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Characterising microplastics in personal care products

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

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Abstract: Any uncertainty in determining numbers of microplastics in the environment may be barrier to assessing their impact and may stem from various aspects of methodologies used to quantify them. This paper undertakes a comparison of approaches

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to quantify and characterize microplastics in four personal care products. The aim was to not only 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-FT-IR) 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-FT-IR 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.

Keywords: microplastics; contaminants of emerging concern; personal care products

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INTRODUCTION

The increasing use of plastics together with inappropriate disposal have 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 is 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 waste water 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 has 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 standardisation in methodology and reporting of microplastics are 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 microplastics analysis and harmonisation of results globally (Toussaint et al. 2019).

To illustrate, characterisation of microplastics in facial scrubs, undertaken by a number of independent groups has yielded size distributions of 4.1 to 1240 µm 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.

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 infra-red (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 criticised for the very high concentrations of microplastics used which may not reflect real-world scenarios (Burton, 2017; Lenz et al., 2016).

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 paper by Wong and colleagues (Wong et al., 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 hypothesised 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 this work is to report data on the numbers of particles counted using different techniques using four personal care products (PCPs) as discrete samples with 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, this study reports the sensitivity of the different analytical techniques in the analysis of ultra-pure laboratory grade water (blank samples) and its implications for microplastics studies.

EXPERIMENTAL METHODS

Two types of PCPs were chosen for this study, toothpastes and facial scrubs. According to the labelling all products contained polyethylene. The toothpastes selected were Colgate “Max White One Luminous” (TP1) and “Advanced White Go Pure” (TP2). The two 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 multi-technique approach. Size measurements were determined using laser diffraction (LD), light microscopy and imaging flow cytometry (IFC), and the number of microplastics per 100 g of product was determined. The µ-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 as 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 pre-filtering samples (70 µm) to protect the instrument.

Separation of microplastics from the samples

The plastic particles used in this work were not reference materials, as 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 was subsequently dispersed in 1 ml of water at 50-60°C. To achieve density separation, 50 ml of sodium chloride solution (140 g/L) at 50-60°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. The 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 ~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, due to the presence of other matrix components. To facilitate removal of plastics from the remaining matrix components, a centrifugation step was introduced.

The residual 15 ml volume was transferred to 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. The sodium pyrophosphate was used as a dispersant to prevent the microplastics from agglomerating as it has been reported to inhibit bond formations between (Sehly et al. 2015; Silva et al. 2015). Centrifugation was carried out at 1700×g, after which the top layer of the solution (around 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 dispersal agent helped minimise loss of particles. Blank samples with no PCP added were also extracted through the whole process.

Characterisation 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 2,500 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, UK). 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 analyser and collected during the washing cycle. Recovery of microplastics from the instrument was confirmed by checking background readings.

Characterisation 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 50-60°C, into a 50 mL beaker. Pipetting from the bottom, the liquid below the floating polyethylene particles was then reduced until 10ml remained. The suspension was then agitated, and a 1 ml aliquot was transferred to a Sedgewick Rafter cell (SRC), etched with a 50 x 20 x 1 mm grid. The temperature of water used facilitated the transfer of microplastics to the SRC. Size and particle count measurements were determined at 200x magnification with an Olympus BX 51 calibrated eyepiece binocular microscope with QCapture Pro 5.1 imaging software. The longest length of the first 100 particles counted in 6 randomly selected transects were measured and recorded for each slide. This was based on an statistical assessment of the technique (Renner, 2018). To determine particle size distribution, the data from the 100 particles from each replicate was pooled (n=300 particles from each sample were measured). Statistical analysis was conducted using R to undertake an analysis of variance (two-sample assuming unequal variance) with a post-hoc t-test.

Imaging flow cytometry. For the second pathway, 1 mL of the hot (50-60°C) agitated sample was pre-filtered (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 20x magnification, with a 120 µm × 256 µm field of view using brightfield and the 488 nm laser set at 100 mW. To ensure 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 focussing) 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-minute maximum acquisition time, whichever occurred first. Filter sterilised water was run on the system between samples to prevent cross-contamination. It was noted that in all samples (except for FS2), that 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 non-homogenous sample.

The IDEAS version 6.2.65.0 software was used to analyse 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 analysed using the gradient root mean square (RMS) function. Using the gated single event for the in-focus population, a histogram of aspect ratio vs normalised 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 mid-IR range of between 700 and 4,000 cm^{-1}, the spectral results were based on reflectance in imaging mode with two co-added scans per pixel, an aperture size of 25 µm^{2} and at 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 four products contained particles with sizes from the low micron range up to hundreds of microns (Figure 2) with no particles observed in blanks. The cut-off 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. 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 around 200 µm. Another study has also shown size distributions from 10 - 3,000 µm for particles in PCPs with one sample extending to around 2,500 µm (Godoy et al. 2019). The particle size distribution indicated all samples exhibited high peaks between 200 – 300 µm (TP1), 300 µm (TP2), 200 – 400 µm (FS1) and 300 µm (FS2). These peaks were fairly consistent with those reported in a study on the characterisation of microplastics in cosmetics (Napper et al. 2015; Godoy et al. 2019).

Particle size distribution and number by microscopy

Microplastics in all personal care products analysed were recorded in the size range of 5 - 483 µm (Table 2). In contrast, another study using microscopy has reported size ranges of 3 - 178 µm and another, 60 - 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 comparisons of means at the 95% family-wise confidence level, showed that the sizes of particles in TP1-TP2 and FS1-FS2 pairings were not significantly different but 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 200x magnification was conducted. However, visual characterisation resulted in a narrower size distribution with a larger size at the lower end (5 µm cut-off) 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 four 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 to 0.5 µm), it is, however, important to note that the sample was pre-filtered through a 70 µm filter which excluded the higher range. However, it was apparent that imaging flow cytometry, with its ability to count and visualise each particle, observed many smaller particles than the other techniques, and there is some evidence that these were present in very large numbers in one sample.

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, are 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 ultra-pure laboratory grade water possibly reflects the high sensitivity of the imaging flow cytometry technique when compared to other analytical techniques used in this study. The other techniques did not detect contaminants in blank samples. Although their presence and source is not easily explained in ultra-pure 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 sub-micron 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 work also stated that “it is impossible to separate unstained PE particles from the background noise in the blank sample (milli-Q water with 10% DMSO)”, possibly explaining findings observed in this work. It was found that staining with Nile Red and using 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 due to their large surface area to volume ratio (Taylor et al. 2016) may become a significant route of exposure to environmental chemicals (REF). 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 and Bakir 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, non-destructive nature and ability to cross reference samples with already established libraries of functional groups for different polymers makes 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 2020). 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 also appears to have limitations related to the detection of false positives due to artifacts from staining (Kaile et al. 2020). The staining approach may be a pre-requisite 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 maybe a further challenge to the approach.

Overall, there is a consistent recognition in the literature around the resolution of issues for the methods of detection, sampling, analysis and characterization of microplastics. Authors such as 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 is again expressed as "development and application of standardized analytical methods are urgently needed 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 four personal care products, this research 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 also report what they find differently. Although showing promise, automated approaches such as flow cytometry still require further development before being applied to field samples. In order for microplastic research to be useful, is important that scientists have the necessary tools to accurately measure microplastics in the environment, and the need for standardised methods and reference materials is frequently highlighted in the literature.

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

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Figure captions:

GRAPHICAL ABSTRACT CAPTION: Different methods give different results for analysis of plastic particles from facial scrubs and toothpastes.

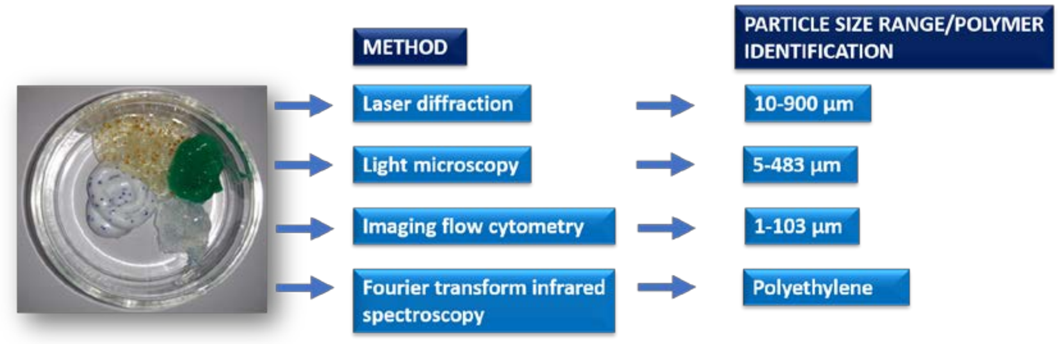

FIGURE 1: Schematic diagram of the approach used to characterise microplastics in personal care products showing separation and centrifugation, followed by particle size analysis, microscopy and µ-FTIR. Samples were re-extracted for analysis by imaging flow cytometry.

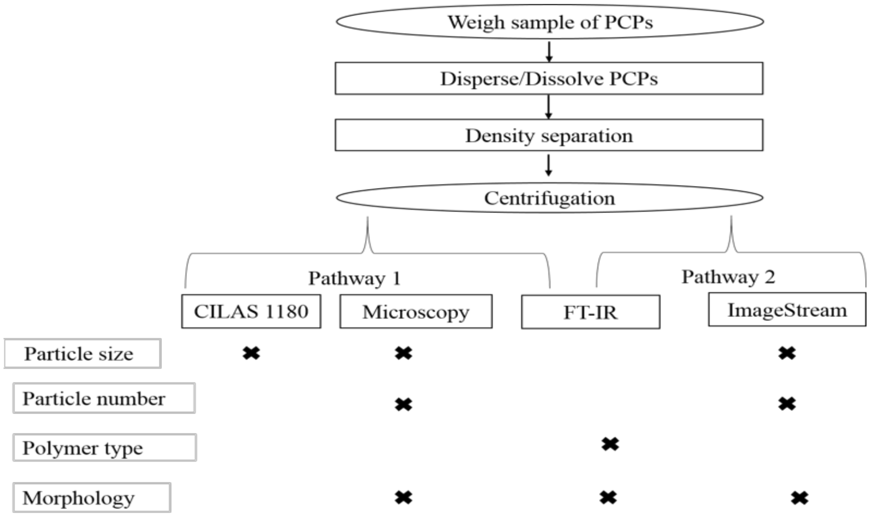

FIGURE 2: The size distribution and cumulative frequency graphs for microplastics recovered from the personal care products using the particle size analyser. Most particles were in the 200 to 900 µm size range, with differences apparent between samples. There was a tail to the smaller size range in all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

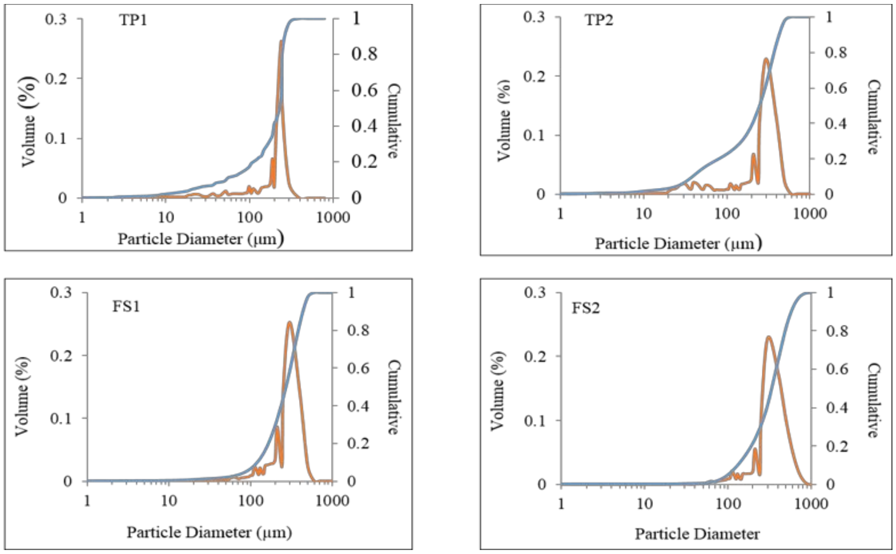

Table 1. Analysis of the use of recovery studies and blank samples in papers in the review by Wong et al., 2020. Four tables in the review listed a total of 31 (No. column) relevant papers, which reported on the occurrence of microplastics in freshwater, freshwater organisms, soil and terrestrial fauna.

Tables in Wong et al. (2020)	No.[a]	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. Some papers had data included in more than one table. Table 4 in Wong et al. (2020), included some data on macro-plastics which are excluded from this analysis.

Table 2: Sizes and numbers of the microplastics in PCPs as determined by microscopy.

Product	Minimum (μm)	Maximum (μm)	Number 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

Table 3: Comparison of mean particle size (μm) between laser diffraction and microscopy

Product	Laser Diffraction	Microscopy
TP1	351	72
TP2	310	97
FS1	276	143

FS2	279	146

Table 4: Size (µm) and number of particles detected using imaging flow cytometry based on the diameter features. Numbers per 100g have been blank adjusted and ND is where the blank was greater than sample values.

Product	Min	Max	Mean	Number per 100 g
TP 1	1.5	19.8	7.5	ND
TP 2	1.5	20.7	7.7	ND
FS 1	2	33.7	8.2	ND
FS 2	1.1	31.1	6.55	3.8×10^9