

1 Alcohol pre-treatment to eliminate the interference
2 of micro additive particles in the identification of
3 microplastics using Raman spectroscopy

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22 **ABSTRACT**

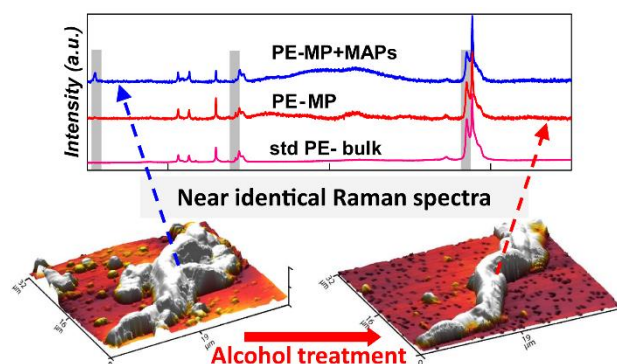
23 Raman spectroscopy is an indispensable tool in the analysis of microplastics smaller than 20
24 μm . However, due to its limitation, Raman spectroscopy may be incapable of effectively
25 distinguishing microplastics from micro additive particles. To validate this hypothesis, we
26 characterized and compared the Raman spectra of six typical slip additives with polyethylene
27 and found that their hit quality index values (0.93-0.96) are much higher than the accepted
28 threshold value (0.70) used to identify microplastics. To prevent this interference, a new
29 protocol involving an alcohol treatment step was introduced to successfully eliminate additive
30 particles and accurately identify microplastics. Tests using the new protocol showed that three
31 typical plastic products (polyethylene pellets, polyethylene bottle caps and polypropylene food
32 containers) can simultaneously release microplastic-like additive particles and microplastics
33 regardless of plastic type, daily-use scenario or service duration. Micro additive particles can
34 also adsorb onto and modify the surfaces of microplastics in a manner that may potentially
35 increase their health risks. This study not only reveals the hidden problem associated with the
36 substantial interference of additive particles in microplastic detection, but also provides a cost-
37 effective method to eliminate this interference and a rigorous basis to quantify the risks
38 associated with microplastic exposure.

39 **KEYWORDS:** microplastic, micro additive particles, Raman spectroscopy, alcohol pre-
40 treatment, hit quality index

41 **Synopsis**

42 Alcohol pre-treatment eliminates the potential misidentification of the chemical additives and
43 microplastic particles released during plastic degradation by enabling particle separation and
44 individual analysis.

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48 **1. INTRODUCTION**

49 Microplastics (MPs, solid-polymer-containing particles, European Chemical Agency¹) are a
 50 growing global concern,²⁻⁴ especially MPs <10 μm in size because they can translocate from
 51 the gut cavity to the lymph and circulatory systems, causing systemic exposure and
 52 accumulation in the tissues of humans and animals.⁵ Currently, Raman spectroscopy is an
 53 indispensable tool⁶⁻¹¹ to identify and characterize the chemical composition of single MP
 54 particles below 20 μm , including nano-sized MP down to 50 nm¹⁰ (NMPs, <1000 nm^{12, 13}).
 55 Raman spectroscopy is non-destructive, highly accurate and generates spectra due to the
 56 interaction of light with local bond vibrations.⁶ Despite these advantages, it is incapable of
 57 distinguishing between materials with subtle differences in chemical structure, since some
 58 fingerprint vibrations such as carbonyl group (C=O) are weakly detected.¹⁴

59 However, distinguishing between a target substances and reference materials with similar
 60 chemical structures is essential in MP studies. Modern plastics are a complex cocktail of
 61 polymers, chemical additives and residual monomers.¹⁵ On average, nonfiber plastics contain
 62 93% polymer resin and 7% additives by mass.¹⁶ Typical organic additives consist of small
 63 molecules, which are substantially different from the polymers (macromolecules) comprised
 64 of repeating monomer units. The potential risks of these additives heavily depend on their
 65 physicochemical properties. For instance, the additive butylated hydroxytoluene (BHT) can

66 primarily target liver, increasing liver weight and enzyme activity¹⁷ while the additive
67 erucamide has low toxicity to human health.¹⁸ While polymer-based MPs <10µm can
68 translocate and accumulate in tissues, such as liver and kidney, the specific risk of MPs to
69 human health is still unknown.⁵ For these reasons, it is crucial to separately determine the levels
70 of additives and MPs for accurate exposure assessments and effective management of their
71 potential risks.

72 Many additives are insoluble in water while some (i.e., slip additives) are designed to naturally
73 migrate to the surface of plastics.^{19,20} Consequently, the latter additives are likely to be released
74 into water, especially during the initial stages of plastic degradation. More importantly, some
75 additives have very similar chemical structure to that of their parent polymers. For example,
76 behenamide ($\text{CH}_3(\text{CH}_2)_{20}\text{CONH}_2$) is a typical slip additive widely used in polyethylene (PE)
77 plastic (i.e., bottle water caps^{21,22}). It comprises a long saturated alkyl chain terminated at one
78 end by an amide group (i.e., adjoining carbonyl and amine groups). PE polymer, on the other
79 hand, is comprised exclusively of long saturated alkyl chains. Given the strong Raman signal
80 associated with these saturated alkyl chains (i.e., $\nu(\text{C-H})$) and relatively weak Raman signals
81 from carbonyl and amine groups,^{14,23} it is extremely difficult to distinguish between pure micro
82 additives particles (MAPs) derived from a slip additive such as behenamide and PE MPs from
83 the parent plastic.

84 It is well known in food safety studies that plastic packaging is a significant source of plastic
85 additives that migrate to contacted food.^{19,24-27} However, there are very few reports of additive
86 particle release in MPs studies. Here, we hypothesize that MAPs released from plastics are
87 potentially misassigned as MPs in MP studies due to the limitations of Raman spectroscopy.
88 To test this hypothesis, we first characterised the Raman spectra of six typical additives and PE
89 in bulk form and as micron-sized particles and found that their hit quality index values (0.93-
90 0.96) are much higher than the accepted threshold value (0.70) used to identify MPs in

91 microplastic studies. To prevent this interference, we introduced a new protocol that includes
92 an alcohol rinse step that successfully separates interfering MAPs from the MPs and allows
93 them to be separately analysed. We then investigated three typical plastic products (i.e., PE
94 pellets, PE caps and Polypropylene-PP food containers) using the new protocol to confirm the
95 simultaneous release of MAPs and MPs regardless of the plastic type, daily use scenario or
96 service duration. This study not only reveals the hidden problem associated with the
97 interference of additives in the detection of MPs, but also provides a cost-effective method to
98 prevent this problem.

99

100 **2. MATERIALS AND METHODS**

101 **2.1. Precautions for contamination prevention**

102 During the experiments, the following steps were followed to avoid any potential MP
103 contamination: borosilicate 3.3 glassware were chosen for sample preparation; thoroughly cleaned
104 particle-free nitrile gloves and cotton-based laboratory coats were worn during experiments.
105 All water samples were covered by glass lids and all filtered samples were stored in glass
106 containers. A blank control sample was analysed every ten samples by filtering 100 mL DI
107 water. PE MPs were detected in blank control samples with the average levels of 107 MPs per
108 liter while no PP MPs were found in control studies.

109 **2.2. Studies on standard MPs, MAPs and real-world plastic products**

110 **2.2.1. Preparation of standard MPs and MAPs samples**

111 To investigate standard PE MPs, 5 mg of additive-free standard PE spheres (Cospheric, size
112 range of 3-16 μm) were dispersed in 1 liter of DI water (25 °C) and recaptured using a gold-
113 coated polycarbonate (PC) membrane filter (APC, the pore size of 0.8 μm , herein referred to
114 as the filter). The captured standard PE MPs were then characterized using multiple techniques,
115 such as Raman spectroscopy and atomic force microscopy (AFM). Following the same method,

116 standard micro-sized particles samples of behenamide (Merck), stearamide (TCI), erucamide
117 (TCI), oleamide (TCI), stearic acid (Merck) and erucic acid (TCI) were also prepared and
118 investigated.

119 To validate the newly developed protocol, samples were prepared by mixing standard stock PE
120 spheres and stearic acid additives in DI water and tested. The standard stock PE sphere solution
121 was prepared by evenly dispersing around 10 mg standard PE spheres (in powder form) in 1
122 liter DI water via sonication. The PE sphere concentration (around 3000 spheres per mL) in
123 this stock solution was determined by membrane filtration and Raman determination using
124 established protocols.^{7, 28, 29} Test water samples were then prepared by mixing 1mL stock PE
125 sphere solution and 100 mg stearic acid additive in 1 liter DI water (containing around 3000
126 PE spheres and 100 mg stearic acid additive per liter). The water samples were shaken at a
127 speed of 150 rpm for 2 hrs (25 °C) before filtration and test.

128 **2.2.2. Plastic food containers study**

129 PE and PP are the most widely used plastics, accounting for 33% and 21% of global market
130 share, respectively.²⁰ In this study, PP-based food containers, PE-based bottle caps and
131 Standard PE pellets were chosen to investigate the potential interference of chemical additives
132 in the identification of MPs released from plastic products.

133 Brand-new PP-based food containers (purchased from local stores) were cleaned thoroughly
134 after removing the packaging. Mimicking daily-use scenarios, the clean containers were filled
135 with 500 mL DI water and microwaved for 5 mins. After that, the containers were covered by
136 the lid and shaken at a speed of 150 rpm for 5 mins to mimic the shaking actions during users'
137 holding, moving and eating. After cooldown, the water samples were filtered using the filter.
138 Raman determination was performed following typical MPs protocol²⁹⁻³¹ to characterise and
139 quantify the particles on the filter. The filter was then placed onto a glass holder and rinsed
140 using ~30 mL ethanol (HPLC grade, in glass bottle, Fisher Chemical). After the rinse, the

141 remaining particles on the filter were again characterised and quantified using Raman
142 spectroscopy.

143 To determine the sources of MAPs, the container experiments were repeated 50 times
144 following the same protocol detailed above. Detailed chemical analysis (before and after
145 ethanol rinse) was undertaken on the water samples from the 10th and 50th run.

146 **2.2.3. Standard PE pellets study**

147 For standard PE pellets (low density, Merck, nominal size of 5 mm), 5g of pellets were placed
148 in 80 mL DI water in a glass bottle, which was then shaken at a speed of 150 rpm for 4 hrs (25
149 °C), consistent with previous reports.^{32,33} The pellets were removed from water sample using
150 a stainless steel mesh (mesh size of 1 mm). The particles releases into the water samples were
151 captured using the filter and characterized using multiple techniques. Similar to the container
152 tests, the particles were analysed before and after an ethanol rinse. Before filtration, the water
153 samples were also tested using TOC analyser to determine the concentration of organic Carbon
154 (C) and Nitrogen (N). In addition, the ethanol filtrate from the rinse was drop casted on a gold
155 coated substrate, air dried and tested using Fourier-transform infrared spectroscopy (FTIR).

156 **2.2.4. Bottle water caps study**

157 Bottled water was purchased from local stores and the PE-based water bottle caps (n=4) were
158 carefully rinsed using DI water and placed in 100 mL DI water in a glass flask. The flask was
159 then shaken at a speed of 150 rpm for 4 hrs (25 °C). The particles released into the water
160 samples was captured using the filter and characterized using multiple techniques. Similar to
161 the container tests, the filter was investigated before and after an ethanol rinse. In addition, GC-
162 MS tests were also conducted to determine the composition of alcohol-dissolved MAPs
163 released from the PE cap samples.

164 **2.3. Alcohol treatment to eliminate interference of MAPs on MPs detection**

165 Two types of alcohol treatments were conducted: alcohol rinse and in-situ ethanol test. During
166 an alcohol rinse, the filter containing captured particles was placed onto a glass holder and
167 rinsed using ~30 mL alcohol (ethanol or methanol). In the case of in-situ ethanol tests, a target
168 particle or a specific region of the filter was exposed to 1 drop of ethanol (20 μL) and was
169 immediately re-imaged after air-drying (Videos. S1-2, detailed in supporting material).

170 **2.4. Characterization and determination of MPs and MAPs**

171 **2.4.1 MPs and MAPs determination using Raman spectroscopy in our lab**

172 A calibrated Raman spectrometer (Renishaw InVia) equipped with a charge-coupled device
173 (CCD), an upright microscope (NT-MDT) and a 532nm laser (Coherent Inc.) was used to
174 identify and quantify MPs captured on the filter surface. For typical particle detection, the
175 accumulation was set to 3 times and exposure time set to 10 seconds, which is similar to the
176 typical test setting for MP determination.^{7, 28, 29} For some particles with weak signals, the
177 accumulation time was increased to obtain clear spectra. The spectra were measured in the
178 range of 500-3500 cm^{-1} . Referring to previous studies,^{30, 31, 34} the HQI value of 0.70 was set as
179 the threshold for identifying a particle as a *potential* MP. The HQI was obtained by conducting
180 Pearson correlation analysis (OriginPro 8.6) between the target particle and the standard
181 polymer. If necessary, the Raman spectra background of target particles were subtracted before
182 analysis (LabSpec 5). Given the high similarity between MAPs and MPs, in-situ ethanol tests
183 and manual spectra checks were further conducted to confirm whether particles were MPs. To
184 identify a MP, around 20 μL ethanol was drop-casted on the target particle. After air drying, if
185 the morphology changed substantially, additional ethanol was added until there is no significant
186 morphology change. Then, the particle's Raman spectra were collected and compared with the
187 known fingerprint spectra of standard parent polymers. For example, for *potential* PE MPs,
188 characteristic peaks at around 1415, 1440 and 1460 cm^{-1} were checked, which are associated
189 with the CH_2 vibrations in PE and the level of PE polymer crystallinity.^{35, 36} To quantify the

190 MPs levels, four representative spots (typically 2 spots in the middle area and 2 spots close to
191 the edge of the filter with a total tested area of 1.5 mm²) were analysed. After Raman test, the
192 total number of MPs in tested area were obtained using software ImageJ (US National Institutes
193 of Health). Finally, the MPs level per liter was calculated based on the water sample volume,
194 total area of filter, tested filter area and confirmed MP numbers. Following this protocol, the
195 recovery rate of tests involving standard PE microplastic spheres can reach 93.8%, as
196 previously detailed.^{37, 38}

197 **2.4.2. MPs and MAPs determination using other technologies**

198 Scanning electron microscope (SEM, Zeiss Ultra Plus), FTIR instrument (PerkinElmer), TOC
199 analyser (Shimadzu, TOC-L), AFM (NT-MDT) and GC-MS (Shimadzu) were conducted to
200 characterize and determine MPs and MAPs, the test conditions were detailed at supporting
201 material.

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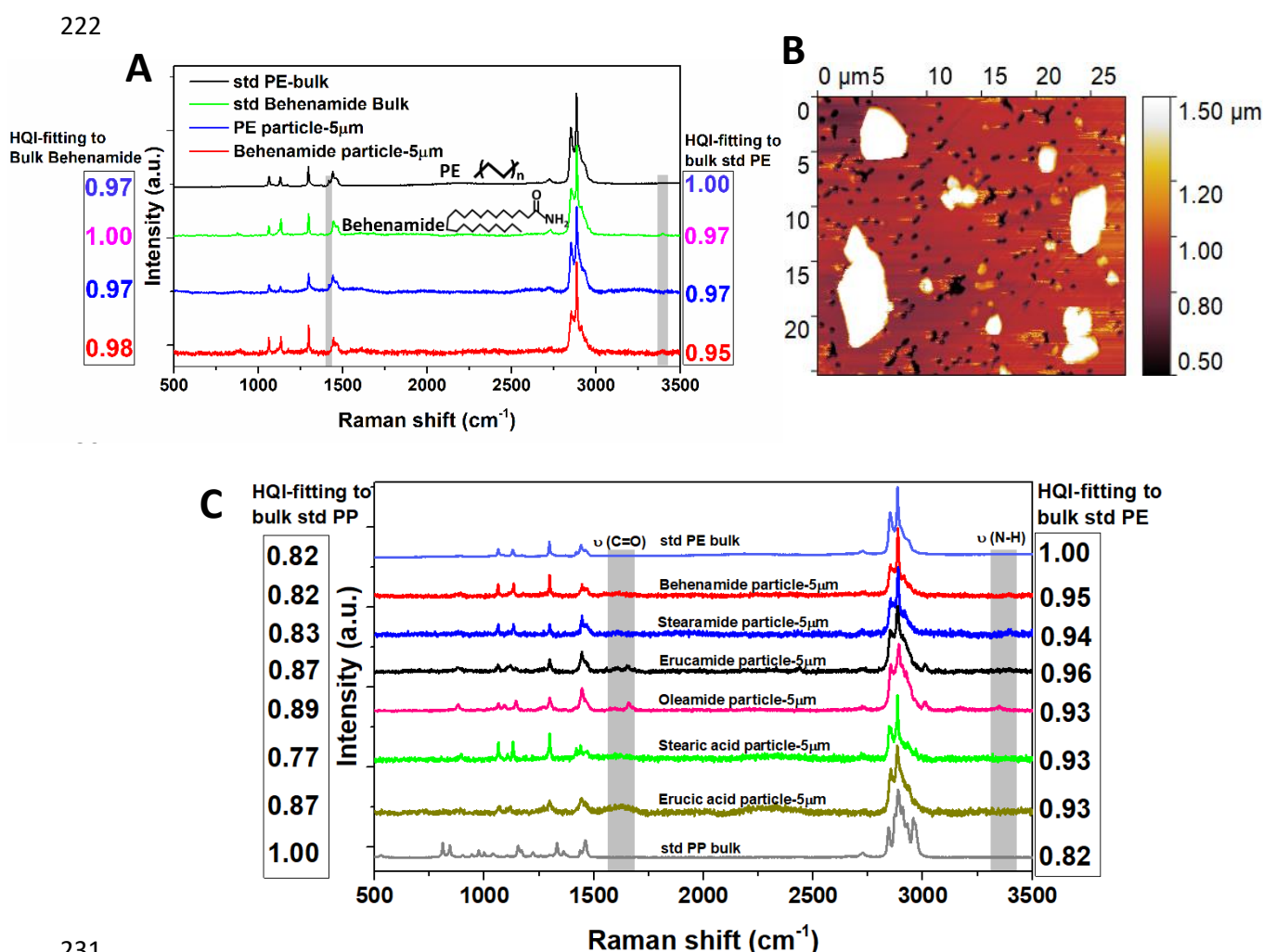
203 **3. RESULTS**

204 **3.1 Challenge of Raman spectroscopy to distinguish MAPs and MPs**

205 We begin with a Raman analysis of standard PE and behenamide (one typical slip additive used
206 in plastic, Figure 1A). The Raman spectra of these two bulk materials showed minor
207 differences. PE showed a small peak at 1415 cm⁻¹ associated with the vibration of CH₂,^{36, 39}
208 while there is a minor peak in the behenamide spectrum at around 3415 cm⁻¹ associated with
209 the NH-amine vibration. However, even for the bulk materials, these differences are too small
210 to distinguish behenamide from PE. The HQI between the bulk behenamide and PE is 0.97,
211 much higher than the typically accepted threshold value (0.70).^{30, 31, 34}

212 Behenamide powder and standard PE spheres were then dispersed in DI water and recaptured
213 using an Au-coated filter. Interestingly, a high number of irregular shaped micro-sized solid
214 particles were obtained from the behenamide sample (Figure 1B), which are remarkably similar

215 to the shape of MPs reported in previous publications.^{40, 41} When the particles size decreases to
 216 the micro scale, the two small Raman peaks differentiating the materials in the bulk spectra
 217 become even weaker and close to the signal-noise ratio level under typical Raman test setting
 218 used in MP studies.^{7, 28} For a 5- μm particle of behemamide, the HQI compared to PE bulk can
 219 reach 0.95, as its spectrum is very similar to that of a standard PE sphere (HQI=0.97, compared
 220 to PE bulk). Evidently, it is very difficult on the basis of Raman to distinguish between
 221 behenamide MAPs and PE MPs.



231
 232 **Figure 1.** (A) Raman spectra of PE and behenamide in bulk and micro sized particles. The standard
 233 bulk PE sheet obtained from Goodfellow. (B) AFM image of behenamide micro particles on filter
 234 surface. (C) Raman spectra of MAPs from six typical slip additives and standard PE and PP sheets.

235

236 In addition to the behenamide test, five typical slip additives were also separately dispersed in
237 DI water, re-captured and tested using Raman spectroscopy. In comparison to standard PE, the
238 HQIs for all additive particles were in the 0.93-0.96 range (Figure 1C), much higher than the
239 accepted threshold value (0.70). Moreover, the HQI of these additives compared to standard
240 PP are around 0.8-0.9 and so that these particles may also be easily *mis-assigned* as PP MPs.
241 This confirms that this interference problem is common across a wide range chemical additives
242 typically incorporated into plastics. Improvement in detection and analysis may be achieved
243 by specifically analysing narrow frequency ranges associated with characteristic peaks (e.g.,
244 1000-1500 cm^{-1} to distinguish between PE and slip additives, Figure S1), enhancing the
245 equipment sensitivity, modifying test protocols and analysis algorithms, but it is very difficult
246 for Raman to distinguish between chemical additives and MPs based on currently used MP test
247 protocol.²⁹⁻³¹

248 While most additives are insoluble in water (solubility <0.0005 g/100 g (0-60 °C) for stearic
249 acid in water,⁴² Figure S2), they can be dissolved and extracted using alcoholic solvents
250 (solubility 2.3 g/100 g (20°C) - 400 g/100 g (60°C) for stearic acid in ethanol⁴²⁻⁴⁵). Tests using
251 filter-captured oleamide particles show that they are dissolved and removed in a few minutes
252 after exposure to just one drop (20 μL) of ethanol (Video. S1). Crucially, exposure to ethanol
253 has no impact on MPs, as evidenced by the stability of 5 μm standard PE sphere after repeated
254 in-situ exposure tests to ethanol (Figure S3). This is consistent with a previous report that most
255 polymers are highly resistant to ethanol and methanol.⁴⁶

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257 **3.2 MAP release and its interference in detection of MPs from plastic products**

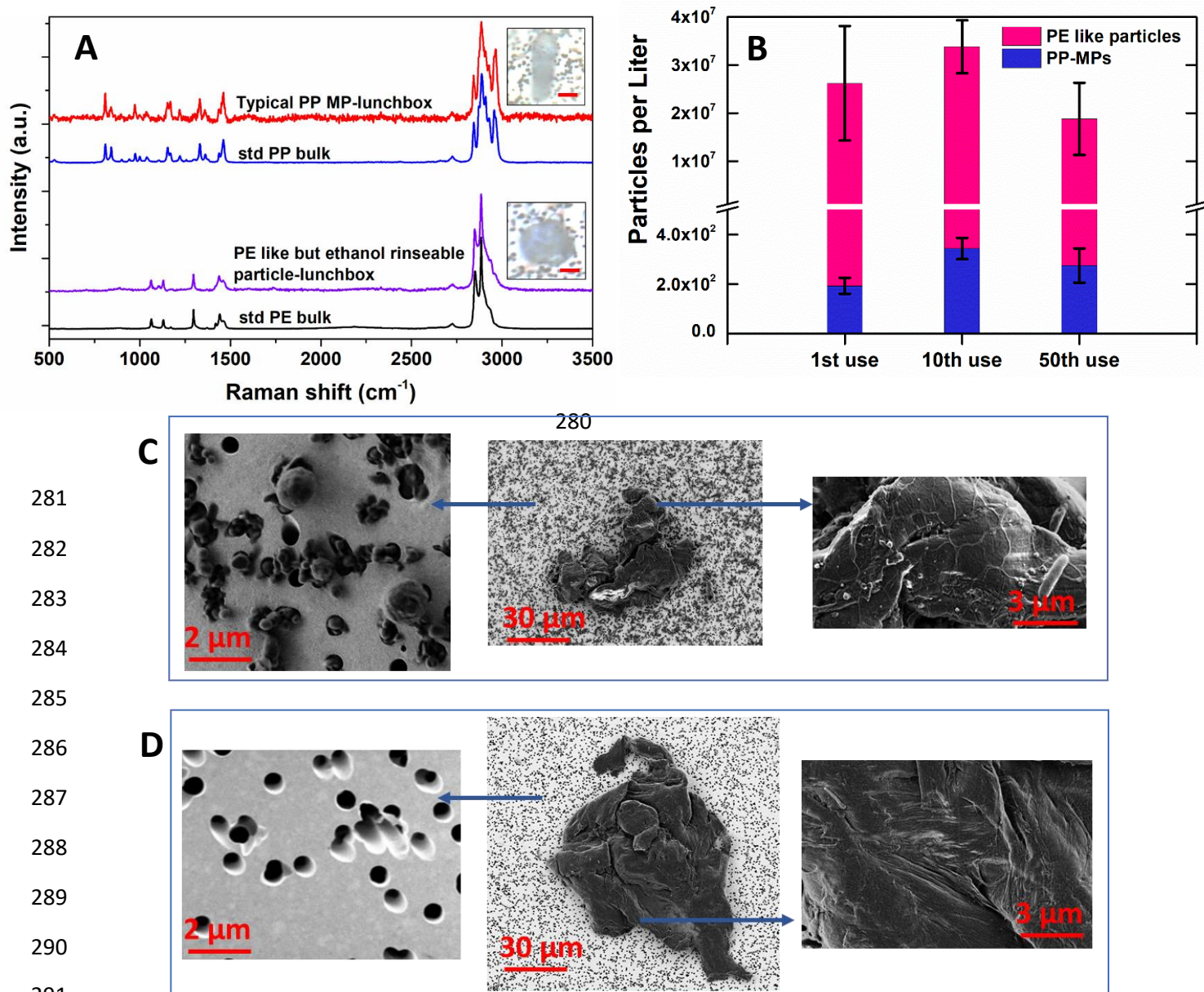
258 PP-based food containers were investigated to check for MAP interference under real-world
259 conditions. Raman-based chemical determination following the typical MPs protocol²⁹⁻³¹

260 confirmed high quantities ($26,000,000 \pm 11,900,000$ per liter) of PE-like MPs released into the
261 water samples during the first use. The HQIs of most tested particles compared to standard PE
262 were over 0.95 (Figure 2A, B). It is also noticeable that the HQIs of tested particles compared
263 to standard PP can reach 0.85-0.89 and so these particles could be *mis-assigned* as PP MPs
264 since they originated from a PP-based container. This high MP release level is comparable to
265 previous reports on plastic containers (releasing over 1 million MPs per liter^{26,47} or 1.2-7.6 mg
266 MPs from a single container⁴⁸). However, most of these confirmed MPs dissolved away when
267 the filter was placed onto a glass holder and rinsed using ethanol. FTIR tests of the dissolved
268 particles showed a significant peak associated with carbonyl (C=O) group (1740 cm^{-1}) and
269 library fitting indicates they are slip additives such as stearic acid (Figure S4). After the ethanol
270 rinse, a Raman analysis of the filter confirmed that the PE-like particles (HQIs > 0.95) were
271 completely removed. The remaining particles (230 ± 30 PP MPs per liter) exhibited HQIs
272 values compared to standard PP that were higher than 0.95, exhibiting clear characteristic peaks
273 at around 810 and 840 cm^{-1} associated with CH_2 rocking, C-C stretching and crystallinity of
274 PP.⁴⁹ Evidently, MAPs can substantially interfere with the determination of MP levels, in this
275 case up to 5 orders of magnitude difference in levels.

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278



292 **Figure 2.** (A) Typical Raman spectra of PE-like particle (from plastic food container), PP MP (from
 293 plastic food container), standard PE and PP bulk sheet, respectively. The standard bulk PE and PP sheet
 294 obtained from Goodfellow. The scale bars in the inserted images are 5 μm . (B) The quantities of PE-
 295 like particles and PP MPs during first to 50th test of the plastic food container. (C) SEM images of
 296 particles release from standard PE pellets before (upper panel) and (D) after (lower panel) ethanol rinse.
 297 All particles were on Au coated PC filter membrane.

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300

301 To check whether this interference is a short-lived effect due to the initial condition of the
302 manufactured product and/or the facile depletion of MAPs from the product surface region, the
303 food containers were repeatedly used 50 times and the water samples from the 10th and 50th run
304 were chemically analysed. Without the ethanol rinse, the PE-like MPs were always higher than
305 18 million per liter during the 50 runs. This result contrast strongly with the low MPs release
306 levels after the ethanol rinse, only 390 ± 40 and 340 ± 70 of PP MPs released during 10th and
307 50th use, respectively. On this basis, there is a consistent level of MAPs released and the
308 interference phenomenon persists over time. This is consistent with the ability of slip additives
309 to continuously migrate to the surface, even though they are generally blended inside the bulk
310 polymer.⁵⁰

311 Distinguishing between PE-like MAPs and PE MPs released from PE parent plastics is even
312 more difficult for Raman spectroscopy. Due to their widespread use in MPs studies,^{32, 33, 51, 52}
313 commercial standard PE pellets were investigated by shaking in DI water (25 °C). Two types
314 of particles were released: small ball-like particles (1-3 μm) and large irregular fragment (50-
315 100 μm) (Figure 2C). Raman determination confirmed both types of particles are PE MPs, with
316 HQIs compared to standard PE for the small ball-like particles and large fragments of 0.89 and
317 0.88, respectively (Figure S5). However, most of the small ball-like particles were ethanol
318 soluble. FTIR filtrate tests showed significant peaks associated with carbonyl (C=O) (1750 cm^{-1})
319 and amide (N-H₂) groups ($3200\text{-}3500\text{ cm}^{-1}$) while TOC-TN analyser confirmed that the
320 carbon to nitrogen molar ratio (C/N) of these ball-like particles is around 22:1. These results
321 indicates they are likely behenamide or erucamide. As to the large irregular fragments, the
322 quantity and size distribution remained unchanged after the ethanol rinse. Moreover, Raman
323 spectra of the insoluble large fragments exhibited of HQI of around 0.9 compared to standard
324 PE, which indicates that these fragments are *real* PE MPs.

325 Raman-based tests of bottle water caps (a typical PE product) also confirmed that high
326 quantities of PE-like particles are released in the water samples. These PE-like particles were
327 easily dissolved by alcohols, which confirmed that they are MAPs (Video. S2). GC-MS tests
328 showed that the retention time of the main peak from the MAPs released from cap sample was
329 20.5 min (Figure S6). Mass-to-charge ratio (m/z) analysis show that the chemical composition
330 of the main peak matched that of oleamide (Figure S6, with similarity of 94/100). Evidently,
331 the release of MAPs is a pervasive phenomenon regardless of plastic types, daily use scenario
332 and service duration.

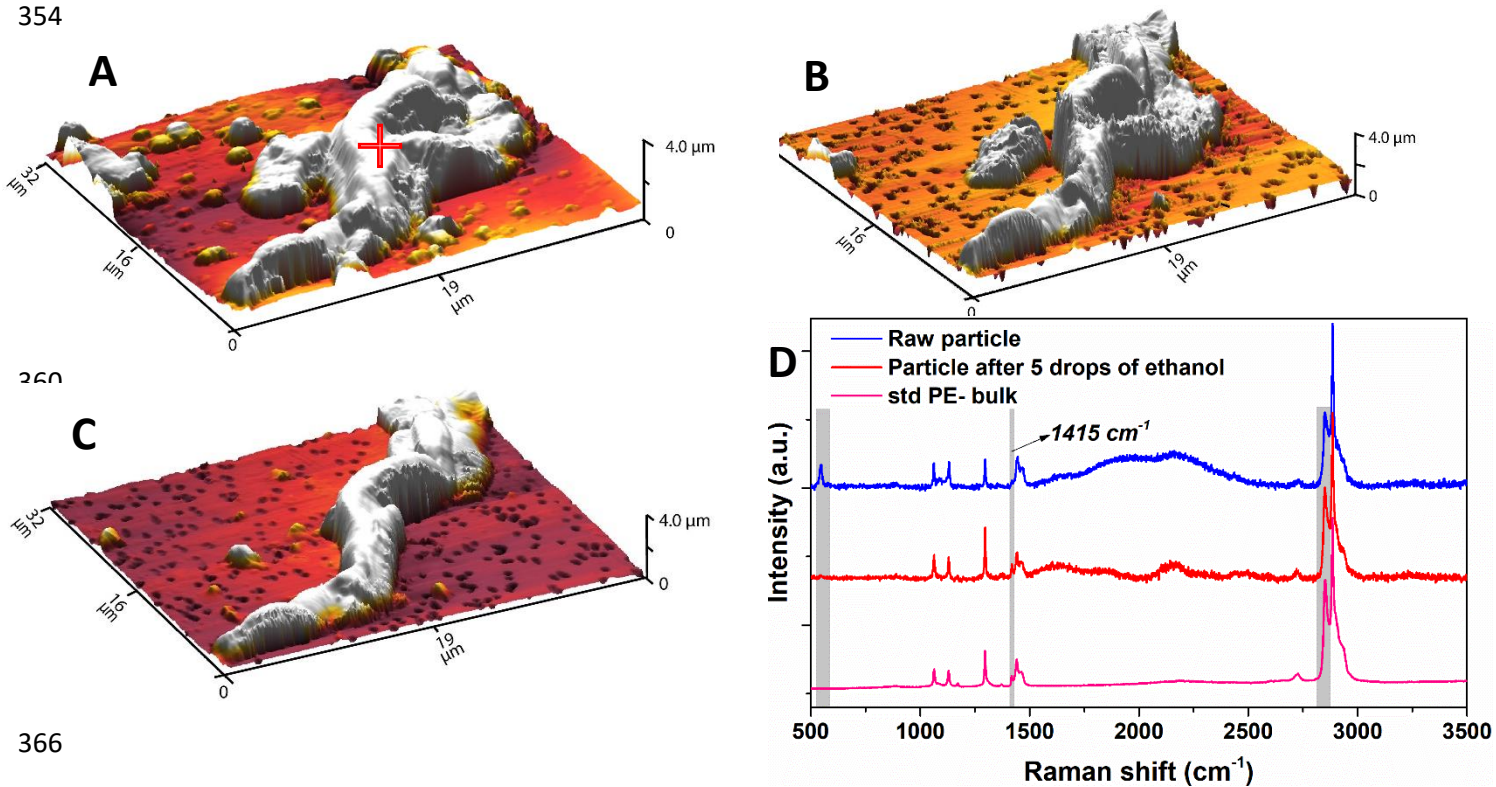
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334 **3.3 MAPs modify the surface of MPs**

335 MAPs can attach and modify the surfaces of MPs. Figure 3 shows a large irregular shaped
336 particle (labelled + in Figure 3A) released from a bottled water cap. Raman spectrum from the
337 labelled particle is similar to that of the parent polymer (PE). However, the peak at 1415 cm^{-1}
338 associated with the vibration of CH_2 from PE is very weak, which indicates this particle may
339 be a MAP or MAP/MP mixture. After exposure to 1 drop of ethanol (Figure 3B, in-situ test),
340 the particle topography was substantially changed, with the reduction on the right side of the
341 particle and the emergence of a separate smaller particle on the left. Following additional
342 ethanol treatment, the big irregular fragment was transformed into a fiber-like shape, with the
343 loss of material on the left and right (Figure 3C). After 5 drops of ethanol, further ethanol
344 treatment resulted in no significant change in the fiber shape. Raman analysis of the ethanol-
345 exposed particle showed that the PE vibration of CH_2 at 1415 cm^{-1} is visible, which indicates
346 that the mixed additive coating has been removed from the surface of the PE particle. Similarly,
347 for the case of PE pellets samples, it is evident that smaller particles can attach onto the surface
348 of large MPs (Figure 2C, D). After the ethanol rinse, the attached particles are completely
349 removed, which indicates they were likely MAPs. Organic surface coatings on MPs can

350 substantially enhance the cellular internalization capacity of MPs.^{53, 54} Recent studies have also
351 shown that MP shape is a critical determinant of health risks in humans.⁵⁵ Evidently, alcohol
352 pre-treatment can reveal/remove MAPs attached on MP surfaces, which enables the *real*
353 morphology and risks of MP to be determined.

354



366

367 **Figure 3.** In-situ test of micro-plastic and additives mixture particle release from bottle water cap. (A)
368 Raw particles captured using Au-coated PC filter. (B) Particle changes after the drop and dry of 1 drop
369 of ethanol. (C) Particle changes after the drop and dry of 5 drops of ethanol. (D) Raman spectra of the
370 raw particle, particle after 5 drops of ethanol and standard PE, respectively.

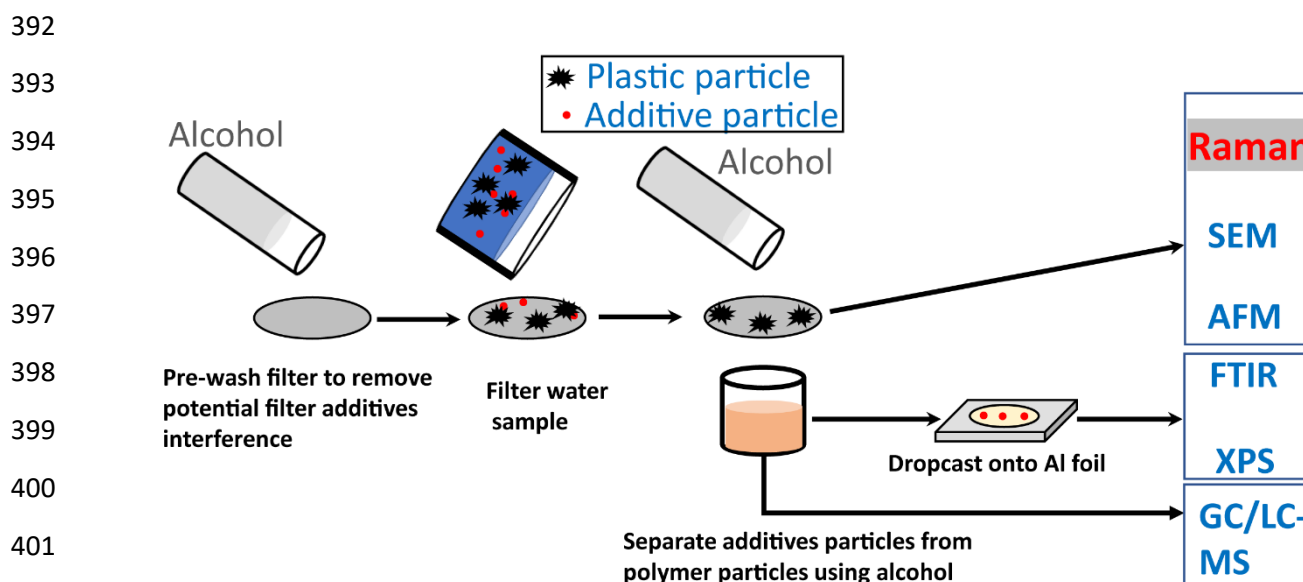
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372 **3.4 Protocol to test MAPs and MPs separately.**

373 To effectively separate MAPs and MPs and prevent misassignment, an alcohol rinse step was
374 incorporated into a modified protocol (Figure 4). The filter was first washed using alcohol
375 solvent to remove any possible additive contamination in the filter. After that, the filter was
376 used to capture the MPs and MAPs particles from the water sample. The captured particles
377 were then rinsed by alcohol. Tests using real samples (food containers, PE pellets samples,
378 etc.) confirmed that a rinse with ~30 mL ethanol/methanol for 30 mins is sufficient to remove

379 the interfering MAPs. Finally, the residual particles on filter surface were analysed using
380 Raman spectroscopy while the alcohol filtrate containing the dissolved MAPs was analysed
381 using other techniques (i.e., GC-MS).

382 To validate this protocol, a water sample was prepared by mixing standard PE spheres (3-16
383 μm) and stearic acid in DI water. The PE sphere level was 3000 particles per liter, which is
384 similar to the MP level previously reported.⁵⁶ Following the proposed protocol, the PE spheres
385 were collected and counted yielding a recovery rate of 93.8%, which is comparable to previous
386 report.⁵⁷ In addition, the presence of stearic acid dissolved in the methanol rinse was also
387 successfully identified using GC-MS (Figure S7). As to the topography of PE spheres, the
388 original round and smooth surfaces were initially modified by MAP attachment but fully
389 recovered to original smooth shape after the methanol rinse (Figure S8). Hence, the developed
390 protocol can successfully and effectively separate additives from MPs and avoid
391 misassignment, while facilitating the characterisation of the *true* topography of MPs.



403 **Figure 4.** Protocol to separate additives particles and plastic particles.

404

405

406 **4. DISSCUSSION**

407 **4.1 The challenges to avoid MAPs in MPs detection**

408 Accurately mimicking real world conditions is critical to quantitatively determine the levels of
409 MPs released from plastics, including their true morphology. For example, in the manufacturers'
410 instructions for microwaveable PP food containers, the contents (water and/or food) are
411 suggested to be thoroughly heated and allowed cool down before consuming. During this cool
412 down period the plastic container will continue to release MPs and MAPs at a rate and level
413 following which an accurate assessment of the risk to the consumer is possible, including the
414 formation of MAPs/MPs composite particles where MAPs become attached to the surfaces of
415 MPs (Figure 3). This is crucial to accurately assess MPs translocation inside the human body
416 and for a rigorous risk assessment of MPs to human health.⁵³

417 In this paper, we identified that plastics can release up to five orders of magnitude higher levels
418 of MAPs than MPs (Figure 2). Moreover, the small quantities of released MPs may be blended
419 or even covered by MAPs (Figure 3), making it extremely difficult or even impossible to find
420 and cleanly detect MPs using Raman. There are two possible contributions to this behaviour:
421 MAPs are initially released at the surface as mobile molecular species or clusters that aggregate
422 into a very small particles (less than 20 μm) when they come into contact with water (Figure
423 2). Another possible contribution is the migration of additives directly from the bulk to
424 creep/cover the plastic surface.^{22, 25, 50, 58} Our studies showed that an initial prewash of the
425 plastic part/product in ethanol had no substantial effect on the subsequent MAP release,
426 demonstrating that there is facile transport to the surface, consistent with the dominant release
427 of MAPs over MPs. Longitudinal studies will be required to determine the exact profile of the
428 MAP release and whether the level wanes after extended use, beyond the 50 product cycles
429 reported in this work (Figure 2B)

430 Although typical slip additives were characterized here, the potential interference from other
431 additives is also expected given that over 400 additives are used in just 10 types of plastics.⁵⁹
432 For example, BHT is a typical antioxidant widely used in PP. The HQI of BHT to PP is around
433 0.8, higher than the accepted threshold value. This indicates that water-insoluble BHT could
434 potentially interfere in the determination of PP MPs.

435 Great care must also be taken in the analysis of environmental and other organic-rich samples
436 such as the ground/marine water, sediments and soils samples. Particle separation based on
437 density differences are widely used in MP study.⁶⁰ However, this method is not able to separate
438 MAPs from MPs efficiently as these MAPs have similar density to LDPE (Table S1). In
439 addition, the melting point of behenamide and stearamide are similar to PE while others are
440 lower than that of PE. This indicates that the temperature-based separation method may only
441 be useful for certain additives. As to digestion, most studies (~ 60%) did not involve a digestion
442 process to remove organic material during sample isolation and preparation,⁶ which means that
443 there is potential blending and interference of MAPs during MP detection. Only a small portion
444 of these studies involve a sample digestion process aimed at removing environmentally-
445 introduced organic matter but even this will likely fail to remove MAPs effectively. For
446 example, digestion of behenamide using three typical chemicals (H₂O₂, HCl, and NaOH, 1 M,
447 60 °C 24 hours, following typical digestion processes^{57, 61-63}), fails to remove behenamide
448 MAPs.

449 In addition to micro-sized particles, the interference of nano-sized MAPs (NMAPs, < 1000 nm)
450 is also likely. Raman spectroscopy is the most widely used non-destructive technique for NMPs
451 characterisation.⁸⁻¹¹ Our case study confirmed the high quantities of NMAPs are released from
452 PE plastic (Figure S9). However, the potential interference of NMAPs in NMPs determination
453 is expected to be even greater given that the signal/noise ratio decreases as the MPs size
454 decreases (Figure 1). Hence, the prevention of NMAPs interference is also essential.

455 **4.2 The misassignment of MPs and MAPs**

456 Given the similarity between some MAPs and MPs, the high quantity of MAPs release and the
457 lack of preventative steps (~ 60% of MPs studies did not involve a digestion pre-treatment⁶), it
458 is likely that MAPs interference played a significant role in many previous MPs studies. For
459 some reported PE MPs confirmed by Raman analysis, there was no vibration of CH₂ at around
460 1420 cm⁻¹ ^{7, 9, 10, 28} but instead the presence of an extra peak at around 3100 cm⁻¹.^{7, 28} In addition,
461 a peak around 1750 cm⁻¹ associated with carbonyl group was also observed in the case of other
462 confirmed PE MPs.²⁸ These minor changes in the Raman spectra are generally attributed to
463 environmental weathering and/or the inclusion of additional compounds and pigments.²⁸
464 However, it is evident that pure MAPs can account for these Raman features (Figures 1-2).
465 HQI analysis based on a full spectrum comparison is widely used in MPs studies but it is
466 difficult to distinguish and account for minor differences (Figures 1-3). Additionally, in some
467 instances just a few characteristic vibrational bands associated with the specific polymer were
468 used for MPs determination and chemical spectra mapping.⁶⁴⁻⁶⁶ However, comparing MAPs of
469 typical slip additives to standard PP using the spectra range between 2500-3200 cm⁻¹ (Figures
470 1 and 2), the characteristic peaks at 2720, 2850 and 2880 cm⁻¹ perfectly match that of PP. The
471 comparison to standard PP using this narrow spectral range yields HQIs for typical slip
472 additives' particles (i.e., oleamide and erucic acid) of over 0.9, much higher than the accepted
473 threshold value.

474 MAP misassignment clearly occurred in studies of plastic food containers. There were over a
475 million MPs per liter released in the present study and in previous reports.^{26, 47, 48} However,
476 after an ethanol rinse, the MPs level dropped by 5 orders of magnitude in this study. Similarly,
477 while large quantities of MP were reported to be released from plastic baby feeding bottles^{3, 37}
478 our re-assessment based on the new alcohol protocol showed that the levels of MPs decreased
479 from over 1 million per liter to around 100-100,000 per liter, which depends on multiple factors,

480 such as the brand type and duration of use. Beyond the cases mentioned above, the problem of
481 unintentional misassignment of MAPs in previous MPs studies is significant and primarily due
482 to the unanticipated limitations of the detection technologies and sampling protocols used.
483 Evidently, a re-assessment of the MPs release levels is required, especially in instances where
484 extremely high levels were reported.

485 The misassignment of MPs and MAPs can also result in an inaccurate risk assessment and false
486 management given their different physicochemical properties and toxicity. Firstly, while MAPs
487 are particles, they are easily broken down into their constituent molecules. MPs are highly
488 recalcitrant macromolecules that are inert, with very poor biodegradability.⁶⁷ For instance,
489 there is only negligible weight loss observed when PE was kept in moist soil for 12-32 years.⁶⁷
490 In contrast, many MAPs have good biodegradability (European Chemicals Agency). For
491 instance, over 70% of stearic acid mixed in soils can be biodegraded to CO₂ within 3 months.⁶⁸
492 The toxicity of MPs and MAPs to human health can be substantially different. For MAPs, it
493 was reported that BHT chronically damages the liver, such as increase the liver weight and
494 enzymes activity.^{17, 69} The chronic exposure to erucic acid can lead to myocardial lipidosis,
495 though this effect is reversible and transient.⁷⁰ As for erucamide, oleamide, behenamide,
496 stearamide and stearic acid, EU Commission regulation¹⁸ pointed out that they are allowed in
497 the production of food grade plastic materials, which indicates that they should have
498 insignificant risk to humans. An assessment conducted by the Canadian Environment and
499 Health Department also confirmed that erucamide and oleamide have low toxicity to human
500 health.⁷¹ As for MPs, a previous study found that MPs smaller than 10 µm can translocate and
501 accumulate in tissues, such as liver and kidney.⁵ However, the specific risks of MPs to human
502 health is still unknown⁵ although the negative health impact of MPs on animals is widely
503 reported.⁷²⁻⁷⁴ Currently, studies focusing on MPs toxicity characterization use both commercial
504 MP spheres and real irregular MPs generated from bulk plastics. However, there was no

505 consideration given to the potential levels of additives in these MPs samples. The types and
506 concentrations of additives in MPs can be significantly different. For instance, antioxidant
507 additives can range from 0.05% to 3% (by mass) in the same type of plastics.²⁰ It is interesting
508 to note that toxicity studies using the same type of MPs draw contradicting conclusions⁵⁵,
509 which may reflect different levels of additives incorporated in plastics sourced from different
510 manufacturers. Obviously, this requires further investigation. However, it should be noted that
511 the mixing of MAPs and MPs and the coating of the latter by the former may enhance the
512 cellular internalization capacity and alter the toxicity of MPs. Clearly, it is crucial to have an
513 effective method to separate and investigate MPs and MAPs to accurately assess such risks.

514 **4.3 Cost-effective method to distinguish between MAPs and MPs**

515 To date, there is still no consensus on a standard MP test method.⁷⁵ Researchers and regulators
516 are making huge efforts to develop reliable MP detection methodologies. Recently, the
517 California Water Boards developed a MP analytical method that involves spiking water
518 samples with typical polymer particles (PE, PS, etc.) and conducting a cross-laboratory
519 validation.⁷⁶ However, due to the omission of MAPs, this approach will likely be incapable of
520 accurately determining MPs in real-world water samples that will unavoidably contain
521 significant quantities of polymer-like MAPs.

522 Our proposed protocol with an alcoholic solvent rinse step achieved effective separation of
523 interfering MAPs from MPs and enabled quantitative chemical analysis of each separately.
524 Interestingly, 50% ethanol treatment was previously employed in an MPs study to destroy the
525 foam generated during filtration⁵⁶, which indicates that ethanol treatment may have multiple
526 advantages in MPs detection. While here we used Au-coated PC filter, the alcohol rinse is also
527 suitable for other types of filters, such as aluminium oxide filter in our preliminary test.
528 However, alcoholic solvents may not be effective for all MAPs used in plastics or other organic
529 matter introduced from the surrounding environment. Further improvements are possible and

530 achievable. Increasing the ethanol temperature is an effective method to substantially increase
531 the solubility of many organic additives^{42, 45} (Figure S2). It should be noted that directly adding
532 ethanol into water samples at room temperature is not effective for removing MAPs due to the
533 extremely low solubility of additives in water/ethanol mixture regardless of the mixing ratio.⁴⁵
534 However, raising the temperature or changing the solvent can effectively increase the
535 solubility. In addition, directly adding isopropanol into water samples at room temperature in
536 the ratio of 50/50 (volume) should be feasible to remove MAPs interference.⁷⁷ Other solvents
537 such as acetone and polysorbate 80 are also potential choices. While there are numerous solvent
538 combinations, it is critical to ensure the solvents do not damage the target MPs.

539 Taken together, we have demonstrated that additives and polymers, the two major components
540 of plastics, can simultaneously release micron-sized particles, which substantially interfere
541 with an accurate determination of MPs release levels using Raman spectroscopy. Alcoholic
542 solvent pre-treatment is proved to be a cost-effective method to enable the separation, detection
543 and analysis of MPs and MAPs. By tuning the solvent types, pre-treatment conditions and
544 filtration methods, researchers can systematically explore the release quantity/size, the
545 attachment/detachment process and morphology changes of MAPs and MPs under different
546 conditions. Crucially, the approach provided here can be used to re-assess the accuracy of
547 previous MPs studies and avoid potential misleading results in future MPs research.

548

549 **ASSOCIATED CONTENT**

550 **Supporting Information**

551 The Supporting Information associated with this article can be found in another file (9 Figures,
552 1 Table and 2 Videos).

553 Methods for in-situ alcohol treatment; methods for particle characterization using SEM, AFM,
554 FTIR, etc; videos for in-situ alcohol treatment; Raman, FTIR and GC/MS test results for

555 additives and microplastics, respectively; stearic acid solubility in water and ethanol,
556 respectively; In-situ ethanol test of standard PE sphere; SEM images of PE coated by additives
557 and rinsed by ethanol, respectively; nano-sized additives particles; information summary of
558 typical additives and polymers.

559

560 **AUTHOR INFORMATION**

561 **Author Contributions**

562 **J.J.W. L.X.** and **J.J.B** conceptualized and supervised the study, reviewed and edited the manuscript,
563 **D.L** performed the experiment, analysed the data and wrote the original manuscript, **E.S** performed the
564 experiment (FTIR and SEM), **Y.S** and **L.Y** prepared samples and performed the experiment (Raman),
565 and all authors provided comments on the manuscript.

566 **Notes**

567 The authors declare that they have no known competing financial interests.

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588 REFERENCES

- 589 1. European Chemical Agency. ECHA public consultation on the restriction dossier for
590 microplastics intentionally added to products. [https://ec.europa.eu/growth/news/echa-public-](https://ec.europa.eu/growth/news/echa-public-consultation-restriction-dossier-microplastics-intentionally-added-products-2019-03-28_en)
591 [consultation-restriction-dossier-microplastics-intentionally-added-products-2019-03-28_en](https://ec.europa.eu/growth/news/echa-public-consultation-restriction-dossier-microplastics-intentionally-added-products-2019-03-28_en)
592 (accessed Jan 2022)
- 593 2. Law, K. L.; Thompson, R. C., Microplastics in the seas. *Science* **2014**, *345*, (6193), 144-145.
- 594 3. Su, Y.; Hu, X.; Tang, H.; Lu, K.; Li, H.; Liu, S.; Xing, B.; Ji, R., Steam disinfection releases micro
595 (nano) plastics from silicone-rubber baby teats as examined by optical photothermal infrared
596 microspectroscopy. *Nature Nanotechnology* **2021**, 1-10.
- 597 4. Rillig, M. C.; Lehmann, A., Microplastic in terrestrial ecosystems. *Science* **2020**, *368*, (6498),
598 1430-1431.
- 599 5. Vethaak, A. D.; Legler, J., Microplastics and human health. *Science* **2021**, *371*, (6530), 672-
600 674.
- 601 6. Prata, J. C.; da Costa, J. P.; Duarte, A. C.; Rocha-Santos, T., Methods for sampling and
602 detection of microplastics in water and sediment: a critical review. *TrAC Trends in Analytical*
603 *Chemistry* **2019**, *110*, 150-159.
- 604 7. K  ppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.-J.;
605 Voit, B., Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or
606 both? *Analytical and bioanalytical chemistry* **2016**, *408*, (29), 8377-8391.
- 607 8. Cai, H.; Xu, E. G.; Du, F.; Li, R.; Liu, J.; Shi, H., Analysis of environmental nanoplastics:
608 progress and challenges. *Chemical Engineering Journal* **2020**, 128208.
- 609 9. Ivleva, N. P., Chemical analysis of microplastics and nanoplastics: Challenges, advanced
610 methods, and perspectives. *Chemical Reviews* **2021**, *121*, (19), 11886-11936.
- 611 10. Gillibert, R.; Balakrishnan, G.; Deshoules, Q.; Tardivel, M.; Magazz  , A.; Donato, M. G.;
612 Marag  , O. M.; Lamy de La Chapelle, M.; Colas, F.; Lagarde, F., Raman Tweezers for Small
613 Microplastics and Nanoplastics Identification in Seawater. *Environmental science & technology* **2019**,
614 *53*, (15), 9003-9013.
- 615 11. Xu, G.; Cheng, H.; Jones, R.; Feng, Y.; Gong, K.; Li, K.; Fang, X.; Tahir, M. A.; Valev, V. K.;
616 Zhang, L., Surface-Enhanced Raman Spectroscopy Facilitates the Detection of Microplastics < 1 μm in
617 the Environment. *Environmental science & technology* **2020**, *54*, (24), 15594-15603.
- 618 12. Gigault, J.; Ter Halle, A.; Baudrimont, M.; Pascal, P.-Y.; Gauffre, F.; Phi, T.-L.; El Hadri, H.;
619 Grassl, B.; Reynaud, S., Current opinion: what is a nanoplastic? *Environmental pollution* **2018**, *235*,
620 1030-1034.

621 13. Hartmann, N. B.; Hüffer, T.; Thompson, R. C.; Hassellöv, M.; Verschoor, A.; Daugaard, A. E.;
622 Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; Herrling, M. P.; Hess, M. C.; Ivleva, N. P.; Lusher, A. L.;
623 Wagner, M., Are We Speaking the Same Language? Recommendations for a Definition and
624 Categorization Framework for Plastic Debris. *Environmental science & technology* **2019**, *53*, (3),
625 1039-1047.

626 14. Farber, C.; Li, J.; Hager, E.; Chemelewski, R.; Mullet, J.; Rogachev, A. Y.; Kourouski, D.,
627 Complementarity of raman and infrared spectroscopy for structural characterization of plant
628 epicuticular waxes. *ACS Omega* **2019**, *4*, (2), 3700-3707.

629 15. Galloway, T. S.; Cole, M.; Lewis, C., Interactions of microplastic debris throughout the marine
630 ecosystem. *Nature Ecology & Evolution* **2017**, *1*, (5), 1-8.

631 16. Geyer, R.; Jambeck, J. R.; Law, K. L., Production, use, and fate of all plastics ever made.
632 *Science advances* **2017**, *3*, (7), e1700782.

633 17. Bernauer, U.; Bodin, L.; Chaudhry, Q.; Coenraads, P.; Dusinska, M.; Ezendam, J.; Gaffet, E.;
634 Galli, C.; Granum, B.; Panteri, E. *SCCS OPINION on Butylated Hydroxytoluene (BHT)-SCCS/1636/21-*
635 *Preliminary version*; 2021; p 35.

636 18. Regulation, E., No 10/2011 (2011). *Commission Regulation of 14 January 2011 on*
637 *Plastic Materials and Articles Intended to Come into Contact with Food* **2011**.

638 19. Cooper, I.; Tice, P. A., Migration studies on fatty acid amide slip additives from plastics into
639 food simulants. *Food Additives & Contaminants* **1995**, *12*, (2), 235-244.

640 20. Hahladakis, J. N.; Velis, C. A.; Weber, R.; Iacovidou, E.; Purnell, P., An overview of chemical
641 additives present in plastics: Migration, release, fate and environmental impact during their use,
642 disposal and recycling. *Journal of hazardous materials* **2018**, *344*, 179-199.

643 21. Winkler, A.; Santo, N.; Ortenzi, M. A.; Bolzoni, E.; Bacchetta, R.; Tremolada, P., Does
644 mechanical stress cause microplastic release from plastic water bottles? *Water research* **2019**, *166*,
645 115082.

646 22. Dulal, N.; Shanks, R.; Gengenbach, T.; Gill, H.; Chalmers, D.; Adhikari, B.; Martinez, I. P., Slip-
647 additive migration, surface morphology, and performance on injection moulded high-density
648 polyethylene closures. *Journal of colloid and interface science* **2017**, *505*, 537-545.

649 23. Yvon, H. J. Raman spectroscopy for analysis and monitoring.
650 [https://static.horiba.com/fileadmin/Horiba/Technology/Measurement_Techniques/Molecular Spec](https://static.horiba.com/fileadmin/Horiba/Technology/Measurement_Techniques/Molecular_Spectroscopy/Raman_Spectroscopy/Raman_Academy/Raman_Tutorial/Raman_bands.pdf)
651 [troscopy/Raman Spectroscopy/Raman Academy/Raman Tutorial/Raman bands.pdf](https://static.horiba.com/fileadmin/Horiba/Technology/Measurement_Techniques/Molecular_Spectroscopy/Raman_Spectroscopy/Raman_Academy/Raman_Tutorial/Raman_bands.pdf)

652 24. Helmroth, E.; Rijk, R.; Dekker, M.; Jongen, W., Predictive modelling of migration from
653 packaging materials into food products for regulatory purposes. *Trends in Food Science &*
654 *Technology* **2002**, *13*, (3), 102-109.

655 25. Sheftel, V. O., *Indirect food additives and polymers: migration and toxicology*. CRC Press:
656 2000.

657 26. He, Y.-J.; Qin, Y.; Zhang, T.-L.; Zhu, Y.-Y.; Wang, Z.-J.; Zhou, Z.-S.; Xie, T.-Z.; Luo, X.-D.,
658 Migration of (non-) intentionally added substances and microplastics from microwavable plastic food
659 containers. *Journal of hazardous materials* **2021**, *417*, 126074.

660 27. Gerhard, M. N.; Schymanski, D.; Ebner, I.; Esselen, M.; Stahl, T.; Humpf, H.-U., Can the
661 presence of additives result in false positive errors for microplastics in infant feeding bottles? *Food*
662 *Additives & Contaminants: Part A* **2021**, 1-13.

663 28. Erni-Cassola, G.; Gibson, M. I.; Thompson, R. C.; Christie-Oleza, J. A., Lost, but found with
664 Nile red: a novel method for detecting and quantifying small microplastics (1 mm to 20 µm) in
665 environmental samples. *Environmental science & technology* **2017**, *51*, (23), 13641-13648.

666 29. Tong, H.; Jiang, Q.; Hu, X.; Zhong, X., Occurrence and identification of microplastics in tap
667 water from China. *Chemosphere* **2020**, *252*, 126493.

668 30. Karami, A.; Golieskardi, A.; Choo, C. K.; Larat, V.; Galloway, T. S.; Salamatinia, B., The
669 presence of microplastics in commercial salts from different countries. *Scientific Reports* **2017**, *7*, (1),
670 1-11.

- 671 31. Schymanski, D.; Goldbeck, C.; Humpf, H.-U.; Fürst, P., Analysis of microplastics in water by
672 micro-Raman spectroscopy: release of plastic particles from different packaging into mineral water.
673 *Water research* **2018**, *129*, 154-162.
- 674 32. Da Costa, J. P.; Nunes, A. R.; Santos, P. S.; Girao, A. V.; Duarte, A. C.; Rocha-Santos, T.,
675 Degradation of polyethylene microplastics in seawater: Insights into the environmental degradation
676 of polymers. *Journal of Environmental Science and Health, Part A* **2018**, *53*, (9), 866-875.
- 677 33. Song, Y. K.; Hong, S. H.; Jang, M.; Han, G. M.; Jung, S. W.; Shim, W. J., Combined effects of UV
678 exposure duration and mechanical abrasion on microplastic fragmentation by polymer type.
679 *Environmental science & technology* **2017**, *51*, (8), 4368-4376.
- 680 34. Woodall, L. C.; Sanchez-Vidal, A.; Canals, M.; Paterson, G. L.; Coppock, R.; Sleight, V.; Calafat,
681 A.; Rogers, A. D.; Narayanaswamy, B. E.; Thompson, R. C., The deep sea is a major sink for
682 microplastic debris. *Royal Society open science* **2014**, *1*, (4), 140317.
- 683 35. Strobl, G.; Hagedorn, W., Raman spectroscopic method for determining the crystallinity of
684 polyethylene. *Journal of Polymer Science: Polymer Physics Edition* **1978**, *16*, (7), 1181-1193.
- 685 36. Pigeon, M.; Prud'Homme, R. E.; Pezolet, M., Characterization of molecular orientation in
686 polyethylene by Raman spectroscopy. *Macromolecules* **1991**, *24*, (20), 5687-5694.
- 687 37. Li, D.; Shi, Y.; Yang, L.; Xiao, L.; Kehoe, D. K.; Gun'ko, Y. K.; Boland, J. J.; Wang, J. J.,
688 Microplastic release from the degradation of polypropylene feeding bottles during infant formula
689 preparation. *Nature Food* **2020**.
- 690 38. Li, D.; Yang, L.; Kavanagh, R.; Xiao, L.; Shi, Y.; Kehoe, D. K.; Sheerin, E. D.; Gun'ko, Y. K.;
691 Boland, J. J.; Wang, J. J., Sampling, Identification and Characterization of Microplastics Release from
692 Polypropylene Baby Feeding Bottle during Daily Use. *JoVE* **2021**, e62545.
- 693 39. Naylor, C. C.; Meier, R. J.; Kip, B. J.; Williams, K. P.; Mason, S. M.; Conroy, N.; Gerrard, D. L.,
694 Raman spectroscopy employed for the determination of the intermediate phase in polyethylene.
695 *Macromolecules* **1995**, *28*, (8), 2969-2978.
- 696 40. World Health Organization. Microplastics in drinking-water. **2019**.
- 697 41. Choi, J. S.; Jung, Y.-J.; Hong, N.-H.; Hong, S. H.; Park, J.-W., Toxicological effects of irregularly
698 shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon*
699 *variegatus*). *Marine pollution bulletin* **2018**, *129*, (1), 231-240.
- 700 42. Ralston, A.; Hoerr, C., The solubilities of the normal saturated fatty acids. *The Journal of*
701 *Organic Chemistry* **1942**, *7*, (6), 546-555.
- 702 43. Calvo, B.; Cepeda, E. A., Solubilities of stearic acid in organic solvents and in azeotropic
703 solvent mixtures. *Journal of Chemical & Engineering Data* **2008**, *53*, (3), 628-633.
- 704 44. Heryanto, R.; Hasan, M.; Abdullah, E. C.; Kumoro, A. C., Solubility of stearic acid in various
705 organic solvents and its prediction using non-ideal solution models. *ScienceAsia* **2007**, *33*, 469-472.
- 706 45. Noubigh, A., Stearic acid solubility in mixed solvents of (water+ ethanol) and (ethanol+ ethyl
707 acetate): Experimental data and comparison among different thermodynamic models. *Journal of*
708 *Molecular Liquids* **2019**, *296*, 112101.
- 709 46. Scientific Thermofisher. chemical compatibility guide.
710 <https://tools.thermofisher.com/content/sfs/brochures/D20480.pdf>
- 711 47. Liu, G.; Wang, J.; Wang, M.; Ying, R.; Li, X.; Hu, Z.; Zhang, Y., Disposable plastic materials
712 release microplastics and harmful substances in hot water. *Science of The Total Environment* **2021**,
713 151685.
- 714 48. Fadare, O. O.; Wan, B.; Guo, L.-H.; Zhao, L., Microplastics from consumer plastic food
715 containers: Are we consuming it? *Chemosphere* **2020**, *253*, 126787.
- 716 49. Nielsen, A. S.; Batchelder, D.; Pyrz, R., Estimation of crystallinity of isotactic polypropylene
717 using Raman spectroscopy. *Polymer* **2002**, *43*, (9), 2671-2676.
- 718 50. Wakabayashi, M.; Kohno, T.; Kimura, T.; Tamura, S.; Endoh, M.; Ohnishi, S.; Nishioka, T.;
719 Tanaka, Y.; Kanai, T., New bleeding model of additives in a polypropylene film under atmospheric
720 pressure. *Journal of applied polymer science* **2007**, *104*, (6), 3751-3757.

- 721 51. Paço, A.; Duarte, K.; da Costa, J. P.; Santos, P. S.; Pereira, R.; Pereira, M.; Freitas, A. C.;
722 Duarte, A. C.; Rocha-Santos, T. A., Biodegradation of polyethylene microplastics by the marine
723 fungus *Zalerion maritimum*. *Science of the Total Environment* **2017**, *586*, 10-15.
- 724 52. Holmes, L. A.; Turner, A.; Thompson, R. C., Adsorption of trace metals to plastic resin pellets
725 in the marine environment. *Environmental Pollution* **2012**, *160*, 42-48.
- 726 53. Ramsperger, A.; Narayana, V.; Gross, W.; Mohanraj, J.; Thelakkat, M.; Greiner, A.; Schmalz,
727 H.; Kress, H.; Laforsch, C., Environmental exposure enhances the internalization of microplastic
728 particles into cells. *Science Advances* **2020**, *6*, (50), eabd1211.
- 729 54. Ramsperger, A.; Jasinski, J.; Völkl, M.; Witzmann, T.; Meinhart, M.; Jérôme, V.; Kretschmer,
730 W.; Freitag, R.; Senker, J.; Fery, A., Supposedly identical microplastic particles substantially differ in
731 their material properties influencing particle-cell interactions and cellular responses. *Journal of*
732 *hazardous materials* **2022**, *425*, 127961.
- 733 55. Danopoulos, E.; Twiddy, M.; West, R.; Rotchell, J. M., A rapid review and meta-regression
734 analyses of the toxicological impacts of microplastic exposure in human cells. *Journal of hazardous*
735 *materials* **2021**, 127861.
- 736 56. Oßmann, B. E.; Sarau, G.; Holtmannspötter, H.; Pischetsrieder, M.; Christiansen, S. H.; Dicke,
737 W., Small-sized microplastics and pigmented particles in bottled mineral water. *Water research*
738 **2018**, *141*, 307-316.
- 739 57. Zhao, S.; Danley, M.; Ward, J. E.; Li, D.; Mincer, T. J., An approach for extraction,
740 characterization and quantitation of microplastic in natural marine snow using Raman microscopy.
741 *Analytical methods* **2017**, *9*, (9), 1470-1478.
- 742 58. Gall, M.; Schweighuber, A.; Buchberger, W.; W Lang, R., Plastic bottle cap recycling—
743 characterization of recycle composition and opportunities for design for circularity. *Sustainability*
744 **2020**, *12*, (24), 10378.
- 745 59. European Chemicals Agency (ECHA), Plastic additives initiative (accessed December 07).
746 <https://echa.europa.eu/et/plastic-additives-initiative>
- 747 60. Quinn, B.; Murphy, F.; Ewins, C., Validation of density separation for the rapid recovery of
748 microplastics from sediment. *Analytical Methods* **2017**, *9*, (9), 1491-1498.
- 749 61. Hurley, R. R.; Lusher, A. L.; Olsen, M.; Nizzetto, L., Validation of a method for extracting
750 microplastics from complex, organic-rich, environmental matrices. *Environmental science &*
751 *technology* **2018**, *52*, (13), 7409-7417.
- 752 62. Sujathan, S.; Kniggendorf, A.-K.; Kumar, A.; Roth, B.; Rosenwinkel, K.-H.; Nogueira, R., Heat
753 and bleach: a cost-efficient method for extracting microplastics from return activated sludge.
754 *Archives of environmental contamination and toxicology* **2017**, *73*, (4), 641-648.
- 755 63. Avio, C. G.; Gorbi, S.; Regoli, F., Experimental development of a new protocol for extraction
756 and characterization of microplastics in fish tissues: first observations in commercial species from
757 Adriatic Sea. *Marine environmental research* **2015**, *111*, 18-26.
- 758 64. Adams, J. K.; Dean, B. Y.; Athey, S. N.; Jantunen, L. M.; Bernstein, S.; Stern, G.; Diamond, M.
759 L.; Finkelstein, S. A., Anthropogenic particles (including microfibers and microplastics) in marine
760 sediments of the Canadian Arctic. *Science of The Total Environment* **2021**, *784*, 147155.
- 761 65. Renner, G.; Nellessen, A.; Schwieters, A.; Wenzel, M.; Schmidt, T. C.; Schram, J., Data
762 preprocessing & evaluation used in the microplastics identification process: A critical review &
763 practical guide. *TrAC Trends in Analytical Chemistry* **2019**, *111*, 229-238.
- 764 66. Oßmann, B. E.; Sarau, G.; Schmitt, S. W.; Holtmannspötter, H.; Christiansen, S. H.; Dicke, W.,
765 Development of an optimal filter substrate for the identification of small microplastic particles in
766 food by micro-Raman spectroscopy. *Analytical and bioanalytical chemistry* **2017**, *409*, (16), 4099-
767 4109.
- 768 67. Ghatge, S.; Yang, Y.; Ahn, J.-H.; Hur, H.-G., Biodegradation of polyethylene: a brief review.
769 *Applied Biological Chemistry* **2020**, *63*, (1), 1-14.
- 770 68. Ward, T.; Larson, R., Biodegradation kinetics of linear alkylbenzene sulfonate in sludge-
771 amended agricultural soils. *Ecotoxicology and environmental safety* **1989**, *17*, (1), 119-130.

- 772 69. European Food Safety Authority, Scientific Opinion on the re - evaluation of butylated
773 hydroxytoluene BHT (E 321) as a food additive. *EFSA Journal* **2012**, *10*, (3), 2588.
- 774 70. EFSA Panel on Contaminants in the Food Chain.; Knutsen, H. K.; Alexander, J.; Barregård, L.;
775 Bignami, M.; Brüschweiler, B.; Ceccatelli, S.; Dinovi, M.; Edler, L.; Grasl - Kraupp, B., Erucic acid in
776 feed and food. *EFSA Journal* **2016**, *14*, (11), e04593.
- 777 71. Environment and Climate Change Canada.; Health Canada. *Draft Screening Assessment Fatty*
778 *Amides Group*; 2018.
- 779 72. Mattsson, K.; Johnson, E. V.; Malmendal, A.; Linse, S.; Hansson, L.-A.; Cedervall, T., Brain
780 damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the
781 food chain. *Scientific reports* **2017**, *7*, (1), 11452.
- 782 73. Jin, Y.; Lu, L.; Tu, W.; Luo, T.; Fu, Z., Impacts of polystyrene microplastic on the gut barrier,
783 microbiota and metabolism of mice. *Science of the total environment* **2019**, *649*, 308-317.
- 784 74. Lu, L.; Wan, Z.; Luo, T.; Fu, Z.; Jin, Y., Polystyrene microplastics induce gut microbiota
785 dysbiosis and hepatic lipid metabolism disorder in mice. *Science of the total environment* **2018**, *631*,
786 449-458.
- 787 75. European Commission.; Centre, J. R.; Ramaye, Y.; Stroka, J.; Cella, C.; Held, A.; Robouch, P.;
788 La Spina, R.; Sirio Fumagalli, F.; Méhn, D.; Bianchi, I.; Seghers, J.; Geiss, O.; Emteborg, H.; Gilliland, D.;
789 Jacobsson, U.; Stefaniak, E.; Sokull-Klüttgen, B.; Belz, S., Current status of the quantification of
790 microplastics in water : results of a JRC/BAM interlaboratory comparison study on PET in water.
791 Publications Office: 2021.
- 792 76. California water boards. Microplastics.
793 https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/microplastics.html
- 794 77. Eurofins Lancaster laboratories. *Solubility for Common Extractable Compounds*; 2019.

795