Environmental Toxicology and Chemistry—Volume 41, Number 4—pp. 880–887, 2022
Received: 26 June 2020 | Revised: 27 July 2020 | Accepted: 28 March 2021

Environmental Chemistry

A Comparison of Different Approaches for Characterizing Microplastics in Selected Personal Care Products

Kofi O. Renner,[a] Helen A. Foster,[b] Edwin J. Routledge,[a] and Mark D. Scrimshaw[b,*]

[a]Department of Life Sciences, College of Health, Medicine and Life Sciences, Brunel University London, Uxbridge, United Kingdom
[b]Department of Biological and Environmental Sciences, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom

Abstract: Any uncertainty in determining numbers of microplastics in the environment may be a barrier to assessing their impact and may stem from various aspects of methodologies used to quantify them. We undertook a comparison of approaches to quantify and characterize microplastics in 4 personal care products. The aim was not only to determine how many particles were present but to assess any differences due to the methods used. Counting of extracted microplastics was undertaken using particle size analysis, light microscopy, and imaging flow cytometry. Micro–Fourier transform infrared spectroscopy (μ-FTIR) was used to characterize the particles in each product. The mean size distribution of microplastics differed depending on the method employed, and it was apparent that imaging flow cytometry was affected by high background noise that may require staining of plastics to overcome. The application of μ-FTIR confirmed polyethylene as the microplastic in each product. Methodological challenges encountered in the study and the literature have highlighted the need for standardization of methods for determining microplastics. *Environ Toxicol Chem* 2022;41:880–887. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Microplastics; Contaminants of emerging concern; Personal care products

INTRODUCTION

The increasing use of plastics together with inappropriate disposal has resulted in a global dispersal of plastics evidenced by widespread litter, although the ultimate sink for plastics is frequently the oceans (Briassoulis et al. 2019; Erni-Cassola et al. 2019). The impacts of large plastic debris (macroplastics) to the environment are widely known (Ryan 2018; Beaumont et al. 2019). There is increasing concern about the emergence of microplastics and their impact on the environment (Anderson et al. 2017; Rezania et al. 2018; Kazour et al. 2019). Microplastics are intentionally used in some personal care products (PCPs), which consequently find their way directly into aqueous environments via wastewater-treatment plants (Alimi et al. 2018; Kazour et al. 2019; Wolff et al. 2019); or they occur as secondary microplastics produced through physical, chemical, or biological degradation of large plastic debris (Germanov et al. 2018; Lehtiniemi et al. 2018; Wolff et al. 2019). Their ubiquity, rate of dispersion, and likelihood of ingestion by organisms increase the risk to global ecosystems (Sharma and Chatterjee 2017; de Souza Machado et al. 2018; Windsor et al. 2019). Data on the occurrence of microplastics have been generated following the generic approach of sample collection, separation of microplastics from the matrix, filtration, counting, and identification of particles (Li et al. 2018; Nguyen et al. 2019). It has been highlighted that a lack of standardization in methodology and reporting of microplastics is a challenge for risk assessment (Renner 2018; Henry et al. 2019). In particular, it has been reported that the lack of internationally accepted standard reference materials highlights the challenges in microplastic analysis and harmonization of results globally (Toussaint et al. 2019).

To illustrate, characterization of microplastics in facial scrubs, undertaken by a number of independent groups, has yielded size distributions of 4.1 to 1240 and 3 to 178 μm (Hintersteiner and Himmelsbach 2015; Praveena et al. 2018). These data were all obtained by microscopy, with no cross-referencing to other methods of assessing particle size. Particle size measurements of facial scrubs using laser diffraction reported a size distribution of 2 to 2500 μm, suggesting that current laser diffraction technology can measure a wider particle size range than conventional light microscopy (Napper et al. 2015). Furthermore, none of these studies reported the use of international reference standard materials to validate their data.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
* Address correspondence to mark.scrimshaw@brunel.ac.uk
Published online 5 April 2021 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5057

© 2021 The Authors

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Approaches such as pyrolysis gas chromatography coupled with mass spectrometry have been used for the identification of the polymeric composition of microplastics (Ceccarini et al. 2018; Käppler et al. 2018). Spectroscopic techniques, including Raman and Fourier transform infrared (FTIR), have also been used for the identification of microplastics in a range of matrices and have gained acceptance because of the increase in the accuracy of the techniques (Imhof et al. 2016; Cabernard et al. 2018; Silva et al. 2018). Micro-FTIR (μ-FTIR) has the benefit of increased spatial resolution, allowing for the identification of infrared bands to identify the type of plastic of which smaller particles are composed (Silva et al. 2015; Corami et al. 2020).

Given the increasing body of evidence for widespread microplastic contamination of water, sediment, and animal tissues and negative health impacts under controlled laboratory conditions, the precision with which scientists can detect and quantify microplastics in environmental samples will become ever more critical to our understanding of the risks they pose (Silva et al. 2018). Without greater technical competency in this area, uncertainty about the main sources and categories of microplastics that impact the environment and the concentrations and size ranges of microplastics with highest relevance for ecotoxicological studies will continue. As a consequence of this uncertainty, current ecotoxicology studies are criticized for the very high concentrations of microplastics used, which may not reflect real-world scenarios (Lenz et al. 2016; Burton 2017).

Some of the current quality control issues related to the analysis of samples for microplastics, expected to be routine for those accustomed to working with chemicals, are highlighted in a review by Wong and colleagues (2020). Factors considered were the use of reference standards, calculation of recoveries of microplastics, and evaluation of blank samples. Out of a total of 31 papers in the review which had counted microplastics in freshwater and freshwater organisms, soil, and terrestrial organisms, none reported using standard reference materials in the methodology section. Recovery studies were only undertaken by those reporting the presence of microplastics in soil. Those that undertook recovery studies were all done by authors working with soil (Table 1). Overall, just 12% of papers reported doing any work to determine the recovery of microplastics through their extraction, separation, and counting procedures. Table 1 also highlights that not all studies routinely undertake evaluation for contamination, with 25% of the papers evaluated either omitting or not reporting the use of procedural blank samples in their approaches.

Without such safeguards, we hypothesized that large differences in reported measures of microplastics in the literature were likely to be a consequence of differences in methodological approaches. In the absence of analytical standards for microplastics, the aim of the present study is to report data on the numbers of particles counted using different techniques with 4 PCPs as discrete samples and a uniform matrix for comparisons. This approach enabled us to highlight differences in quantification that are directly attributable to different methodologies and confirms the need for international standards in microplastics studies. Furthermore, the present study reports the sensitivity of the different analytical techniques in the analysis of ultrapure laboratory-grade water (blank samples) and its implications for microplastics studies.

EXPERIMENTAL METHODS

Two types of PCPs were chosen for the present study, toothpastes and facial scrubs. According to the labeling, all products contained polyethylene. The toothpastes selected were Colgate "Max White One Luminous" (TP1) and "Advanced White Go Pure" (TP2). The 2 facial scrubs were the Palmolive brands "Clean and Clear Morning Energy Skin Energising Daily" (FS1) and "Blackhead Clearing Oil Free Daily" (FS2). Following the separation of microplastics from the PCPs, their size distribution and number were determined using a multitechnique approach. Size measurements were determined using laser diffraction, light microscopy, and imaging flow cytometry; and the number of microplastics per 100 g of product was determined. Micro-FTIR was used to determine the polymer type, and the approach was as outlined in Figure 1.

Following sample preparation, particle size distribution was determined by laser diffraction (CILAS 1180; Quantachrome), then by counting and measurement with microscopy with triplicate samples. Particle size analysis by laser diffraction was based on the equivalent spherical diameter measurement (Slotwinski et al. 2014). Imaging flow cytometry (Amnis ImageStream Mark II; Merck Millipore) was used with duplicate samples because triplicates were not available for all samples taken through previous stages of analysis. This technique also generated a size distribution and number of particles. However, this required prefiltering samples (70 μm) to protect the instrument.

TABLE 1: Analysis of the use of recovery studies and blank samples in papers in the review by Wong et al. (2020)[a]

Tables in Wong et al. (2020)	No.[b]	Undertook recovery studies	Used blank samples
Table 1. Global studies of microplastic pollution in freshwater environment	17	None	58.8%
Table 2. Occurrence of microplastics in freshwater organisms	7	None	100%
Table 3. Summary of abundance of microplastics in various type of soil	6	66.7%	66.7%
Table 4. Summary of the presence of microplastics in terrestrial fauna	3	None	66.7%

[a]Four tables in the review listed a total of 31 ("No." column) relevant papers, which reported on the occurrence of microplastics in freshwater, freshwater organisms, soil, and terrestrial fauna.
[b]Some papers had data included in more than one table. Table 4 in Wong et al. (2020) included some data on macroplastics, which are excluded from this analysis.

 © 2021 The Authors

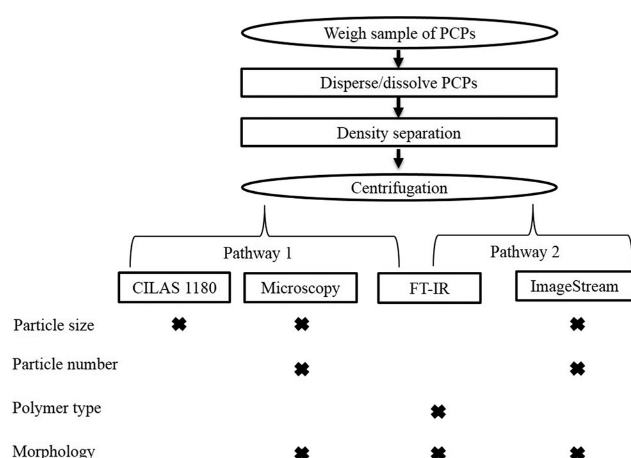

FIGURE 1: Schematic diagram of the approach used to characterize microplastics in personal care products showing separation and centrifugation, followed by particle size analysis, microscopy and micro–Fourier transform infrared. Samples were reextracted for analysis by imaging flow cytometry. PCPs = personal care products; FT-IR = Fourier transform infrared.

Separation of microplastics from the samples

The plastic particles used in the present study were not reference materials because the aim was not to validate methods but to assess if different ways of counting the same particles gave the same results. For all samples, 0.5 g wet weight of each product was accurately weighed on an analytical balance and subsequently dispersed in 1 mL of water at 50 to 60 °C. To achieve density separation, 50 mL of sodium chloride solution (140 g/L) at 50 to 60 °C was then added to the dispersion in a glass beaker. The resulting solution was mixed for 7 min with a glass rod and left to settle for 5 min. Polyethylene particles (now floating on the surface) were collected by decanting 15 mL into a clean glass beaker. The density separation process was repeated by adding another 50 mL of salt solution to the residual approximately 35 mL to ensure complete extraction of the microplastics. Another 15 mL was decanted from the second solution. This density separation resulted in an approximate volume of 30 mL sodium chloride solution with the suspended microplastics on the surface. The volume of this solution was then reduced by pipetting 15 mL of sodium chloride solution from the bottom of the beaker. At this stage the extract was cloudy because of the presence of other matrix components. To facilitate removal of plastics from the remaining matrix components, a centrifugation step was introduced.

The residual 150-mL volume was transferred to a 50-mL high-density polypropylene centrifuge tube, after which 30 mL of the hot sodium chloride solution and 5 mL of sodium pyrophosphate solution (10%, room temperature) were added. Sodium pyrophosphate was used as a dispersant to prevent the microplastics from agglomerating because it has been reported to inhibit bond formation (Sehly et al. 2015; Silva et al. 2015). Centrifugation was carried out at 1700 *g*, after which the top layer of the solution (~10 mL) containing microplastics was decanted into a clean 50-mL beaker. For all density separation steps the temperature of the water (50–60 °C) and the dispersal agent helped to minimize the loss of particles. Blank samples with no PCP added were also extracted through the whole process.

Characterization of microplastic particles

Particle size analysis by laser diffraction. Following centrifugation, particle size analysis was undertaken using the CILAS 1180, with an operational range of 0.04 to 2500 µm. This involved running background scans to ensure a consistent baseline particle count was reached between sample runs. The extracted microplastics were transferred from the 50-mL beaker into the CILAS 1180 tank with high purity, 18MΩ, water (MilliQ; Millipore). This was followed by 30 mL of 10% sodium pyrophosphate solution and 50 mL of 10% methanol (to reduce surface tension), after which measurements for size distribution were conducted. After analysis, the sample was discharged from the particle size analyzer and collected during the washing cycle. Recovery of microplastics from the instrument was confirmed by checking background readings.

Characterization by light microscopy. Samples collected from the CILAS 1180 were transferred onto a 1.2-µm glass microfiber (GF/C) filter (Whatman) under vacuum and then washed off with 30 mL of ultrapure water at 50 to 60 °C into a 50-mL beaker. Pipetting from the bottom, the liquid below the floating polyethylene particles was then reduced until 10 mL remained. The suspension was then agitated, and a 1-mL aliquot was transferred to a Sedgewick Rafter cell, etched with a 50 × 20 × 1 mm grid. The temperature of water used facilitated the transfer of microplastics to the Sedgewick Rafter cell. Size and particle count measurements were determined at ×200 magnification with an Olympus BX 51 calibrated eyepiece binocular microscope with QCapture Pro 5.1 imaging software. The longest of the first 100 particles counted in 6 randomly selected transects were measured and recorded for each slide. This was based on a statistical assessment of the technique (Renner 2018). To determine particle size distribution, the data from the 100 particles from each replicate were pooled ($n = 300$ particles from each sample were measured). Statistical analysis was conducted using R to undertake an analysis of variance (2-sample assuming unequal variance) with a post hoc *t* test (R Development Core Team 2019).

Imaging flow cytometry. For the second pathway, 1 mL of the hot (50–60 °C) agitated sample was prefiltered (70 µm, to protect the instrument flow path from larger particles) and transferred to a 1.5-mL microcentrifuge tube. The tube was placed onto the Amnis ImageStream with data acquired using the Inspire software. For each sample, image capture was conducted at ×20 magnification, with a 120 × 256 µm field of view using brightfield and the 488 nm laser set at 100 mW. To ensure that only particles in the sample were captured, the calibration speed beads (1-µm plastic spheres that are purposefully included in the carrier buffer to assist with instrument

© 2021 The Authors

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

focusing) were turned off. Image acquisition was defined using the brightfield channel with data collection parameters of either 10 000 particles in focus or a 10-min maximum acquisition time, whichever occurred first. Filter-sterilized water was run on the system between samples to prevent cross-contamination. It was noted that in all samples (except for FS2) larger microplastic particles appeared to settle quickly in the microcentrifuge tube prior to loading of the sample. Although the sample was then run immediately through the ImageStream (and particles were visible on the monitor), there was a short delay in actual data acquisition by the instrument. During this delay, larger particles were visible on the real-time image display but were not recorded, and following commencement of data acquisition, there was an apparent decline in both the size and number of particles with time, consistent with uptake of a nonhomogenous sample.

The IDEAS, Ver 6.2.65.0, software (IDEAS 2020) was used to analyze the microplastics data. The initial step gated for single particles, which were defined via brightfield area versus aspect ratio (minor axis divided by major axis). Subsequently, particles that were in focus were analyzed using the gradient root mean square function. Using the gated single event for the in-focus population, a histogram of aspect ratio versus normalized frequency was plotted to identify elongated and circular particles. Finally, the diameter feature, which provides the diameter of the circle that has the same area of the object, was used to determine the size distribution for all particles.

Polymer identification. Ten fragments of microplastic from each sample were imaged using the μ-FTIR equipped with a focal plane array detector. Using the midinfrared range of between 700 and 4000 cm^{-1}, the spectral results were based on reflectance in imaging mode with 2 co-added scans per pixel, an aperture size of 25 μm^2, and a spectral resolution of 16 cm^{-1}. This method of spectra acquisition provides information about the identification of a polymer within minutes (Tagg et al. 2015; Corami et al. 2020). Spectra of polyethylene standards were collected and used for reference. A random selection of 10 particles from each sample was taken for FTIR identification.

RESULTS AND DISCUSSION

Particle size distribution by laser diffraction

Laser diffraction analysis indicated that all 4 products contained particles with sizes from the low micron range up to hundreds of microns (Figure 2), with no particles observed in blanks. The cutoff at the larger size range was steep in all cases and occurred between 300 and 900 μm. At the lower end, distributions were more extended, and this was more pronounced in the toothpastes. Although the numbers of small particles were low and do not show on the graphs, the raw data demonstrated their occurrence in the PCPs at 0.2 to 0.5 μm. Sample TP1 exhibited the narrowest particle size distribution in the major peak (200–400 μm) and FS2, the broadest (250–900 μm). All samples had a minor peak at approximately

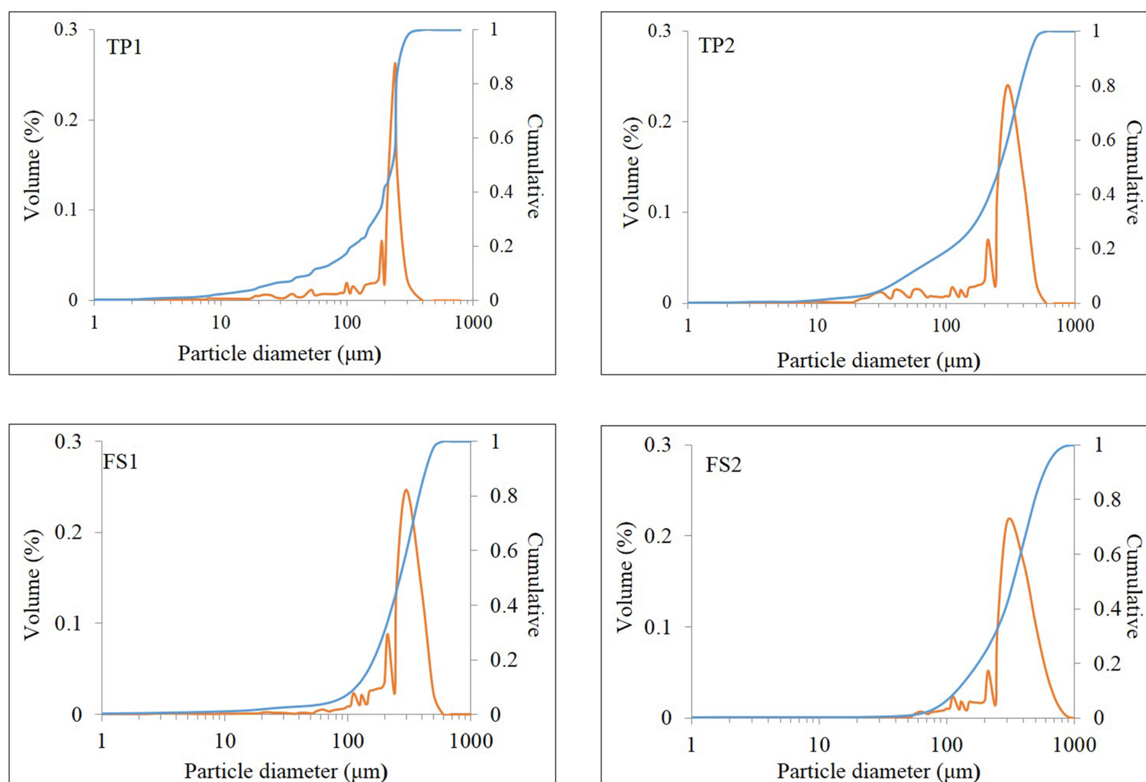

FIGURE 2: The size distribution and cumulative frequency graphs for microplastics recovered from the personal care products using the particle size analyzer. Most particles were in the 200 to 900 μm size range, with differences apparent between samples. There was a tail to the smaller size range in all products. TP1 peaked at 250 μm, TP2 and FS1 at 300 μm, and FS2 at 400 μm, with more of a tail toward the larger particles observed. TP = toothpaste; FS = facial scrub.

 © 2021 The Authors

200 μm. Another study has also shown size distributions from 10 to 3000 μm for particles in PCPs, with one sample extending to approximately 2500 μm (Godoy et al. 2019). The particle size distribution indicated that all samples exhibited high peaks between 200 and 300 μm (TP1), 300 μm (TP2), 200 to 400 μm (FS1), and 300 μm (FS2). These peaks were fairly consistent with those reported in a study on the characterization of microplastics in cosmetics (Napper et al. 2015; Godoy et al. 2019).

Particle size distribution and number by microscopy

Microplastics in all PCPs analyzed were recorded in the size range of 5 to 483 μm (Table 2). In contrast, another study using microscopy has reported size ranges of 3 to 178 μm and another, 60 to 800 μm of microplastics (Chang 2015; Praveena et al. 2018). There was a statistically significant difference between the size of microplastics in products ($p < 0.001$). A post hoc Tukey's multiple comparison of means at the 95% family-wise confidence level showed that the sizes of particles in TP1–TP2 and FS1–FS2 pairings was not significantly different but that all other pairings showed significant differences in size. No particles were observed in the blanks.

The light microscopy technique allowed for the direct imaging of the microplastics in all samples, and a 2-D measurement of visible microplastics at ×200 magnification was conducted. However, visual characterization resulted in a narrower size distribution with a larger size at the lower end (5 μm cutoff) of the distribution compared to particle size analysis. Although microscopy did not identify the smaller particles, it did result in lower mean particle sizes in the PCPs compared to laser diffraction with the CILAS data (Table 3). It has been reported that microscopy tends to result in smaller particle size value averages compared to laser diffraction techniques, as a consequence of the different physical properties of the particles being measured and the magnification of the microscope used (De Cleyn et al. 2019).

As well as conducting size analysis, microscopy was used to count the number of microplastic particles present in the original PCPs, expressed as number per 100 g. The number of microplastics in the 4 PCPs varied from 10.4×10^6 particles per 100 g in a tube of toothpaste to 24.9×10^6 per 100 g in a facial wash. In comparison, using the same technique, another paper has reported between 1.4×10^5 to 2.8×10^6 particles per 100 mL (Napper et al. 2015).

Particle size distribution and number using imaging flow cytometry

The analysis with the diameter feature showed that the particle size ranged from 1.1 to 33.7 μm, with FS2 and FS1 having the smallest and largest particles, respectively (Table 4). At the lower end, this compares well with laser diffraction (0.2–0.5 μm); it is, however, important to note that the sample was prefiltered through a 70-μm filter, which excluded the higher range. However, it was apparent that imaging flow cytometry, with its ability to count and visualize each particle, revealed many smaller particles than the other techniques; and there is some evidence that these were present in very large numbers in one sample.

TABLE 2: Sizes and numbers of the microplastics in personal care products as determined by microscopy

Product	Minimum (μm)	Maximum (μm)	No. per 100 g
TP1	5	483	12.6×10^6
TP2	5	403	10.3×10^6
FS1	40	407	10.6×10^6
FS2	20	423	24.9×10^6

TP = toothpaste; FS = facial scrub.

The blank samples (filtered laboratory-grade water) run through the imaging flow cytometer exhibited higher particle counts (in the range 10^6–10^9) than the microplastics present in all products, except for FS2. The number of particles per 100 g of product reported in Table 4 were blank-corrected by subtracting blank values from the value for the sample. Only FS2 exhibited particle numbers well above background count, with 3.7×10^9 particles per 100 g. The detection of particles in ultrapure laboratory-grade water possibly reflects the high sensitivity of the imaging flow cytometry technique when compared to other analytical techniques used in the present study. The other techniques did not detect contaminants in blank samples. Although their presence and source are not easily explained in ultrapure water, this finding illustrates the importance of reporting the results of blank samples, a notable absence in all microplastics studies. The evidence from FS2 of a high number of small particles in the low micron range is supported by observations reported in facial scrubs of 300 billion particles per gram in the submicron size range (Hernandez et al. 2017). It is apparent that analysis of PCPs using imaging flow cytometry showed 150 times more microplastics than other techniques in one sample. Although the number of microplastics was not determined in all samples, the technique offers a possible means of analyzing microplastics in the smaller micron range, which, because of their environmental implications, may be important (Li et al. 2018; Ma et al. 2019).

Flow cytometry has been investigated for the analysis of material scraped from plastic surfaces in laboratory studies by Kaile et al. (2020). The approach involved the use of staining and detection of fluorescent particles to help differentiate the microplastics. The study also stated that "it is impossible to separate unstained polyethylene particles from the background noise in the blank sample (milli-Q water with 10% dimethyl

TABLE 3: Comparison of mean particle size between laser diffraction and microscopy

Product	Laser diffraction (μm)	Microscopy (μm)
TP1	351	72
TP2	310	97
FS1	276	143
FS2	279	146

TP = toothpaste; FS = facial scrub.

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4: Size and number of particles detected using imaging flow cytometry based on the diameter features[a]

Product	Minimum (μm)	Maximum (μm)	Mean (μm)	No. per 100 g
TP1	1.5	19.8	7.5	ND
TP2	1.5	20.7	7.7	ND
FS1	2	33.7	8.2	ND
FS2	1.1	31.1	6.55	3.8×10^9

[a]Numbers per 100 g have been blank-adjusted.
TP = toothpaste; FS = facial scrub; ND = not determined (blank was greater than sample values).

sulfoxide)," possibly explaining the findings in the study. It was found that staining with Nile red and using the fluorescence of the particles made analysis of 4 out of 9 plastics studied possible but that challenges remained in relation to staining and aggregation of the dye. In a very different type of application, imaging flow cytometry has been used to study the uptake of microplastics by phagocytic cells, where it was able to differentiate cells that had taken up fluorescent polystyrene microplastic beads (Park et al. 2020).

On entering the environment, larger microplastics particles are likely to be ingested by organisms, resulting in blockages along the digestive tract, injury, and mortality (Taylor et al. 2016). By contrast, the smaller microplastics may adsorb toxic chemicals from the environment and, because of their large surface area to volume ratio may become a significant route of exposure to environmental chemicals (Taylor et al. 2016). In addition, the sorption and transfer of toxic chemicals by ingestion of prey and smaller nanoparticles may be potential sources of harm to living organisms (Koelmans et al. 2016; Burton 2017).

Identification of the polymers using μ-FTIR

Samples of particles from the PCPs were confirmed as polyethylene, based on the regions of absorbance indicative of the stretching of C-H (3000–2770 cm^{-1}) and bending of C-H (1500–1450 cm^{-1}) bonds present in particles. The reliability, nondestructive nature, and ability to cross reference samples with established libraries of functional groups for different polymers make it a useful tool for microplastics studies (Pinto da Costa et al. 2019; Zarfl 2019).

Future research directions for quantifying microplastics

The issue of a lack of standardization of sampling and detection methods has been highlighted in a "critical review of current understanding and identification of future research needs" in relation to microplastics in the environment (Akdogan and Guven 2019). Microscopy is a common approach; however, it is laborious to undertake, and quantification can be subjective depending on the user and magnification. Automation using flow cytometry remains a challenge and appears to have limitations related to the detection of false positives as a result of artifacts from staining (Kaile et al. 2020). The staining approach may be a prerequisite for analysis using automated methods; however, issues around the ability of stains as used by Kaile et al. (2020), to work effectively on weathered particles from the environment rather than on particles made in the laboratory, may be a further challenge to the approach.

Overall, there is a consistent recognition in the literature of the need to resolve the issues with the methods of detection, sampling, analysis, and characterization of microplastics. Wong et al. (2020) highlighted this "particularly in terms of their accuracy, reliability, simplicity and efficiency" and concluded that standardization was required. Similar views regarding standardization and a need to increase the speed of processing focusing on automation have been expressed in relation to sampling river systems (Campanale et al. 2020). In a review of studies related to the ingestion and trophic transfer of plastic particles, the view in relation to quantification was again expressed as an urgent need for the "development and application of standardized analytical methods… to better understand spatial and temporal trends" (Gouin 2020). It is clear that counting and characterization underpin our understanding of the sources, fate, behavior, and effects of microplastics in the environment but that standardization is needed to ensure parity of findings from measurements conducted around the world.

CONCLUSIONS

Based on the analysis of 4 PCPs, the present study shows that no single technique will offer a complete solution to the characterization of microplastics. The different techniques have apparent trade-offs with respect to sensitivity, specificity, and precision because the human eye and instrumental detectors see differently and report what they find differently. Although showing promise, automated approaches such as flow cytometry still require further development before being applied to field samples. For microplastic research to be useful, it is important that scientists have the necessary tools to accurately measure microplastics in the environment, and the need for standardized methods and reference materials is frequently highlighted in the literature.

Acknowledgment—The authors acknowledge the support from staff of the Experimental Techniques Centre at Brunel University London for the use of the μ-FTIR and C. Probst and P.J. Chana from Amnis Corporation, for their advice on using the ImageStream.

Author Contributions Statement—K. Renner, H. Foster, E. Routledge, and M. Scrimshaw all contributed to experimental conception and design; K. Renner and H. Foster performed experimental work and data analysis; K. Renner, H. Foster, and M. Scrimshaw wrote the manuscript; E. Routledge reviewed the manuscript.

Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (mark.scrimshaw@brunel.ac.uk).

 © 2021 The Authors

REFERENCES

Akdogan Z, Guven B. 2019. Microplastics in the environment: A critical review of current understanding and identification of future research needs. *Environ Pollut* 254:113011.

Alimi OS, Farner Budarz J, Hernandez LM, Tufenkji N. 2018. Microplastics and nanoplastics in aquatic environments: Aggregation, deposition, and enhanced contaminant transport. *Environ Sci Technol* 52:1704–1724.

Anderson PJ, Warrack S, Langen V, Challis JK, Hanson ML, Rennie MD. 2017. Microplastic contamination in Lake Winnipeg, Canada. *Environ Pollut* 225:223–231.

Beaumont NJ, Aanesen M, Austen MC, Börger T, Clark JR, Cole M, Hooper T, Lindeque PK, Pascoe C, Wyles KJ. 2019. Global ecological, social and economic impacts of marine plastic. *Mar Pollut Bull* 142:189–195.

Briassoulis D, Pikasi A, Briassoulis C, Mistriotis A. 2019. Disintegration behaviour of bio-based plastics in coastal zone marine environments: A field experiment under natural conditions. *Sci Total Environ* 688:208–223.

Burton GA. 2017. Stressor exposures determine risk: So, why do fellow scientists continue to focus on superficial microplastics risk? *Environ Sci Technol* 51:13515–13516.

Cabernard L, Roscher L, Lorenz C, Gerdts G, Primpke S. 2018. Comparison of Raman and Fourier transform infrared spectroscopy for the quantification of microplastics in the aquatic environment. *Environ Sci Technol* 52:13279–13288.

Campanale CS, Savino I, Pojar I, Massarelli C, Uricchio VF. 2020. A practical overview of methodologies for sampling and analysis of microplastics in riverine environments. *Sustainability* 12:6755.

Ceccarini A, Corti A, Erba F, Modugno F, La Nasa J, Bianchi S, Castelvetro V. 2018. The hidden microplastics: New insights and figures from the thorough separation and characterization of microplastics and of their degradation byproducts in coastal sediments. *Environ Sci Technol* 52:5634–5643.

Chang M. 2015. Reducing microplastics from facial exfoliating cleansers in wastewater through treatment versus consumer product decisions. *Mar Pollut Bull* 101:330–333.

Corami F, Rosso B, Bravo B, Gambaro A, Barbante C. 2020. A novel method for purification, quantitative analysis and characterization of microplastic fibers using micro-FTIR. *Chemosphere* 238:124564.

De Cleyn E, Holm R, Van, den Mooter G. 2019. Size analysis of small particles in wet dispersions by laser diffractometry: A guidance to quality data. *J Pharm Sci* 108:1905–1914.

de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. 2018. Microplastics as an emerging threat to terrestrial ecosystems. *Glob Chang Biol* 24:1405–1416.

Erni-Cassola G, Zadjelovic V, Gibson MI, Christie-Oleza JA. 2019. Distribution of plastic polymer types in the marine environment; A meta-analysis. *J Hazard Mater* 369:691–698.

Germanov ES, Marshall AD, Bejder L, Fossi MC, Loneragan NR. 2018. Microplastics: No small problem for filter-feeding megafauna. *Trends Ecol Evol* 33:227–232.

Godoy V, Martín-Lara MA, Calero M, Blázquez G. 2019. Physical–chemical characterization of microplastics present in some exfoliating products from Spain. *Mar Pollut Bull* 139:91–99.

Gouin T. 2020. Toward an improved understanding of the ingestion and trophic transfer of microplastic particles: Critical review and implications for future research. *Environ Toxicol Chem* 39:1119–1137.

Henry B, Laitala K, Klepp IG. 2019. Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. *Sci Total Environ* 652:483–494.

Hernandez LM, Yousefi N, Tufenkji N. 2017. Are there nanoplastics in your personal care products? *Environ Sci Technol Lett* 4:280–285.

Hintersteiner I, Himmelsbach M. 2015. Characterization and quantitation of polyolefin microplastics in personal-care products using high-temperature gel-permeation chromatography. *Anal Bioanal Chem* 407:253–1259.

IDEAS. 2020. *Image Data Exploration and Analysis Software*. Luminex Corporation, Washington, USA. [cited 2021 April 29]. Available from: https://www.luminexcorp.com

Imhof HK, Laforsch C, Wiesheu AC, Schmid J, Anger PM, Niessner R, Ivleva NP. 2016. Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. *Water Res* 98:64–74.

Kaile N, Lindivat M, Elio J, Thuestad G, Crowley QG, Hoell IA. 2020. Preliminary results from detection of microplastics in liquid samples using flow cytometry. *Front Mar Sci* 7:552688.

Käppler A, Fischer M, Scholz-Böttcher BM, Oberbeckmann S, Labrenz M, Fischer D, Eichhorn K-J, Voit B. 2018. Comparison of μ-ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. *Anal Bioanal Chem* 410:5313–5327.

Kazour M, Terki S, Rabhi K, Jemaa S, Khalaf G, Amara R. 2019. Sources of microplastics pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill. *Mar Pollut Bull* 146:608–618.

Koelmans AA, Bakir A, Burton GA, Janssen CR. 2016. Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. *Environ Sci Technol* 50:3315–3326.

Lehtiniemi M, Hartikainen S, Näkki P, Engström-Öst J, Koistinen A, Setälä O. 2018. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. *Food Webs* 17:e00097.

Lenz R, Enders K, Nielsen TG. 2016. Microplastic exposure studies should be environmentally realistic. *Proc Natl Acad Sci USA* 113:E4121–E4122.

Li J, Liu H, Paul, Chen J. 2018. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res* 137:362–374.

Ma J, Zhao J, Zhu Z, Li L, Yu F. 2019. Effect of microplastic size on the adsorption behavior and mechanism of triclosan on polyvinyl chloride. *Environ Pollut* 254:113104.

Napper IE, Bakir A, Rowland SJ, Thompson RC. 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar Pollut Bull* 99:178–185.

Nguyen B, Claveau-Mallet D, Hernandez LM, Xu EG, Farner JM, Tufenkji N. 2019. Separation and analysis of microplastics and nanoplastics in complex environmental samples. *Acc Chem Res* 52:858–866.

Park Y, Abihssira-García IS, Thalmann S, Wiegertjes GF, Barreda DR, Olsvik PA, Kiron V. 2020. Imaging flow cytometry protocols for examining phagocytosis of microplastics and bioparticles by immune cells of aquatic animals. *Front Immunol* 11:203.

Pinto da Costa J, Reis V, Paço A, Costa M, Duarte AC, Rocha-Santos T. 2019. Micro(nano)plastics—Analytical challenges towards risk evaluation. *Trends Analyt Chem* 111:173–184.

Praveena SM, Shaifuddin SNM, Akizuki S. 2018. Exploration of microplastics from personal care and cosmetic products and its estimated emissions to marine environment: An evidence from Malaysia. *Mar Pollut Bull* 136:135–140.

R Development Core Team. 2019. *R: A Language and Environment for Statistical Computing*, Ver 3.6.0. R Foundation for Statistical Computing, Vienna, Austria.

Renner K. 2018. Particle size analysis, quantification, and identification of microplastics in selected consumer products: A critical comparison of methods and analytical techniques. PhD thesis. Brunel University London, Uxbridge, UK.

Rezania S, Park J, Md Din MF, Mat Taib S, Talaiekhozani A, Kumar Yadav K, Kamyab H. 2018. Microplastics pollution in different aquatic environments and biota: A review of recent studies. *Mar Pollut Bull* 133:191–208.

Ryan PG. 2018. Entanglement of birds in plastics and other synthetic materials. *Mar Pollut Bull* 135:159–164.

Sehly K, Chiew HL, Li H, Song A. 2015. Stability and ageing behaviour and the formulation of potassium-based drilling muds. *Appl Clay Sci* 104:309–317.

Sharma S, Chatterjee S. 2017. Microplastic pollution, a threat to marine ecosystem and human health: A short review. *Environ Sci Pollut Res* 24:21530–2154.

Silva AB, Bastos AS, Justino CIL, da Costa JP, Duarte AC, Rocha-Santos TAP. 2018. Microplastics in the environment: Challenges in analytical chemistry—A review. *Anal Chim Acta* 1017:1–19.

Silva JHS, Deenik JL, Yost RS, Bruland GL, Crow SE. 2015. Improving clay content measurement in oxidic and volcanic ash soils of Hawaii by increasing dispersant concentration and ultrasonic energy levels. *Geoderma* 237–238:211–233.

© 2021 The Authors

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Slotwinski JA, Garboczi EJ, Stutzman PE, Ferraris CF, Watson SS, Peltz MA. 2014. Characterization of metal powders used for additive manufacturing. *J Res Natl Inst Stand Technol* 119:461–493.

Tagg AS, Sapp M, Harrison JP, Ojeda JJ. 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. *Anal Chem* 87:6032–6040.

Taylor M, Gwinnett C, Robinson L, Woodall LC. 2016. Plastic microfibre ingestion by deep-sea organisms. *Sci Rep* 6:33997.

Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, Rio-Echevarria IM, Van, den Eede G. 2019. Review of micro- and nanoplastic contamination in the food chain. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 36:639–673.

Windsor FM, Tilley RM, Tyler CR, Ormerod SJ. 2019. Microplastic ingestion by riverine macroinvertebrates. *Sci Total Environ* 646:68–74.

Wolff S, Kerpen J, Prediger J, Barkmann L, Müller L. 2019. Determination of the microplastics emission in the effluent of a municipal waste water treatment plant using Raman microspectroscopy. *Water Res X* 2:100014.

Wong JKH, Lee KK, Tang KHD, Yap P-S. 2020. Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. *Sci Total Environ* 719:137512.

Zarfl C. 2019. Promising techniques and open challenges for microplastic identification and quantification in environmental matrices. *Anal Bioanal Chem* 411:3743–3756.

 © 2021 The Authors

15528618, 2022, 4, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5057 by Test, Wiley Online Library on [20/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License