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When Good Intentions Go Bad—False Positive Microplastic Detection Caused by Disposable Gloves

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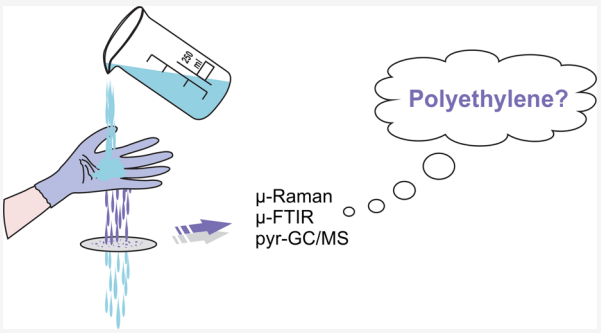
ABSTRACT: Apart from being considered a potential threat to ecosystems and human health, the ubiquity of microplastics presents analytical challenges. There is a high risk of sample contamination during sampling, sample preparation, and analysis. In this study, the potential of sample contamination or misinterpretation due to substances associated with disposable laboratory gloves or reagents used during sample preparation was investigated. Leachates of 10 different types of disposable gloves were analyzed using Raman microspectroscopy (μ-Raman), Fourier-transform infrared microspectroscopy (μ-FTIR), and pyrolysis–gas chromatography/mass spectrometry (pyr–GC/MS). There appeared to be polyethylene (PE) in almost all investigated glove leachates and with all applied methods. Closer investigations revealed that the leachates contained long-chain compounds such as stearates or fatty acids, which were falsely identified as PE by the applied analytical methods. Sodium dodecyl sulfate, which is commonly applied in microplastic research during sample preparation, may also be mistaken for PE. Therefore, μ-Raman, μ-FTIR, and pyr–GC/MS were further tested for their capability to distinguish among PE, sodium dodecyl sulfate, and stearates. It became clear that stearates and sodium dodecyl sulfates can cause substantial overestimation of PE.



1. INTRODUCTION

For the greatest part of the global population, plastics are indispensable materials of daily use.[1] Due to ever-growing production, as well as high persistence, plastic has become ubiquitous.[2] Since the early 2000s, increasing numbers of studies focus on the toxicological effects and quantification of small plastic particles, so-called "microplastics" (MP), in the environment. MP are defined as particulate synthetic or heavily modified natural polymers for which the largest dimension is in the size range between 1 and 1000 μm.[3,4] So far, methods for sampling, sample preparation, and analysis of MP are still under development. As a result, reported findings of MP can differ by several orders of magnitude, even for the same matrix, and must be interpreted with caution.[5]

Raman microspectroscopy (μ-Raman) and Fourier-transform infrared microspectroscopy (μ-FTIR), as well as pyrolysis–gas chromatography/mass spectrometry (pyr–GC/MS), are among the most commonly applied analytical methods for identification and quantification of MP.[6−8] With μ-Raman and μ-FTIR, particles down to 1 and 10 μm, respectively, can be detected.[7,8] With both methods, the size and number of particles, as well as the chemical identity of the particles, can be determined. Chemical identification is performed for μ-Raman based on inelastic scattering of photons at the sample. These scattered photons reflect the molecular vibrations. For μ-FTIR, the identification is based on molecular vibrations induced by the absorption of infrared light by the sample. For both methods, resulting vibrational spectra are characteristic for individual materials and allow polymer types to be distinguished.[9] Mass spectrometric methods, e.g. pyr–GC/MS, are complementary to spectroscopic methods, as they render information on polymer mass concentrations instead of particle numbers. During pyr–GC/MS, samples are thermally decomposed and the decomposition products are separated by gas chromatography and analyzed with mass spectrometry.[10]

Irrespective of the analytical method, care must be taken to minimize MP contamination during sampling, sample preparation, and analysis.[5] Common precautions taken against plastic contamination are the use of laminar flow cabinets, fume hoods, or air treatment devices to prevent plastic input from the air.[11,12] Further measures against contamination include the avoidance of plastic utensils to prevent direct

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contact between samples and plastic items, the application of extensive cleaning procedures for all applied utensils, the wearing of disposable gloves and cotton laboratory coats to avoid introduction of plastic particles via hands and clothes, as well as the use of particle-free water for all cleaning and sample preparation steps.[12−14] Despite precautions, contamination cannot be prevented entirely due to the ubiquitous nature of microplastics particles.[12] Therefore, additional examination of blank samples is required in order to assess the level of contamination and to establish a limit of quantification (LOQ).

In this research, contamination induced by the use of sodium dodecyl sulfate (SDS) as well as disposable gloves is investigated. SDS is an anionic surfactant that is commonly applied during sample preparation, as it aids the decomposition of animal and plant residues and increases the contact surface for subsequent treatment steps.[15] Disposable gloves are frequently worn during sample handling for personal protection or in order to prevent sample contamination. The production of disposable gloves comprises the washing and drying of hand-shaped porcelain formers that are dipped into a coagulant and a compounded latex. The coagulant promotes fast film buildup and is made of polyvalent metal salt, organic acid, or organic acid salt dissolved in water, methanol, or ethanol.[16] The current state of the art is the addition of stearates to the coagulant as a mold-release agent.[17] As described by Akabane, the compounded latex is usually made of natural rubber, polyisoprene rubber, acrylonitrile butadiene rubber, or chloroprene rubber combined with a variety of compounding chemicals (cross-linkers, vulcanization accelerators, vulcanization activators, and antiaging additives). After the dipping of formers into the coagulant and compounded latex, subsequent steps are leaching (residual chemicals and proteins are leached from the glove surface), beading (strengthening/rolling of the cuff), and vulcanization (a curing process where the gloves are heated to gain elasticity and strength). Powdered gloves are additionally treated with talc, silica, or cross-linked starch to prevent adhesion and enable easy donning, while powder-free gloves are either chlorinated or provided with a polymer coating (e.g., acryl, polyurethane).[16] Although there are already a number of publications on the topic of disposable gloves causing unwanted residues in samples,[18−20] the risk and impact of MP samples being contaminated due to the use of disposable gloves has not yet been addressed in MP research, to the best of our knowledge.

Preliminary examinations showed that disposable gloves may cause substantial overestimation of polyethylene (PE) in samples. Therefore, the aims of this study are (i) to determine a potential overestimation of PE particles or mass found in MP samples due to the utilization of powder-free disposable gloves; (ii) to analyze the reasons of this overestimation; (iii) to determine the capability of μ-Raman, μ-FTIR, and pyr−GC/MS to distinguish among PE, SDS, and stearates; and (iv) to discuss options for overcoming potential bias in mass and particle number for PE in MP samples.

2. MATERIALS AND METHODS

2.1. Minimization of Contamination. *Measures for Reducing Contamination.* Three laboratories, each specialized in a different method for MP analysis, were involved in the present study. At all three laboratories, precautions to reduce sample contamination were taken: 100% cotton lab coats were worn by staff while handling samples, application of plastic tools was omitted, and samples were covered with aluminum foil, glass, or stainless steel lids during storage. To further minimize air-borne contamination, the whole procedure of sample processing and analysis was performed in laminar flow cabinets at the laboratory focused on μ-Raman analysis. The laboratory focused on μ-FTIR analysis conducted sample processing in a laminar flow cabinet and analysis in a room equipped with an air-cleaner including a HEPA filter [for more details on air-cleaning equipment, see Table S1 of the Supporting Information (SI)]. At the pyr−GC/MS laboratory, no technical equipment to prevent air-borne contamination was used, because previous long-term investigations had shown no relevant MP input by air.[21]

Protocol for Cleaning of Hands, Glassware, and Utensils. At all three laboratories, a strict and uniform protocol was followed for the washing of hands as well as for the cleaning of glassware and utensils prior to and during sample handling. All glassware and utensils were successively cleaned in a dishwasher, with tap water, with distilled water, and with ultrapure water (for more details on specifications, see Table S2, SI) and were finally submitted to 3-fold ultrasonification with ultrapure water. Hands were washed in a three-step procedure, first using tap water and soap, then using only tap water, and finally using ultrapure water. Between the first two washing steps, hands were dried with paper towels. After the final washing step, hands were either allowed to air-dry within the laminar flow cabinets (μ-FTIR laboratory) or were dried with Kimtech Science Precision Wipes at the μ-Raman and pyr−GC/MS laboratories.

2.2. Sample Selection and Distribution. In order to investigate the potential overestimation of PE particles or mass caused by the use of common laboratory powder-free gloves, seven types of nitrile gloves (N1−N7) from different manufacturers, as well as one type of latex gloves (L1), one type of neoprene gloves (Neo1), and one type of vinyl gloves (V1), were acquired in packages of 100 gloves each. Furthermore, three identical looking granular samples were obtained for determining the capability of μ-Raman, μ-FTIR, and pyr−GC/MS to distinguish among sodium stearate (G-S; Alfa Aesar by Thermo Fisher GmbH), PE (G-PE; ultrahigh molecular weight, surface-modified, powder, 40−48 μm particle size, ID: 434272-100G, Sigma-Aldrich by Merck KGaA), and SDS (G-SDS; w > 99%, Fluka). Each laboratory was provided with an identical test package, containing ten pairs of gloves (one pair per glove type) as well as three glass vials with anonymized granulate (one vial per granulate type). For each glove type, duplicates were investigated at each of the three laboratories.

2.3. Sample Processing for Glove Leachates. *Gloves.* The gloves were filled with 50 g of sand as ballast and were immersed in separate glass beakers containing 200 mL of ultrapure water (Figure 1). By pulling the opening of each glove over the brim of the beaker, it was ensured that only the outside of each glove came into contact with the water. Gloves were leached for 5 h. Subsequently, each glove was removed from its beaker and was rinsed thoroughly from the outside with ultrapure water. The rinsing water and the leachate of each glove were united for filtration and analysis, which differed between the three participating laboratories (see section 2.4).

Blank Values. In order to assess contamination during sample processing and analysis of the glove leachates, triplicate processing blanks were considered at each laboratory. A

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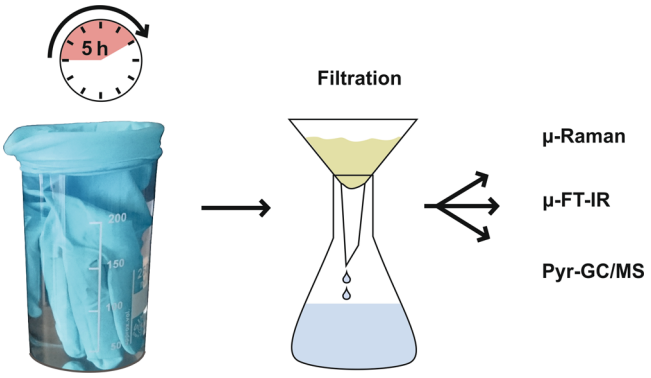

Figure 1. Schematic visualization of sample processing: Sand is filled into a glove, which is submersed in ultrapure water and leached for 5 h. Leachates are filtered and analyzed.

200 mL portion of ultrapure water was poured into each beaker, which was covered with aluminum foil and was left standing for 5 h. Additionally, three blank samples of a washed hand (see section 2.1), hereafter called "hand blanks", were considered at each laboratory. A 200 mL portion of ultrapure water was poured into a beaker and laboratory staff brought one hand into contact with the water (either by pouring water over the hand or by immersing the hand in the water). For all blank samples, filtration and analysis were performed as described below for the leachates of the gloves at each laboratory.

2.4. Identification and Quantification for Glove Leachates. *μ-Raman.* Glove leachates were vacuum-filtered through a polytetrafluoroethylene membrane (PTFE; sintered, diameter 25 mm, pore size 1−2 μm, Pieper Filter GmbH) using a stainless steel in-line filter holder (Pall Corp.). The beaker and filter holder were rinsed with 200 mL of ultrapure water. For each glove a separate filter was used and stored in a glass Petri dish prior to analysis. Filters were analyzed using a Horiba XploRa Plus Raman microscope system (Horiba Jobin Yvon) equipped with a Syncerity EMCCD camera and a confocal microscope (Olympus BX51, Olympus). The minimal particle size analyzed was 5 μm. μ-Raman analysis was performed for 6.3% of the filtration area (six square subsections of approximately 2 × 2 mm, distributed to best represent the whole filter; for more details, see Figure S1, SI) using the ParticleFinder tool of the software LabSpec (ver. 6, Spectroscopy Suite Software, Horiba Jobin Yvon). Spectra were obtained at 20-fold magnification (numerical aperture of 0.45) with an excitation wavelength of 532 nm (air cooled solid-state laser kit) and 7.5 mW laser power on the sample, 600 gr/mm spectral grating, 100 μm slit, 300 μm pinhole, an acquisition time of 2 s, and without accumulations. The examined wavenumber range was 150−3400 cm^{-1}. All acquired spectra were automatically baseline-corrected during acquisition and were compared with a spectra database established at the laboratory using the software TrueMatch (WITec GmbH). For all particles identified as potential polymers by TrueMatch, spectra attribution was manually verified. By area-weighting, the detected particle number per polymer type was extrapolated to the filtration area. The unit of quantification is particle numbers per filter (#/filter).

μ-FTIR. Glove leachates were vacuum filtered through an aluminum oxide membrane (Whatman Anopore membrane disk with a polypropylene support ring, pore size 0.2 μm, diameter 25 mm, GE Healthcare) using tailor-made glass filtration equipment with a filtration diameter of 10 mm. Rinsing of the funnel and beaker was performed with 25 mL of ultrapure water, due to very low filtration velocity. For each glove, a separate filter was used. Filters were stored and dried in glass Petri dishes in a desiccator prior to measurement. Filters were analyzed using a Bruker Hyperion 3000 μ-FTIR microscope connected to a Tensor 27 IR-spectrometer (both Bruker Optik GmbH). A visual image of the whole filter area placed on a CaF_2 window (Korth Kristalle GmbH) was acquired with a 4× glass objective. Afterward, IR imaging was performed for the whole sample spot (part of the filter substrate onto which glove leachates were filtrated; average area 76 mm^2) with a 15× IR objective in transmission mode. Spectral data were recorded by a 64 × 64 elements focal plane array detector (FPA) with six scans for each FPA field and binning of 4 × 4 pixels. The examined spectral range was 3600−1250 cm^{-1} with a resolution of 8 cm^{-1}. Background measurement was recorded on a sample-free area on the aluminum oxide membrane to overcome self-absorption of the membrane material in the spectroscopic fingerprint area.[22] Spectra analysis of FPA measurements was carried out with the freeware program siMPle[12,23] and a free FTIR reference database.[24] The database results "PE", "chlorinated PE",[25] and "oxidized PE" are summarized and indicated as findings for "PE" in this research. The reference database was extended with spectra provided by S.T. Japan Inc. for re-evaluation (section 3.1).

Since several glove leachates rendered a film instead of particles on the aluminum oxide membrane, μ-FTIR results are presented as coverage with a substance in percent relative to the sample spot area.

pyr−GC/MS. Glove leachates were prepared for pyr−GC/MS analysis by filtration through glass microfiber disks (MGB grade, particle retention 1 μm; Sartorius) using pressurized filtration with stainless steel equipment and rinsing with 200 mL of ultrapure water. For each glove, a separate filter was used. Filters were dried at 40 °C for 24 h and subsequently ground and homogenized in a planet mill (Retsch) using stainless steel grinding tools.

Of each filter, 20 mg (total average weight 0.3 g) was weighed into 80 μL pyrolysis cups (Eco-Cup LF, Frontier Laboratories) and pyrolyzed at 600 °C. The pyrolyzer was operated as one shot. Measurements were performed with a Multi-Shot Pyrolyzer EGA/PY-3030D (Frontier Laboratories) equipped with an Auto-Shot Sampler AS-1020E (Frontier Laboratories). Pyrolysis products were injected with a split of 1:20 into an Agilent 7890B gas chromatograph (Agilent) with a deactivated retention gap (5 m length, 0.25 inner diameter) and a HP-5 ms Ultra Inert column (Agilent) with the dimensions of 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness. Chromatographic separation was performed with the following temperature program: hold at 40 °C for 2 min, increase to 320 °C by 20 °C per min, and hold for 13 min. For detection, an Agilent MSD 5977B (Agilent) in scheduled selected ion monitoring (SIM) mode was used.

To estimate the potential amount of glove-related substances, leading to an overestimation of PE, appropriate amounts of cryo-milled PE (Sigma-Aldrich) were serially diluted in pulverized glass microfiber disks to achieve a calibration range from 0.005 to 5 mg/g. For quantification, characteristic pyrolysis products of PE were monitored by the abundance of their indicator ion (for more details, see Table S3, SI). A 10 μL aliquot of a 27 μg/mL solution of

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polystyrene-d_5 (PSd5, Polymer Source) in dichloromethane (Sigma-Aldrich) was added into the pyrolysis cups as an internal standard and monitored at m/z 109.1 and t_r = 6.94 min. The ratio of peak areas of the characteristic pyrolysis product and styrene-d_5 (relative response) was used for quantification. The unit of quantification was milligram of PE per filter.

2.5. Sample Processing and Identification of Granulate Samples. *μ-Raman.* For the samples G-S, G-PE, and G-SDS, a spatula tip of granulate was placed onto separate sections of a microscope slide and each granulate was analyzed by applying μ-Raman. Single granulate particles were identified manually using the settings described in section 2.4, with exception that the acquisition time was 10 s and the number of accumulations was two. Raman spectra of each granulate were manually compared with the laboratory internal as well as a commercially available spectra database (Bio-Rad) using the software KnowItAll (Bio-Rad). The correlation between spectra and reference spectra was determined by the software on the basis of mean-centering of all spectra and dot product normalization, as well as calculation of the Euclidean hit quality index (HQI). Note, all HQI information presented in this research (for μ-Raman and μ-FTIR spectra) is scaled to range from 0 (no correlation) to 1 (full consistency).

μ-FTIR. For the samples G-S, G-PE, and G-SDS, a spatula tip of each solid substance was placed on the diamond crystal of an attenuated total reflection (ATR) accessory connected to the IR spectrometer. Spectra were recorded in ATR mode within a range of 400−4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans for each sample. The diamond crystal was cleaned before analyzing each sample with 70% isopropyl alcohol. For identification, all obtained spectra were compared with ATR-FTIR Lib complete (462-2 Vol. 1−3, Bruker GmbH) and a free FTIR reference database[24] using the operation "spectrum search" (Opus 7.5.18, Bruker GmbH) with its standard algorithm within the range of 4000−2450 and 2250−400 cm^{-1}. The maximum HQI is assigned "if the position of the absorption band deviates less than the full width at half-maximum (fwhm) and if the compared fwhm as well as the relative intensity deviates less than a factor of 2".[26] Further, an aluminum oxide membrane was prepared for identification of granulates G-S, G-PE, and G-SDS by μ-FTIR. Three areas were manually marked on the membrane, and a spatula tip of each substance was strewn on one section each. Measurement and data analysis of an area of 64 mm^2 of the membrane were carried out with the settings and program described above. On the basis of the previous identification of the samples G-S, G-PE, and G-SDS in ATR mode, the free reference database was extended by spectra of stearates and surfactants provided by S.T. Japan Inc. Thresholds, spectral range, and weighting of either raw spectra or derivatives for spectra correlation were adjusted in order to accomplish identification of granulates G-S, G-PE, and G-SDS on the aluminum oxide filters as PE, stearate, or surfactant. This optimized database was also used for re-evaluation of the leachate samples (section 3.1).

pyr−GC/MS. A microspatula tip of the granulates G-S, G-PE, and G-SDS was weighed into 80 μL pyrolysis cups and pyrolyzed at 600 °C. Measurements were performed as described in section 2.4.

3. RESULTS AND DISCUSSION

3.1. PE Measurements of Glove Leachates. To determine the potential contamination of samples due to the use of disposable gloves, leachates from all gloves (N1−N7, L1, Neo1, and V1) were analyzed for MP. The polymers polyamide, polystyrene, polypropylene, and polyethylene terephthalate, as well as indistinguishable acrylates, polyurethane, or varnish, were identified with μ-Raman and μ-FTIR. However, PE was by far the most commonly identified polymer. For this reason, the focus of this research was placed on PE and substances that were falsely identified as PE (false positives).

Within this section, no differentiation is made between PE and substances that are falsely identified as PE, in order to quantify the total potential overestimation of PE in samples due to contamination and misinterpretation. Results for glove leachates are indicated as the mean value from two replicates per glove type. The according range can be derived from Figure 2. Process blanks and hand blanks are indicated as the mean value from three replicates with the according standard deviation (Figure 2).

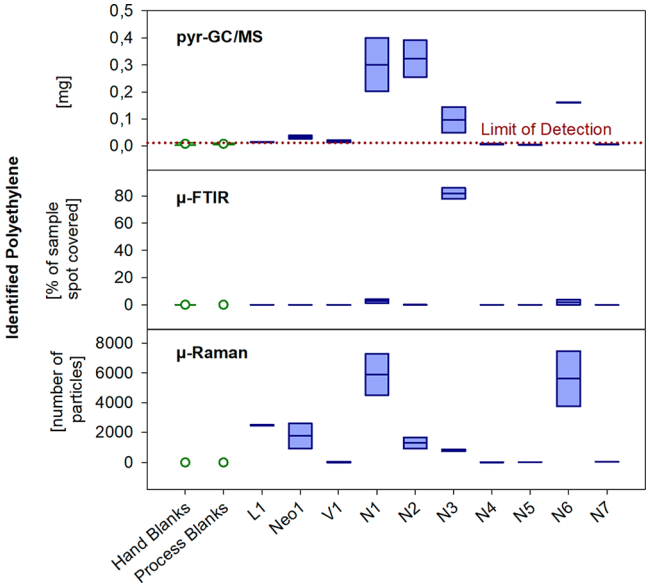

Figure 2. Amount of substances identified as PE in hand blanks (per filter and hand), process blanks (per filter), and 10 different glove types (per filter and glove) using μ-Raman, μ-FTIR, and pyr−GC/MS. For blank values, the mean from three replicates is given (circle) with the standard deviation (error bars). For gloves L1, Neo1, V1, and N1−N7, the mean value from two replicates with the according range (box) is given.

μ-Raman. Analysis rendered PE particles neither on process blanks nor on hand blanks. For leachate duplicates per glove type, a mean of 8 #PE/filter (glove N4) to 5897 #PE/filter (glove N1) was found. High levels of PE particles were identified on gloves L1, Neo1, N1, N2, N3, and N6. The size of PE particles examined across all glove types was in the range between 5 and 20 μm for 82% of the PE particles. Only 18% of the PE particles were in the size range between 20 and 100 μm and none were >100 μm.

μ-FTIR. No PE was identified for process blanks, and the percentage of sample spot covered with PE was at 0.02 ± 0.02% for hand blanks. Distinct PE films were observed for

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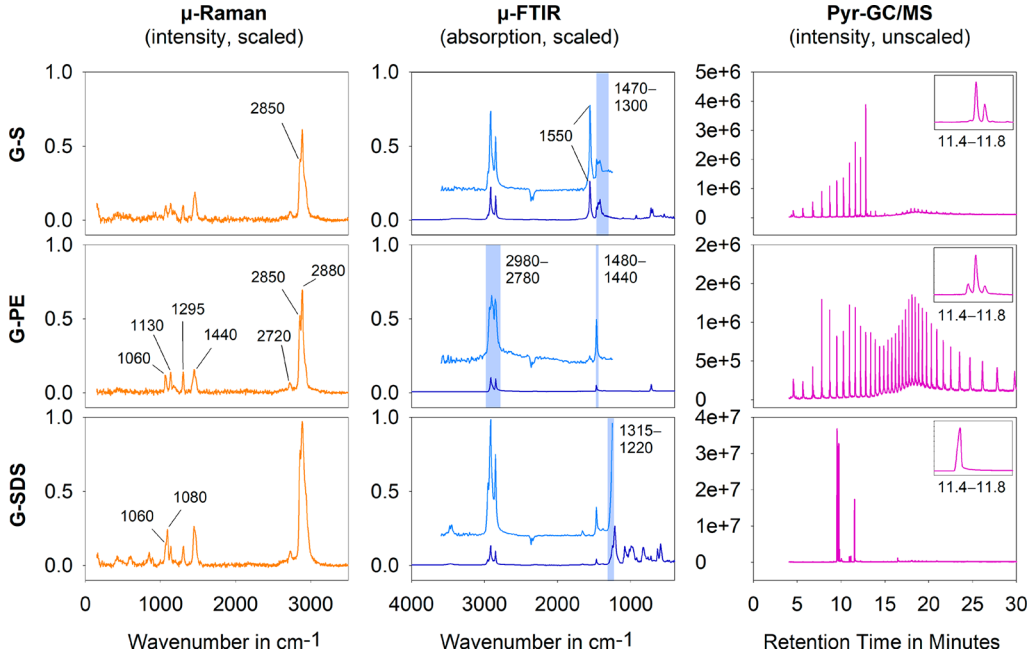

Figure 3. Comparison of the granulates G-S, G-PE, and G-SDS measured with μ-Raman (intensity, scaled by 31 000), μ-FTIR (absorption, scaled by 2; dark blue, ATR mode; light blue, transmission mode on aluminum oxide membrane), and pyr−GC/MS (unscaled, a pyrogram cutout for each granulate at t_r = 11.4−11.8 min is displayed enlarged).

gloves N1 and N6, where 2.70% and 1.89% of the sample spot were covered with PE, respectively. The highest PE values were determined for glove N3, where 82.0% of the sample spot was covered.

pyr−GC/MS. Using 1-pentadecene, hand blanks and processing blanks were below the limit of detection (LOD, 0.012 mg; mean value of process blank plus 3 times standard deviation). The same was true for untreated (only ground/homogenized) filter disks and glove types N4, N5, and N7. Highest concentrations were found for gloves N1, N2, and N6 with a mean of 0.301, 0.324, and 0.161 mg per glove and filter, respectively.

Due to the differing units, results obtained with μ-Raman, μ-FTIR, and pyr−GC/MS can only be compared semi-quantitatively. The most evident similarities are the low PE result for gloves V1, N4, N5, and N7 and the comparably high PE results for N1, N3, and N6 with all applied methods. For gloves L1, Neo1, and N2, however, there is no consistent trend between the methods: glove L1 is below the LOQ (0.023 mg, mean value of process blank plus 10 times standard deviation) for pyr−GC/MS and results in 0.002% PE sample spot coverage with μ-FTIR, while μ-Raman yields high PE particle numbers (on average 2474 #PE/filter). Conversely, Neo 1 and N2 show substantial PE findings with pyr−GC/MS and μ-Raman, but not with μ-FTIR. These intermethod variations may be explained by two factors: (i) differences in the amount of leachable substances on the gloves due to glove-to-glove variations and (ii) variations caused by differences in the applied methods, e.g. differences in the applied filter substrates (material, pore size) and differences in sensitivity of the methods to certain factors. For instance, μ-FTIR requires a sample thickness (particle or film) in the range where signal intensity is sufficiently different from the substrate background. As the film-building tendency of different leachates (originating from different glove types and manufacturers) is likely to differ, PE coverage identified with μ-FTIR may not only depend on the amount of deposited leachate but also on the lateral distribution and height of the filtration residue.

3.2. Distinguishing among PE, SDS, and Stearates.

One of the objectives of this research was to determine the capability of μ-Raman, μ-FTIR, and pyr−GC/MS to distinguish between PE and other substances that may falsely be identified as PE. For this purpose, granulate samples of sodium stearate, PE, and SDS were anonymized and investigated by all three laboratories. Resulting spectra and chromatograms are shown in Figure 3.

μ-Raman. With μ-Raman and the applied measurement settings, it was impossible to distinguish between G-S and G-PE. The spectrum of G-PE had distinct peaks at approximately 2850 and 2880 cm^{-1} (CH_2 stretching) as well as peaks with lower intensity at approximately 1060 and 1130 cm^{-1} (C−C stretching), 1295 cm^{-1} (CH_2 twisting), 1440 cm^{-1} (CH_2 deforming), and 2720 cm^{-1} (overtone).[27,28] G-S showed the same peaks as G-PE, the only noticeable difference being a less distinct peak at 2850 cm^{-1} for G-S than for G-PE. Both spectra matched very well with reference spectra of PE from the database and were identified as such with μ-Raman. For the samples G-S and G-PE, the HQI for PE was 0.96 and 0.98, respectively. The Raman spectrum of G-SDS was identified as SDS with a HQI of 0.98, although PE was also listed as a match with a HQI of 0.97. The spectrum of G-SDS showed the same peaks as G-PE and G-S, with the main difference being an additional low-intensity peak at 1080 cm^{-1} (C−C stretching).[27]

μ-FTIR. By μ-FTIR, the unknown samples G-S, G-PE, and G-SDS were first identified using ATR mode. The correlation between the obtained spectra and reference spectra was calculated with the OPUS operations standard search.[26] Granulate G-S was identified as sodium stearate with a HQI of 0.79. G-PE matched as PE (high density) with a HQI of 0.99. G-SDS showed a high accordance to sodium dodecyl sulfate with a HQI of 0.96. Hence, ATR-FTIR is suitable to

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distinguish these substances. However, μ-FTIR imaging of MP is often carried out in transmission mode with samples immobilized on substrates such as membranes or windows instead of using ATR mode. Commonly applied substrates are for instance aluminum oxide membranes and silicon membranes, as well as zinc selenide, barium fluoride, and calcium fluoride windows.[22,29−31] These substrates differ in their IR transparency (aluminum oxide membrane, 3800−1250 cm^{-1};[22] silicon membranes, 4000−600 cm^{-1};[29] zinc selenide windows, 3750−950 cm^{-1};[30] barium fluoride windows, 71 428−714 cm^{-1}; calcium fluoride windows, 80 000−952 cm^{-1} [31]). Thus, the choice of substrate is relevant for correct identification of polymers, as the according IR-transparent range determines the wavenumber regions that can be measured and used for sample identification.

Measurements using μ-FTIR in transmission mode and an aluminum oxide substrate were performed with a spectral range from 3600 to 1250 cm^{-1}. Typical spectral regions of interest for polyethylene are the C−H bending at 1480−1440 cm^{-1} and C−H stretching at 2980−2780 cm^{-1}.[22] Both G-SDS and G-S showed these absorbance bands. Thus, distinction among sodium stearate, PE, and SDS is only possible by considering further absorption bands. G-SDS shows only a weak signal in the region of 1480−1440 cm^{-1} (C−H deformation) but a strong signal from 1315 to 1220 cm^{-1} (SO_2 stretching) in the ATR spectrum.[32] Although spectra acquisition for aluminum oxide membranes was restricted to 1250 cm^{-1}, a section of strong SO_2 stretching vibration band arising at 1300 cm^{-1} was detected. Apart from the C−H vibration bands, G-S has a characteristic strong signal in the region of 1550 cm^{-1} (CO_2 stretching).[33] In the region of 1470−1370 cm^{-1}, the signals 1470−1430 cm^{-1} (C−H vibrations)[33] and 1420−1300 cm^{-1} (carboxylate vibrations)[33] arise next to each other and result in a signal pattern different from those of both PE and SDS. Therefore, a differentiation among stearate, PE, and SDS by μ-FTIR in transmission mode is feasible if the substrate is transparent at least in the above-mentioned spectral regions for alkyl, SO_2, and CO_2 vibrations.

pyr−GC/MS. pyr−GC separation of PE and subsequent detection with MS results in a homologous series of peak triplets (Figure 3, G-PE). These consist of a weak α,ω-alkadiene peak followed by a dominant 1-alkene peak and a minor *n*-alkane peak, which can be monitored through the whole pyrogram (t_r = 29 min, maximum observed chain length of 42 C atoms), although the triplets merge to one broad peak at high retention times. Sample G-PE could therefore be clearly identified as PE. However, fatty acids also show similar homologous series with varying repetitions of the triple peaks as a function of chain length. In each peak triplet, 1-alkene and *n*-alkane are dominant while α,ω-alkadiene is almost negligible, as it can only originate from thermal decomposition of very long chains, such as PE. For G-S, the homologous series was observed up to t_r = 12.72 min (Figure 3), which reflects 1-heptadecene resulting from decarboxylation of stearic acid during pyrolysis. The chain length of alkyl compounds such as fatty acids determines the number of triplet repetitions, which is a criterion for identification and distinction. Hence, G-S was identified as a stearate. However, the presence of fatty acids of shorter chain lengths such as palmitic acid could not be excluded, as their signals may be overlapping with the dominating derivatives of stearic acid. The pyrogram for G-SDS showed three dominant peaks at 9.46, 9.56, and 9.64 min, which were identified as isomers of dodecene. Further, a peak at 11.43 min was identified as 1-dodecanol. Therefore, a compound with a dodecyl residue, such as SDS, was assumed. However, a detailed identification for G-SDS was not possible with the applied method. More importantly, the pyrogram of G-SDS showed no relevant peak at t_r = 11.41 min used for PE quantification (for more details see Table S3, SI) and therefore would not result in overestimation of PE.

3.3. Determining the Reason for PE Overestimation in Glove Leachates. Investigations of the three granulates proved that is possible to distinguish between PE and stearates with μ-FTIR, if a suited substrate is used and if spectra of stearates are incorporated into the database. As stearates are substances commonly applied in glove manufacturing processes as a mold-release agent,[17] they were suspected of causing the observed elevated PE results for the glove leachates (section 3.1). Therefore, the μ-FTIR database was expanded to include spectra of stearates, and the μ-FTIR glove leachate data was re-evaluated.

This investigation revealed that 89.9 ± 12.6% of the identified PE sample spots of the gloves Neo1, L1, N1, N2, N5, and N6 consisted of stearates. The gloves V1 and N7 contained neither stearates nor PE. For the hand blanks and the process blanks, the identified PE sample spots were composed of 99.9% and 100.0% stearates, respectively. In contrast, gloves N3 and N4 yielded filter coverages of 31.2% and 0.0% stearates, respectively. Whether the remaining percentages of the sample spots are made up of PE (used as stretch modifier or mold-release agent in glove production)[34,35] or other substances falsely identified as PE remains unclear. However, additional investigations with pyr−GC/MS (glass microfiber disks soaked with methanol and wiped over gloves) revealed the presence of free fatty acids or corresponding esters/salts (C12, C16, and C18) for L1, N1, N2, N3, N5, and N6.

3.4. Preventing False Polymer Classification. Gloves are a contamination source for MP, since they are able to release substances that are either actual polymers or misinterpreted as polymers. For instance, stearates resulting from gloves and SDS introduced during sample preparation may easily be mistaken for PE, regardless of whether μ-Raman, μ-FTIR, or pyr−GC/MS is used for identification.

μ-Raman. The differences between Raman spectra of stearate, PE, and SDS are minimal. Differentiation is, if at all, possible only if adequate Raman reference spectra are provided in the database and if the risk of confusion between the substances is known. Possibly, the use of higher gratings, longer acquisition times, or multiple spectra accumulations can improve differentiation among PE, SDS, and stearates. This would however increase the already very long measurement time with μ-Raman and is therefore not feasible when dealing with environmental MP samples. Furthermore, interfering substances (e.g., organic and inorganic compounds) are often present in environmental samples and can cause a high level of noise. Thus, minimal differences between spectra—as observed between the Raman spectra of the pure granulates G-S, G-PE, and G-SDS in Figure 3—are likely to go unrecognized when dealing with environmental samples. Therefore, special attention must be payed to potential misidentification with μ-Raman, if SDS or disposable gloves are used during sample preparation and sample handling. Residues of SDS may be removed prior to analysis by filtration of the sample and thorough rinsing. As washed hands rendered little to no contamination with PE (or false-positives) for all three

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analytical methods (see section 3.1), the application of hand washing procedures may be a suited alternative to using disposable gloves, as long as personal safety precautions permit gloveless handling. In any case, contamination should be quantified by blank value investigations.

μ-FTIR. To avoid misinterpretation or overestimation of PE in IR spectra evaluation, spectra of stearates and surfactants such as SDS should be incorporated into IR databases. Furthermore, only substrates that allow spectra acquisition in the regions of C−H, CO_2, and SO_2 vibration bands (e.g., zinc selenide windows, silicone or aluminum oxide membranes) are recommended for measurements in transmission mode.

pyr−GC/MS. With pyr−GC/MS, misinterpretation of substances like stearates and SDS as PE is less likely than with μ-FTIR or μ-Raman. However, the quantification of PE via 1-pentadecene can lead to an overestimation of PE in the presence of fatty acids. Alternatively, a 1-alkene with a longer chain length (e.g., 1-octadecene) can be chosen to avoid overlapping of pyrolysis products originating from PE and fatty acid, which, however, results in lower sensitivity.

As peak triplets of homologous series of PE tend to merge at higher t_r, it gets harder to differentiate the 1-alkene peak from the weaker peaks of α,ω-alkadiene and *n*-alkane. 1,14-Pentadecadiene can be considered for quantification of PE, as it cannot originate from pyrolysis of fatty acids. Therefore, leachates of studied gloves were additionally evaluated via 1,14-pentadecadiene and 1-octadecene in order to verify the results obtained via 1-pentadecene. 1,14-Pentadecadiene and 1-octadecene rendered significantly lower PE values: for instance, N1 revealed 0.301 mg via 1-pentadecene but 0.077 and 0.027 mg of PE with 1-octadecene and 1,14-pentadecadiene, respectively. Similar effects were observed for N2, N3, and N6, while all other samples yielded PE values below the LOD and LOQ.

It must be assumed that many more synthetic and natural substances exist in laboratories and the environment that may be misinterpreted as polymers (e.g., proteins, fatty acids). These substances are unknown and unexpected as a potential source of contamination. In order to rule out misinterpretation of synthetic and natural substances as polymers in environmental samples, further research is necessary. For contamination stemming from the lab, the determination of (process) blank values is a reliable method to prevent overestimation of MP, both by false-positive identification of substances as polymers as well as by actual polymers. Furthermore, the identification of conspicuous (process) blank values (e.g., for a specific polymer type, sample, or matrix) is the first step toward uncovering and eliminating a contamination source in the laboratory. Gloves causing sample contamination are just one example of the things that can go wrong during microplastic investigations. As the pitfalls in microplastic analysis are manifold and only partially predictable, researchers addressing microplastics quantification are urged to implement rigorous measures for quality assurance, such as minimization of foreseeable sources of contamination, and to strictly perform quality control by means of blank value investigations and recovery experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c03742.

Specifications on the air-cleaning systems (Table S1) and ultrapure water systems (Table S2), details on the μ-Raman evaluation (pages S3−S4 and Figure S1), and characteristic pyrolysis products of PE (Table S3) (PDF)

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Author Contributions

C.S.W. conceived and wrote the manuscript with text contributions by C.F. and K.W., who contributed equally. Presented investigations were mainly carried out by C.S.W. at TZW: DVGW-Technologiezentrum Wasser, C.F. and T.L. at Bundesanstalt für Gewässerkunde, and K.W. at Bayerisches Landesamt für Umwelt. Figures were created by C.S.W. with inspiration by N.Z. The person to first consider MP sample contamination by disposable gloves was P.H., while M.P. subsequently recognized the connection among disposable gloves, PE contamination, and stearates. Y.K.M. identified the risk of confusion between SDS and PE with μ-Raman during previous investigations and helped incorporate her insights into this research. All coauthors critically discussed, commented on, and reviewed the manuscript.

Notes

The authors declare no competing financial interest.

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