.96	52	7.70	36	6.19	34	5.22	42	7	17	6.27	0.90	14	95	1.4	95.1
110-120	46	8.70	38	6.98	28	4.94	30	7.12	36	7	20	6.94	1.34	19	105	1.0	96.1
120-130	32	7.18	38	9.54	28	6.05	26	6.57	31	5	15	7.34	1.33	18	115	0.8	96.9
130-140	32	9.31	26	6.08	18	5.34	22	7.36	25	5	21	7.02	1.50	21	125	0.7	97.7
140-150	20	8.35	16	3.40	6	1.22	22	7.30	16	6	39	5.07	2.89	57	135	0.6	98.3
150-160	22	10.82	6	3.05	16	8.09	12	6.34	14	6	42	7.08	2.82	40	145	0.4	98.7
160-170	10	4.62	14	5.12	10	4.29	12	5.86	12	2	14	4.97	0.59	12	155	0.3	99.0
170-180	4	2.92	12	4.77	10	7.02	8	2.76	9	3	35	4.36	1.72	39	165	0.3	99.3
180-190	8	4.85	4	2.96	2	1.89	6	3.43	5	2	45	3.28	1.06	32	175	0.2	99.5
190-200	4	1.28	4	2.75	4	1.81	4	3.42	4	0	0	2.32	0.83	36	185	0.1	99.6
> 200	16	10.85	8	4.07	14	13.58	16	18.70	14	3	24	11.80	5.28	45	195	0.1	99.7
															> 200	0.3	100.0
Total	4272	115.42	4000	93.98	4602	93.92	3898	115.64	4193	273	7	104.74	10.8	10			

EasyMP™ | Certificate of analysis (COA) | Fiber sample #1

	SS_1		SS_2		SS_3		SS_4		Number of particles (n/mL)			Mass of particles (µg/mL)			Particle size distribution (PSD)		
Length (µm)	n/mL	µg/mL	n/mL	µg/mL	n/mL	µg/mL	n/mL	µg/mL	Mean	SD (n)	RSD (%)	Mean	SD (µg)	RSD (%)	Mean length (µm)	PSD (%)	Cum. PSD (%)
50-100	42	0.75	20	0.39	40	0.74	26	0.51	32	9	29	0.60	0.15	26	75	8.3	8.3
100-150	66	1.91	54	1.57	62	1.76	48	1.37	58	7	12	1.66	0.20	12	125	15.0	23.3
150-200	72	2.94	72	2.87	54	2.16	60	2.36	65	8	12	2.58	0.33	13	175	16.8	40.2
200-250	78	4.00	76	3.80	78	3.98	58	2.98	73	8	12	3.69	0.42	11	225	18.9	59.1
250-300	60	3.72	58	3.62	52	3.25	50	3.15	55	4	7	3.44	0.24	7	275	14.3	73.4
300-350	46	3.37	28	2.07	28	2.04	18	1.33	30	10	34	2.20	0.74	33	325	7.8	81.2
350-400	26	2.23	18	1.52	26	2.22	14	1.19	21	5	25	1.79	0.45	25	375	5.5	86.7
400-450	10	0.97	18	1.73	12	1.17	20	1.91	15	4	27	1.45	0.38	27	425	3.9	90.6
450-500	2	0.21	16	1.75	16	1.72	18	1.95	13	6	49	1.41	0.70	50	475	3.4	94.0
500-550	16	1.90	8	0.97	2	0.24	12	1.48	10	5	54	1.15	0.62	54	525	2.5	96.5
550-600	14	1.84	2	0.26	6	0.78	12	1.58	9	5	56	1.11	0.63	56	575	2.2	98.7
600-650	0	0.00	4	0.57	0	0.00	4	0.56	2	2	100	0.28	0.28	100	625	0.5	99.2
650-700	2	0.31	2	0.32	2	0.31	0	0.00	2	1	58	0.23	0.14	58	675	0.4	99.6
700-750	0	0.00	0	0.00	0	0.00	2	0.32	1	1	173	0.08	0.14	173	725	0.1	99.7
750-800	0	0.00	2	0.35	0	0.00	0	0.00	1	1	173	0.09	0.15	173	775	0.1	99.9
800-850	0	0.00	0	0.00	0	0.00	0	0.00	0	0	N/A	0.00	0.00	N/A	825	0.0	99.9
850-900	2	0.40	0	0.00	0	0.00	0	0.00	1	1	173	0.10	0.17	173	875	0.1	100.0
Total	436	24.57	378	21.81	378	20.38	342	20.69	384	34	9	21.86	1.65	8			

Table 1 - Standard COA datasheets of EasyMP™ samples '*Fragment sample #1*' (top) and *'Fiber sample #1*' (bottom), demonstrating average particle count, mass- and particle size distribution within size groups on the order of 10 and 50 µm, respectively. The MP concentration in the two samples exhibited SD values of 7% and 9%, respectively. The data is also illustrated in Fig. 4. 'SS' is an abbreviation for 'subsample'.

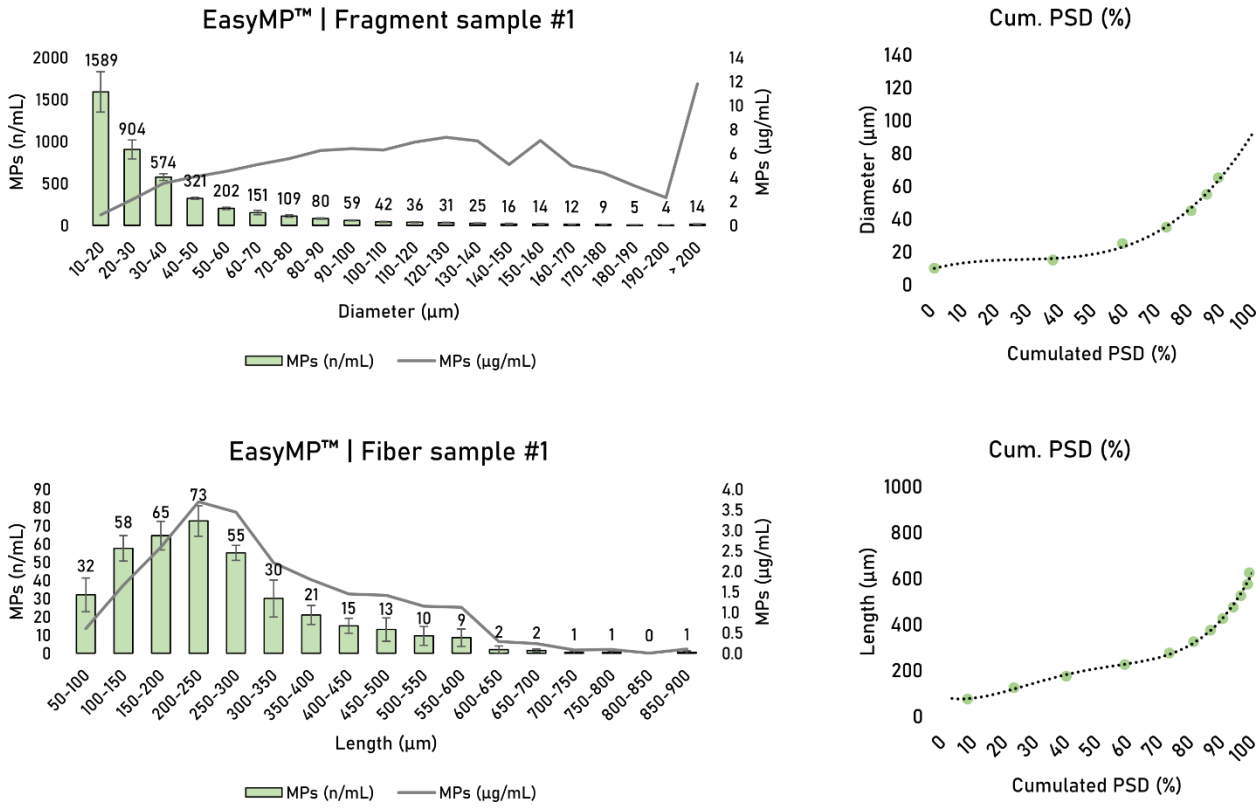

Figure 3 - Graphical illustration of particle concentration in samples '*fragment sample #1*' (top) and '*fiber sample #1*' (bottom). Histogram bars illustrate the number of particles (left y-axis) within each size group, while the line graph represents the mass of particles within that group (right y-axis). Diagrams on the right-hand side illustrate the cumulated particle size distribution (PSD) of the relevant sample.

Among five individual 100 mL EasyMP™ fragment samples [10-100 µm] with concentrations ranging from hundreds to thousands of MPs (n/mL) of different polymer types, including polypropylene (PP), polyurethane (PU), and polyamide 6,6 (PA6,6), mean RSD of the number and mass of fragments, irrespective of size, was estimated at 9 and 13%, respectively. Within size groups on the order of 10 µm, from 10 to 100 µm, mean RSD ranged from 11 to 19% (Table 2). Within size groups, mean RSD increased with decreasing PSD; likely due to decreasing numbers of particles resulting in reduced statistical significance.

Among five individual 100 mL EasyMP™ fiber samples [50-1000 µm] with concentrations in the range of hundreds of fibers (n/mL) of different polymer types, including polyethylene terephthalate (PET), PA6,6, polyacrylonitrile (PAN), and cotton (cellulose), mean RSD of the number and mass of fibers, irrespective of length, was estimated at 9 and 10%, respectively. Within size groups on the order of 50 µm, from 50 to

500 µm, mean RSD ranged from 17 to 51%. Similarly to fragments, mean RSD increased with decreasing PSD.

EasyMP™ | RSD overview | Fragments

					RSD of count of fragments within size groups in µm (%)								
Sample	Polymer type	Fragments (n/mL)	**RSD count (%)**	**RSD mass (%)**	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
#1	PA6,6	4,139	**7**	**10**	15	13	7	4	6	16	13	11	11
#2	PP	12,793	**9**	**4**	9	11	10	15	12	7	10	13	12
#3	PU	340	**9**	**8**	12	17	7	20	23	13	0	26	38
#4	PA6,6	2,893	**15**	**13**	13	14	26	22	22	21	25	18	18
#5	PP	4,469	**9**	**31**	7	8	12	16	20	16	23	22	15
Mean	N/A	N/A	**10**	**13**	11	13	12	15	17	15	14	18	19

EasyMP™ | RSD overview | Fibers

					RSD of count of fibers within size groups in µm (%)								
Sample	Polymer type	Fibers (n/mL)	**RSD count (%)**	**RSD mass (%)**	50-100	100-150	150-200	200-250	250-300	300-350	350-400	400-450	450-500
#1	Cellulose	384	**9**	**8**	29	12	12	12	7	34	25	27	49
#2	PET	490	**8**	**9**	17	12	35	18	4	18	31	12	28
#3	PAN	200	**7**	**7**	41	38	19	36	24	39	20	47	26
#4	PET	142	**9**	**12**	104	12	14	12	35	31	41	36	34
#5	PA6,6	161	**12**	**13**	65	55	27	20	15	59	29	54	66
Mean	N/A	N/A	**9**	**10**	51	26	21	20	17	36	29	35	41

Table 2 - SD of particle count and mass irrespective of size (**in bold**) of fragment and fiber samples where each SD value is calculated from four subsamples. Mean RSD of particle count irrespective of particle size, was estimated at 10 and 9% for fragments and fibers, respectively. For fragments within size groups on the order of 10 µm, mean RSD remained below 20%, while mean RSD of fibers within size groups on the order of 50 µm, was mostly above 20%.

For both fragments and fibers, mean RSD increased with decreasing PSD (Fig. 4). Mean RSD irrespective of particle size was estimated at 10 and 9% for fragments and fibers, respectively; well below the 20% threshold for acceptable error (not encompassing RSD within size ranges) suggested by the EUROqCHARM project.[3] In addition, mean RSD of fragments within size groups on the order of 10 µm, from 10 to 100 µm, was consistently below 20%. For fibers, mean RSD was only above 30% within size groups that constituted less than 10% of the PSD. However, there are currently no established guidelines for RSD within size groups, as this approach has not been previously implemented for RMs.[18]

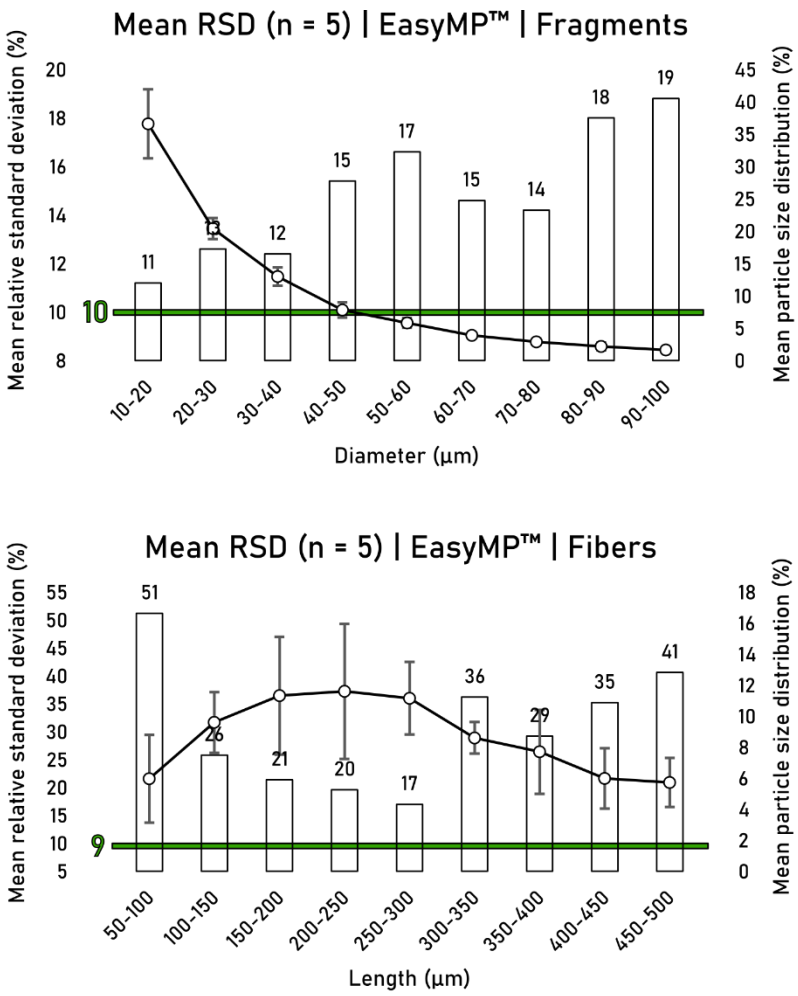

Figure 4 - Mean RSD of particle counts within size groups for both fragments (top) and fibers (bottom), based on RSD values of five individual samples. The green line represents mean RSD irrespective of particle size while the histogram bars represents mean RSD within specific size groups. The line graph represents mean PSD within specific size groups. For both fragments and fibers, an increase in RSD with decreasing PSD was observed.

3.2. Quality control

To prevent external contamination during sample preparation, rigorous quality control measures were adhered to. Sample preparation took place in a laminar flow cabinet situated in a dedicated MP laboratory with restricted access. Surfaces were thoroughly cleaned with a prefiltered (0.45 µm) 50 vol.% ethanol/water solution. All utilized glassware was kiln sterilized at 500°C for 1 h after which they were flushed with prefiltered (0.45 µm) 95 vol.% ethanol, prior to use.

Procedural blanks were prepared by sonicating the filter membranes, which served as substrates for reference materials during micrograph acquisition, in prefiltered (0.45 µm) 95 vol.% ethanol for 10 seconds

prior to sample-spiking, as per the protocol. Microscopic examination of 1 mL of the prefiltered (0.45 µm) 95 vol.% ethanol solution revealed none or negligible numbers of particles on three individual filter membranes. Additionally, all micrograph mosaics were manually inspected and corrected for visual artifacts to prevent visual partitioning or agglomeration of particles, which could lead to under- or overestimation of particle counts.

4. Perspectives

All EasyMP™ RMs are accompanied by COA containing raw data as well as the micrographs from which the data was extracted. EasyMP™ RMs will be manufactured upon request according to the customer's specifications. Customization is an important parameter because experiments that simulate specific environmental conditions may require different concentrations, particle sizes and polymer compositions.[20] For recovery experiments, using colored fragments that maintain their hue at the microscopic scale provides a cost-effective and efficient means of identification, eliminating the need for vibrational microspectroscopy techniques or other chemical identification methods (Fig. 5).

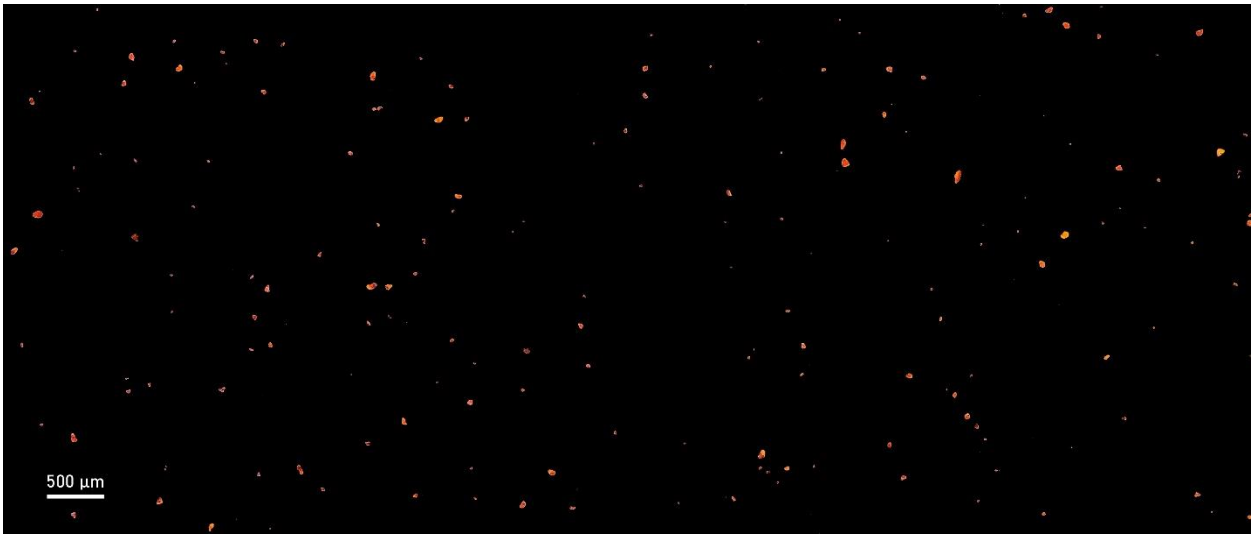

Figure 5 - Photomicrograph mosaic captured under darkfield illumination, of red polyethylene (PE) fragments in the 10-100 µm size range. The application of colored MPs may eliminate the need for chemical identification during recovery experiments.

RMs will be made available for purchase before the end of 2025 under the retail name 'EasyMP™' on www.microplastic.store and www.microplasticsolution.com, with the aim of making 'true-to-nature' RMs globally available at a reasonable cost. For in vivo and -vitro studies, MPs suspended in ultraviolet (UV)-C sterilized grade A water, will also be available for fragments in the 10-100 µm size range. The ten RM samples evaluated in this study were donated to academic and industrial partners and were initially manufactured to meet their required specifications *i.e.* morphology, polymer type and concentration. The

current study presents only self-validated results. For improved reliability, the next step will include validation through an interlaboratory comparison (ILC) study.

5. Conclusions

EasyMP™ microplastic (MP) reference materials (RMs) provide access to both fragments and fibers in known quantities at environmentally relevant concentrations. Based on five samples each and irrespective of particle size, mean relative standard deviation (RSD) of particle counts, was estimated at 10 and 9% for fragments and fibers, respectively; well below the 20% threshold for acceptable error for MP RMs suggested by the EUROqCHARM project.

As a novel approach, RSD within size ranges was also evaluated. For fragments on the order of 10 µm between 10-100 µm, mean RSD remained consistently below 20%, increasing with decreasing particle size distribution (PSD). For fibers on the order of 50 µm from 50-500 µm, mean RSD was mostly above 20%. However, mean RSD was only above 30% within size groups that constituted less than 10% of the PSD. However, there are currently no established guidelines for RSD within size groups, as this approach has not been previously implemented for RMs.

RMs will be manufactured within distinct size ranges but concentrations are determined according to the customer's specifications (quotes for custom size ranges can be issued). This approach facilitates a broader selection of polymer types and color options, including natural-, semisynthetic- and synthetic polymers. Colored MPs that maintain their hue at the microscopic level facilitates visual identification and may eliminate the need for chemical identification.

EasyMP™ RMs including fragments measuring from 10 µm on their longest axis and fibers from a length of 50 µm, will be made commercially available on a global scale before the end of 2025 on www.microplastic.store and www.microplasticsolution.com.

References

1. Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, et al. Lost at Sea: Where Is All the Plastic? Science 2004;304(5672):838–838.

2. Kiran BR, Kopperi H, Venkata Mohan S. Micro/nano-plastics occurrence, identification, risk analysis and mitigation: challenges and perspectives. Reviews in Environmental Science and Bio/Technology 2022;21(1):169–203.

3. Martínez-Francés E, van Bavel B, Hurley R, Nizzetto L, Pakhomova S, Buenaventura NT, et al. Innovative reference materials for method validation in microplastic analysis including interlaboratory comparison exercises. Analytical and Bioanalytical Chemistry 2023;415(15):2907–19.

4. Seghers J, Stefaniak EA, La Spina R, Cella C, Mehn D, Gilliland D, et al. Preparation of a reference material for microplastics in water—evaluation of homogeneity. Analytical and Bioanalytical Chemistry 2022;414(1):385–97.

5. von der Esch E, Lanzinger M, Kohles AJ, Schwaferts C, Weisser J, Hofmann T, et al. Simple Generation of Suspensible Secondary Microplastic Reference Particles via Ultrasound Treatment. Frontiers in Chemistry [Internet] 2020;8. Available from: https://www.frontiersin.org/journals/chemistry/articles/10.3389/fchem.2020.00169

6. Mitrano DM, Diamond ML, Kim JH, Tam KC, Yang M, Wang Z. Balancing New Approaches and Harmonized Techniques in Nano- and Microplastics Research. ACS Sustainable Chem Eng 2023;11(24):8702–5.

7. Way C, Hudson MD, Williams ID, Langley GJ. Evidence of underestimation in microplastic research: A meta-analysis of recovery rate studies. Science of The Total Environment 2022;805:150227.

8. Allen D, Allen S, Le Roux G, Simonneau A, Galop D, Phoenix VR. Temporal Archive of Atmospheric Microplastic Deposition Presented in Ombrotrophic Peat. Environ Sci Technol Lett 2021;8(11):954–60.

9. Boettcher H, Kukulka T, Cohen JH. Methods for controlled preparation and dosing of microplastic fragments in bioassays. Scientific Reports 2023;13(1):5195.

10. Dris R, Gasperi J, Rocher V, Mohamed S, Tassin B. Microplastic contamination in an urban area: A case study in Greater Paris. Environmental Chemistry 2015;12.

11. Negrete Velasco A de J, Rard L, Blois W, Lebrun D, Lebrun F, Pothe F, et al. Microplastic and Fibre Contamination in a Remote Mountain Lake in Switzerland. Water [Internet] 2020;12(9). Available from: https://www.mdpi.com/2073-4441/12/9/2410

12. Stefánsson H, Peternell M, Konrad-Schmolke M, Hannesdóttir H, Ásbjörnsson EJ, Sturkell E. Microplastics in Glaciers: First Results from the Vatnajökull Ice Cap. Sustainability [Internet] 2021;13(8). Available from: https://www.mdpi.com/2071-1050/13/8/4183

13. Hagelskjær O, Le Roux G, Liu R, Dubreuil B, Behra P, Sonke JE. The recovery of aerosol-sized microplastics in highly refractory vegetal matrices for identification by automated Raman microspectroscopy. Chemosphere 2023;328:138487.

14. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Scientific Reports 2019;9(1):8670.

15. Fuller S, Gautam A. A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. Environ Sci Technol 2016;50(11):5774–80.

16. Mills CL, Savanagouder J, de Almeida Monteiro Melo Ferraz M, Noonan MJ. The need for environmentally realistic studies on the health effects of terrestrial microplastics. Microplastics and Nanoplastics 2023;3(1):11.

17. Primpke S, Christiansen SH, Cowger W, De Frond H, Deshpande A, Fischer M, et al. Critical Assessment of Analytical Methods for the Harmonized and Cost-Efficient Analysis of Microplastics. Appl Spectrosc 2020;74(9):1012–47.

18. Hagelskjær O, Crézé A, Le Roux G, Sonke JE. Investigating the correlation between morphological features of microplastics (5–500 µm) and their analytical recovery. Microplastics and Nanoplastics 2023;3(1):22.

19. McColley CJ, Nason JA, Harper BJ, Harper SL. An assessment of methods used for the generation and characterization of cryomilled polystyrene micro- and nanoplastic particles. Microplastics and Nanoplastics 2023;3(1):20.

20. Dehaut A, Himber C, Colin M, Duflos G. Think positive: Proposal of a simple method to create reference materials in the frame of microplastics research. MethodsX 2023;10:102030.

6. Declarations

6.1. Acknowledgements

We thank Jeremy M. Lerner and LightForm®, inc. for their ongoing technical support.

6.2. Author's contribution

O.H. conceptualized and administered the project, led the laboratorial work, produced and interpreted data and led manuscript writing with help from H.M and F.H. J.E.S and G.L.R. secured the funding, supervised the project and provided critical revision of the manuscript.

6.3. Funding

This work is funded by an 80Prime CNRS grant «4DµPlast» (G.L.R, J.E.S.). This publication was supported by ANR-20-CE34-0014 ATMO-PLASTIC (G.L.R, J.E.S.) and the Plasticopyr project within the Interreg V-A Spain-France-Andorra program (G.L.R) as well as observatoire Homme-Milieu Pyrénées Haut Vicdessos - LABEX DRIIHM ANR-11-LABX0010 (G.L.R).

6.4. Competing interests

The authors declare no conflict of interest.

6.5. Availability of data and materials

All data will be made available upon request.

6.6. Abbreviations

MP (microplastic), RM (reference material), SD (standard deviation), RSD (relative standard deviation), PSD (particle size distribution), COA (certificate of analysis), SDS (safety data sheet), PE (polyethylene), PP (polypropylene), PET (polyethylene terephthalate), PAN (polyacrylonitrile), PA6,6 (polyamide 6,6).

6.7. Ethics approval

Not applicable.

6.8. Consent for publication

Not applicable.

7. Figure and table captions

Fig. 1: Excerpt of micrographs captured under darkfield illumination of fragments (top) and fibers (bottom). 'Micrograph mosaic' refers to composites of the original images, while 'Mask overlay' uses distinct colors to highlight the defined individual particles.

Fig. 2: 100 mL EasyMP™ '*Fiber sample #1*', containing cotton (cellulose) fibers from 50-1000 µm in length. The label provides basic information such as particle concentration in both counts (n/mL) and mass (µg/mL). The QR code grants access to a dedicated cloud FOLDER containing the COA and SDS.

Fig. 3: Graphical illustration of particle concentration in samples *'fragment sample #1'* (top) and *'fiber sample #1'* (bottom). Histogram bars illustrate the number of particles (left y-axis) within each size group, while the line graph represents the mass of particles within that group (right y-axis). Diagrams on the right-hand side illustrate the cumulated particle size distribution (PSD) of the relevant sample.

Fig. 4: Mean RSD of particle counts within size groups for both fragments (top) and fibers (bottom), based on RSD values of five individual samples. The green line represents mean RSD irrespective of particle size while the histogram bars represents mean RSD within specific size groups. The line graph represents mean PSD within specific size groups. For both fragments and fibers, an increase in RSD with decreasing PSD was observed.

Fig. 5: Photomicrograph mosaic captured under darkfield illumination, of red polyethylene (PE) fragments in the 10-100 µm size range. The application of colored MPs may eliminate the need for chemical identification during recovery experiments.

Table 1: Standard COA datasheets of EasyMP™ samples '*Fragment sample #1*' (top) and '*Fiber sample #1*' (bottom), demonstrating average particle count, mass- and particle size distribution within size groups on the order of 10 and 50 µm, respectively. The MP concentration in the two samples exhibited SD values of 7% and 9%, respectively. The data is also illustrated in Fig. 4. 'SS' is an abbreviation for 'subsample'.

Table 2: SD of particle count and mass irrespective of size (**in bold**) of fragment and fiber samples where each SD value is calculated from four subsamples. Mean RSD of particle count irrespective of particle size,

was estimated at 10 and 9% for fragments and fibers, respectively. For fragments within size groups on the order of 10 µm, mean RSD remained below 20%, while mean RSD of fibers within size groups on the order of 50 µm, was mostly above 20%.

8. Highlights

- EasyMP™ reference materials (RMs) include microplastic fragments and fibers from 10 and 50 µm, respectively.
- Relative standard deviation (RSD) [n = 5] of particles irrespective of size was determined at 10 and 9% for fibers and fragments, respectively.
- RSD within size groups was also evaluated and was for fragments consistently below 20% but was higher for fibers.
- EasyMP™ RMs will be made commercially available before the end of 2025 on www.microplastic.store and www.microplasticsolution.com