

1 **Small micro- and nanoplastic test and reference materials for research:**

2 **Current status and future needs**

3 Lisbet Sørensen¹, Mathias Hanson Gerace², Andy M. Booth^{1*}

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5 ¹SINTEF Ocean AS, Department of Climate and Environment, Trondheim, Norway

6 ²Norwegian University of Science and Technology (NTNU), Department of Chemistry,

7 Trondheim, Norway

8

9 *Corresponding author:

10 Andy Booth: andy.booth@sintef.no, +47 93089510

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13 **Impact statement**

14 While there has been much focus on the environmental occurrence and impacts of microplastic
15 (MP; 1-5000 μm), small MPs (sMP; $<100 \mu\text{m}$) and nanoplastics (NPs) are hypothesised to be an
16 even greater risk to organisms due to their ability to transfer across biological membranes. High-
17 quality, well-characterised and environmentally relevant test and reference materials are crucial
18 for assessing sMP and NP fate and effects, but these are not currently widely available to the
19 scientific community. This has led to recent efforts to develop methods for the production of
20 more environmentally relevant test/reference materials in sufficient quantities for use in
21 environmental fate and effects assessment, as well as analytical method validation. To our
22 knowledge, the work presented here represents the first summary on the current status of the
23 use and production of environmentally relevant sMP and NP reference materials. The work
24 serves as a basis for describing the current state of the art, highlighting the challenges and
25 limitations associated with the different methods reported alongside the advantages and
26 benefits of each approach. It also provides the reader with a clear overview of the most
27 promising methods and approaches for further development and optimisation. Finally, the work
28 acts as a reference point for identifying and summarising the critical physicochemical properties
29 that should be considered when producing sMP and NP test/reference materials. The impact of
30 this work is considered to have regional and international relevance and reach through the
31 future development and utilisation of more environmentally relevant test and reference
32 materials for use in the fate and effects assessment of sMP and NP. In turn, this will help to
33 improve the accuracy and robustness of risk assessments that form the basis for future
34 mitigation actions and policy development towards plastic pollution, especially in the form of
35 sMP and NP.

36

37 **Abstract**

38 Environmental plastic pollution comprises partially degraded particles representing a continuum
39 of sizes, shapes, polymer types and chemical compositions. Owing to their potential for
40 biological uptake, small microplastic particles (sMP; <100 µm) and nanoplastics (NPs) are
41 considered a potential risk to organisms. Understanding how sMPs and NPs behave in the
42 environment, and how environmental matrices affect their detection, is fundamental to
43 quantifying exposures, assessing hazards, and understanding these risks. For this purpose, high-
44 quality, well-characterised and environmentally relevant test and reference materials are
45 crucial. A current lack of environmentally relevant sMP and NP reference materials has resulted
46 in many studies applying commercially available spherical, homogenous and monodisperse
47 particles, typically produced for specific purposes and without environmental relevance. There
48 is a need for sMP and NP test/reference materials for fate and effects assessments and analytical
49 protocol validation that more accurately represent the sMP and NP present in the environment.
50 To date, feasible methods for producing relevant sMP and NP test materials in sufficient
51 quantities for environmental fate and effects studies remain lacking. The current review
52 provides an overview and comparison of the available methods, highlighting those which show
53 the most promise for producing environmentally relevant sMP and NP with further development
54 and optimisation.

55

56 **Keywords**

57 Degradation; size; additive chemicals; morphology; surface chemistry; environmentally relevant

58

59 **1. The urgent need for environmentally relevant small microplastic and nanoplastic test and**
60 **reference materials**

61 It is estimated that ~10% of all plastic produced enters the global oceans, accounting for 80-85
62 % of the total marine litter load (Coyle *et al.* 2020). High-production polymers such as
63 polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene
64 terephthalate (PET) and polyamide (PA), are used in many consumer products because of their
65 durability and chemical resistance. Under most environment conditions they are considered
66 non-biodegradable over long timescales, implying that they will persist and accumulate in the
67 environment (Hakvåg *et al.* 2023; Tokiwa *et al.* 2009). However, when exposed to a combination
68 of UV-irradiation and mechanical degradation/abrasion, most polymers will undergo
69 physicochemical property changes that lead to fragmentation or 'shedding' (Geyer *et al.* 2017).
70 Such process can occur during the use phase and once the materials reach the natural
71 environment. The consequence is the fragmentation of the material into particles of millimetres,
72 micrometres, or even nanometres in size. Mechanic pressures and abrasion during all life stages,
73 e.g., production, consumer use and waste handling, may also cause release of small- and
74 medium-sized plastic particles from plastics of all types. Importantly, many plastic consumer
75 products contain additive chemicals that are specifically included to protect the material from
76 degradation (e.g., UV stabilizers).

77

78 Many studies have investigated the occurrence and distribution of microplastic (MP; 1-5000 µm)
79 in the environment and in food matrices, where robust, if not standardised, methods for their
80 characterisation and quantification are widely available. The large body of available data
81 suggests that larger MPs in the natural environment are irregular shaped, partially degraded
82 (chemically and physically), a continuum of sizes, shapes, and densities, represent a wide range
83 of polymer types, and acts as a reservoir for thousands of plastic associated chemicals. However,

84 small MPs (sMPs; here defined as $<100\ \mu\text{m}$) and particularly nanoplastics (NPs; $<1\ \mu\text{m}$ or <0.1
85 μm in all dimensions (da Costa *et al.* 2016; Gigault *et al.* 2021; Mitrano *et al.* 2021)) in natural
86 systems have been much less studied, primarily due to significant analytical challenges
87 associated with their combined small size (Gigault *et al.* 2018, 2021). Although direct
88 measurement data of true NP in the environment remains elusive, they are expected to be as
89 equally diverse as MPs (da Costa *et al.* 2016; Koelmans *et al.* 2015; Mattsson *et al.* 2018). Sources
90 of NP release to the environment have been assessed, including primary sources such as NP
91 added to cosmetic products (Hernandez *et al.* 2017) and secondary NP released from surgical
92 masks (Liang *et al.* 2022). The formation of sMP and NP during (simulated) environmental
93 degradation of plastic or larger MP has been indicated through indirect and direct
94 environmental observations (Lambert and Wagner 2016; Sait *et al.* 2021; Tong *et al.* 2022).

95

96 Recent research has suggested that NP may be more hazardous than MP, given the ability of
97 smaller particles to cross biological membranes (Gong *et al.* 2023). It has been demonstrated
98 that the toxicological mechanisms and subsequent observed effects of different size plastic
99 particles differ (Mitrano *et al.* 2021; Yin *et al.* 2021). To date, the vast majority of (s)MP and NP
100 fate and effects studies have been performed with pristine (non-degraded), spherical,
101 monodisperse and near-neutrally buoyant particles (Pradel *et al.* 2023), often without any
102 assessment of the additive chemical composition (Delaeter *et al.* 2022). For example, studies
103 demonstrating membrane transfer and subsequent toxicity have in several cases utilized
104 surface-modified particles meant for drug delivery (Shen *et al.* 2019). Crucially, a few studies
105 have shown significant differences in toxicological effects observed after exposure to spherical
106 and irregular sMP (Xia *et al.* 2022), indicating the importance of particle morphology. As a result,
107 accurate assessment of the environmental fate, effects and risks of sMP and NP is challenging

108 due to the lack of both field observations and laboratory investigations using environmentally
109 relevant test materials (da Costa *et al.* 2016; Pradel *et al.* 2023).

110

111 It is evidently clear that a lack of available sMP and NP test and reference materials,
112 representative of those that may be expected to be released into or formed through plastic
113 degradation in the environment, is limiting progress in understanding the fate, effects and
114 ultimately, the environmental and human health risk of this ubiquitous form of pollution. The
115 current situation is primarily explained by the significant challenges with developing effective
116 and reproducible methods for producing well-characterized reference sMP and NP particles in
117 sufficient quantities (Huber *et al.* 2023; Martínez-Francés *et al.* 2023). This is further compounded
118 by the development of suitable analytical tools representing a major bottleneck with respect to
119 understanding the fate and effects of sMP and NP (Nguyen *et al.* 2019).

120

121 To address the challenges and current limitations with environmental fate and effects
122 assessment, methods to produce significant yields of sMP and NP with a variety of shapes, sizes,
123 polymer types and chemical compositions are needed. While having a repository of available
124 reference materials is desirable for purposes such as analytical method validation and larger
125 comparable studies, we propose that there is an equally large need for transferable methods
126 that can be used to produce test materials from bulk plastics (e.g., specific consumer products)
127 on a case-by-case basis. The aim of the current review is to provide an overview of the currently
128 tested (reported in literature) methods for production of representative sMP and NP materials
129 and suggest which may show the best promise for future development and optimisation. We
130 give focus mainly to top-down methods for producing particles of plausible relevance as
131 fragments of 'real' leaked plastic in the environment.

132

133 2. Methods

134 Peer-reviewed literature was accessed using databases such as Science Direct, Google Scholar,
135 Scopus, ACS Publications, IOP Sciences and through the Norwegian University of Science and
136 Technology Library in the period August 2021 to January 2023. Search strings were “degradation
137 microplastic”, “degradation nanoplastic”, “milling microplastic”, “milling nanoplastic”,
138 “microplastic reference material”, “nanoplastic reference material”. Further literature was also
139 selected cascading from the primary results. Conference proceedings and non-accessible articles
140 were omitted from the review. Papers on nanoplastic synthesis methods were omitted from
141 review. A total of 22 papers were selected for inclusion in the review (Table 1). For the purposes
142 of this review test materials are defined as those that can be made for specific testing
143 applications, but are not widely available, while reference materials are defined as those that
144 are widely available for all to use in studies.

145

146 3. Methods for production of micro- and nanoplastic test and reference materials

147 From a physicochemical perspective, reference materials should be as similar as possible to the
148 sMP and NP particles found in environmental matrices (Crawford and Quinn 2017). The
149 production method must try to imitate the natural weathering processes the plastics undergo,
150 without physicochemically modifying the particle/material in a non-relevant way. It is the
151 produced material from these processes that is referred to as secondary microplastics. As most
152 degradation mechanisms progress, the chemical properties of the particles change through the
153 addition of various functional groups (e.g., hydroxyl, carbonyl, and carboxyl) (Cai *et al.* 2018).
154 This is hugely important as such chemical changes can strongly impact particle environmental
155 behaviour and fate (especially for smaller particles), as well as possibly increasing their chemical
156 reactivity. An essential factor in producing representative sMP and NP test and reference
157 material is therefore to emulate the addition of functional groups and environmentally relevant

158 chemical changes. Given the potentially significant role that plastic associated chemicals play in
159 impacting MP and NP toxicity, it is also the opinion of the authors that test and reference
160 material production should preserve chemical composition also.

161

162 Methods for producing test MP and NP particles can be divided into whether they are produced
163 through bottom-up synthesis procedures (Al-Sid-Cheikh *et al.* 2020; Mitrano *et al.* 2019; Sander
164 *et al.* 2019) or via top-down degradation of larger particles, granules or even environmentally
165 collected items (Kefer *et al.* 2022). While the former may allow relatively fast, controlled and
166 reproducible production of larger quantities of particles, the latter is the desirable approach to
167 mimic environmentally relevant particles (Pradel *et al.* 2023). Bottom-up approaches can be
168 readily used to produce NP, typically resulting in the production of mainly spherical,
169 homogenous and monodisperse particles, i.e., not representative of environmental NPs.
170 Furthermore, they typically do not allow for plastic associated chemical composition of
171 environmental sMP and NP to be replicated. A possible 'intermediary case' is the solvent-
172 dissolution or melting of polymer particles in a solvent followed by extrusion or spray drying of
173 particles in the desired size range (Bhagia *et al.* 2021). Using this approach, it is possible to
174 partially retain the chemical composition of the plastics, but the physical properties (shape,
175 density, porosity, morphology) are not representative of the particles formed from degradation
176 of larger particles or plastic items during use or in the environment. In this review, 17 papers
177 representing top-down production methods have been reviewed and 5 papers describing
178 approaches for re-dissolution of polymers are reviewed. Bottom-up synthesis methods for sMP
179 and NPs were not included, although several are reported, as these are not considered
180 appropriate for producing environmentally relevant particles and are thus out of scope of the
181 review.

182

183 **3.1 Milling and grinding**

184 It is almost impossible in the laboratory to mimic or reproduce all of the degradation processes
185 simultaneously acting up an item of plastic debris in the natural environment. However, some
186 individual degradation mechanisms can be reproduced in the laboratory quite accurately and
187 effectively, with potential for more than one to occur at the same time. A good starting point is
188 to grind or mill pieces of plastic as finely as possible, and this process has generally been shown
189 to produce polydisperse particles with a range of sizes, highly irregular shapes and surface
190 morphologies representative of plastic particles found in the environment (Seghers *et al.* 2022;
191 Takacs 2002). The two primary forms of milling available are rotary milling and ball milling, with
192 the former acting more as a 'cutting' technique and the latter acting as a 'smashing' technique.
193 Crucially, milling approaches allow the plastic associated chemical composition of the start
194 material to be preserved, as long as any subsequent fractionation or particle size range isolation
195 step avoids the use of water or other solvents. For the production of MP >30 µm in size, these
196 methods have proven to be relatively cheap and fast (Seghers *et al.* 2022), although production
197 of large quantities of material can involve some investment of time. It is also important to note
198 that different polymer types respond differently to the process, which impacts final yields and
199 particle size/morphology.

200

201 A critical issue with common grinding and milling techniques, however, is that they are not able
202 to grind the plastics finely enough to produce reasonable yields of sMP and NP (Ciobotaru *et al.*
203 2020; Eitzen *et al.* 2019). While particles in this size range are often reported as being produced,
204 they typically represent yields of <<1%, if they are calculated and reported at all. It was found
205 that approximately half of the reviewed papers applied some form of grinding or milling process
206 to produce sMP and NPs, while most others utilised previously ground or milled particles as a
207 starting material (Table 1). For thermoplastic materials, which may soften in ambient

208 temperatures, a common approach has been to cool down the material (cryomilling), making it
209 more brittle and facilitating the fragmentation process into smaller particles sizes. Cryomilling is
210 conducted at low temperatures (e.g., using liquid nitrogen), which increases the yield of fine
211 structured particles and allows for rapid grain refinement (Suryanarayana 2001). Cryogenic
212 conditions can be applied to both ball and rotary milling, either by pre-cooling the sample to be
213 milled or by pre-cooling the sample holder and maintaining the low temperature throughout
214 the milling process (Eitzen *et al.* 2019). While cryomilling has been shown to be effective for
215 producing larger MP reference materials (>30 μm) in reasonable yields (Kühn *et al.* 2018), the
216 technique is still unable to produce the larger quantities of sMP and NP particles (Eitzen *et al.*
217 2019).

218

219 It has recently been demonstrated that conducting cryomilling and ball milling at ambient
220 temperature in series can help to increase the production of smaller particles, especially NPs
221 (Caldwell *et al.* 2021). Although yields were not explicitly reported, the authors describe stock
222 solutions of the final NP materials at concentrations ranging from 4.8-40.8 $\mu\text{g mL}^{-1}$, which was
223 sufficient for conducting toxicity tests in the study. A combination of dry and wet milling in
224 water, solvent or surfactant has also been shown to improve the yield of lower size ranges
225 (Bhagia *et al.* 2021), and has been applied in most of the studies reviewed herein (Table 1). The
226 approach has achieved the production of non-homogenous (in size and shape) particles for a
227 range of polymers, PS, PP, PE, PET, polybutylene adipate terephthalate (PBAT), and polyether
228 ether ketone (PEEK). Wet high speed friction grinding has recently been investigated as an
229 option for production of MP and sMP, and a significant improvement in yield of PE, PP and PLA
230 particles in the lower size range was obtained by modifying the aqueous grinding media with
231 0.5% guar gum (Bhagia *et al.* 2021). Minimal impact on physicochemical properties was
232 observed by this process.

233

234 **3.2 Ultrasonication**

235 Exposing plastic materials and particles to ultrasonication, where sound waves create
236 mechanical forces that can help to break down plastic particles, has the potential to produce MP
237 and NP test/reference materials. To date, only a few studies have been conducted using this
238 approach, but they have demonstrated promising results, including the production of particles
239 in the nanoscale (von der Esch *et al.* 2020). Ultrasonication is typically conducted by suspending
240 the material to be fragmented in a solvent, where different chemical characteristics can further
241 influence the fragmentation process. For example, using alkaline conditions has been proven to
242 be effective (von der Esch *et al.* 2020). A significant advantage of ultrasonic-based methods is
243 the wide availability of ultrasonication equipment in laboratories globally, increasing the
244 possibility for many researchers to produce their own test materials. These methods are also
245 both cost and time efficient. However, there are still some limitations with the technique,
246 including sonication being a time-demanding method and that it requires further development
247 and harmonisation in order to achieve reproducible results when using different sonicators (von
248 der Esch *et al.* 2020).

249

250 **3.3 Oxidative degradation**

251 An environmentally relevant method for degrading polymers and plastic materials is to expose
252 them to UV radiation. Many studies have investigated environmental UV degradation of plastics
253 in the laboratory under simulated sunlight conditions, typically reporting significant
254 fragmentation and chemical (polymer and additive) composition changes to the start material
255 (Gigault *et al.* 2016; Lambert and Wagner 2016; Sait *et al.* 2021; Sørensen *et al.* 2021). The
256 irradiation causes photo-catalysed oxidative degradation, resulting in broken polymer chains
257 and a reduction of the molecular weight of the particles (Yousif and Haddad 2013). In such

258 studies, the UV wavelengths are usually limited to those present in natural sunlight (e.g. UVA
259 and UVB; >280 nm), but the time scales required to achieve significant degradation typically
260 range from months to years (Sait *et al.* 2021). The process can be sped up by conducting the UV
261 exposure continuously and by using high UV intensities but kept within naturally ranges.
262 However, shorter wavelengths are more effective at degrading plastic materials and exposing
263 them to light containing wavelengths that include the UVC region (200-280 nm) can significantly
264 speed up the degradation process (Doğan 2021; Lee *et al.* 2020)), as can using intensities above
265 natural levels. It should be noted that UVC exposure may lead to the formation different
266 degradation products and oxygenated radicals compared to UVB exposure (Doğan 2021).

267

268 One way of achieving this enhanced degradation rate in the laboratory is to expose the starting
269 polymer material in a UVC-Ozone (UV-O) chamber (Sarkar *et al.* 2021), such as those commonly
270 used for sterilisation purposes. The UV-O exposure facilitates the UV irradiation of the particles
271 while simultaneously oxidizing them via ozone, leading to radical reactions that break down the
272 particles into smaller fragments (Sarkar *et al.* 2021). The application of UV-induced
273 fragmentation for the production of test materials has been investigated for
274 polyhydroxybutyrate (PHB) and PS, in two different studies, producing sMP and NP, respectively
275 (González-Pleiter *et al.* 2019; Sarkar *et al.* 2021). While the approach has been demonstrated to
276 produce sMP and NP particles, yields and production volumes were not reported in either study.
277 Advanced oxidation techniques such as UV-O typically require costly specialist equipment, and
278 while degradation rates may be fast for UV-labile polymers, the method is not applicable to UV-
279 stabile polymers. The applicability of such methods for harmonized production of test and
280 reference materials is therefore likely low.

281

282 **3.4 Laser ablation**

283 Magri et al. (2018) applied laser ablation (irradiation wavelength 248 nm) to create top-down
284 NP fragments of PET in concentrations of up to 300 $\mu\text{g mL}^{-1}$. Small NP size ranges were isolated
285 by filtration (0.2 μm), and the mean diameter found to be ~ 30 nm, one of the smallest sizes
286 among the studies addressed herein. The benefits of this protocol would include not needing to
287 add any particular solvent or stabilizer, while down-sides include the low yield and application
288 of (costly) specialist instrumentation and time consumed. Upon characterization, produced
289 particles were found to be nearly spherical or spherical, and oxidized at the surface compared
290 to the original material. It is hypothesized by the authors that the oxidised surface is
291 representative of (environmentally) degraded PET, but this is not proven. This technique
292 potentially allows the production of NPs without impurities, chemical precursors, and the
293 byproducts associated with bottom-up processes.

294

295 ***3.5 Combined degradation protocols***

296 As highlighted above, combining multiple degradation mechanisms has the potential to be more
297 effective at degrading plastic materials and generating sMP and NP test materials in usable
298 quantities (for example environmental fate and effects assessment). In an attempt to produce
299 sMP particles in the range 1-10 μm , Sarkar et al. (2021) first used cryomilling to produce PS
300 particles of <100 μm , followed by a sequential combination of thermal treatment (70 $^{\circ}\text{C}$)
301 followed by UV(C)-ozone exposure (both dry) and probe sonication in ethanol-water suspension.
302 The application of the post-cryomilling steps increased the number of particles in the 1-10 μm
303 size range, although yields were not determined. While not measured and reported directly by
304 the authors, the size distributions presented indicated that an increase in the NP size fraction
305 was likely also obtained using this method. The produced sMP particles were found to be
306 comparable in size, shape, surface morphology and functionality to particles subjected to
307 environmental weathering. However, the measured surface charge (zeta potential, ZP) was

308 significantly different between the sMPs produced by this accelerated protocol and those
309 produced from environmental weathering. Compared to spherical model PS and
310 environmentally weathered particles, the PS sMPs from the accelerated protocol represented
311 an intermediary ZP. A major downside to the protocol may be the difficulty in scaling up the
312 process to facilitate bulk production of sMP (and potentially NP). Furthermore, the approach
313 was optimized particularly for PS, known to be 'easily' UV-degradable due to its carbon-carbon
314 backbone (Gewert *et al.* 2015; Yousif and Haddad 2013), and so its applicability for other
315 polymer types is unknown.

316

317 Most recently, (Schmitt *et al.* 2023) used a combination of UV exposure and mechanical
318 fragmentation using a custom made air-based fragmentation device to produce high yields of
319 PE MP (~80 %) using a thin film/foil as a starting material. The approach was able to produce PE
320 flakes with irregular shapes, where a decrease in size occurred with longer UV exposure times
321 (up to 2.5 months). Although a minimum average particle size of 110 μm was reported in the
322 study, the relationship between particle size and UV exposure times, combined with the high
323 yields, suggests the approach may have potential for further development and assessment for
324 its applicability in producing sMP and NP materials. A common downside to both protocols
325 presented above is the need for specialist instrumentation that is not readily available in most
326 laboratories (e.g., UV-O chamber and custom-built fragmentors), making them difficult to
327 reproduce in other laboratories without significant cost and commitment. On the positive side,
328 both methods appear to be able to produce polymer fragments of environmental relevance, due
329 to their partially degraded nature.

330

331 ***3.4 Solubilisation and precipitation methods***

332 Several studies have reported the production of nano-sized PET through the dissolution of larger
333 PET particles in a strong solvent, followed by precipitation in (mainly) aqueous media (Elhady *et*
334 *al.* 2022; Johnson *et al.* 2021; Rodríguez-Hernández *et al.* 2019). Another study applied a similar
335 approach for PE, replacing direct precipitation by emulsification with a biosurfactant in seawater
336 (Balakrishnan *et al.* 2019). While the methodologies are reasonably simple and transferable,
337 they are highly similar to conventional bottom-up synthesis approaches that produce mainly
338 spherical particles with a narrow size range, and which have limited environmental relevance.
339 Additional downsides include the use of toxic solvents and the need to remove them before any
340 particles can be utilised in toxicity studies. It also remains unclear and undocumented if such
341 approaches to test/reference material production sufficiently retain the additive chemical
342 composition to the starting material. This is increasingly considered critical for achieving a more
343 accurate and environmentally relevant assessment of sMP and NP.

344

345 Most recently, Peller *et al.* (2022) applied partial solubilization with small volumes of long-
346 chained solvents to successfully transform larger particles of several polymers (PE, PET, and PS)
347 into sMP and NP directly in aqueous media. The relatively simple approach involved the addition
348 of plastic granules, particles or pieces to water, followed by the addition of a small volume (10-
349 30 μL) of long-chain alkanes. Vigorous shaking created a cloudy suspension with average particle
350 sizes of 1.3-4.4 μm for the different materials. Subsequent ultrasound treatment resulted in
351 average particle sizes of 338-724 nm. Differences were observed in the particle morphology,
352 with some materials producing smoother and more spherical particles, and other materials
353 producing more irregular shaped and rougher-surfaced particles. Again, there was no attempt
354 to assess whether the (additive) chemical composition of the resulting particles accurately
355 reflected that of the start material. Given the challenges in producing bulk quantities of sMP and
356 NP particles using mechanical (e.g., cryomilling) and UV-based degradation techniques,

357 dissolution and partial dissolution approaches appear an attractive alternative. The protocols
358 can easily be scaled and facilitate reasonably fast production of particles without the need for
359 specialist instrumentation. However, more work is required to characterise the physicochemical
360 properties of the resulting particles and compare them to those found in the natural
361 environment. Furthermore, there is a need for assessing such techniques for their applicability
362 to a much wider range of polymer types, again reflecting those found in the natural
363 environment.

364

365 **4. Discussion and considerations**

366 **4.1 Yields of sMP and NP**

367 One of the key requirements of test and reference materials is being sufficiently available at a
368 low or reasonable cost. Unfortunately, only a small number of papers report yields or produced
369 mass in a way that allows calculation of yield relative to the starting amount of plastic. Where
370 reported, yields are (on a mass basis) very low ($\ll 1\%$). Dissolution-precipitation methods
371 appear to be much quicker and easier to perform and may have the potential to be scaled up to
372 provide higher sMP and NP yields. In contrast, entirely top-down methods may appear more
373 desirable in terms of their potential for producing sMP and NP with a higher degree of
374 environmental relevance, but are typically costly, time consuming and only able to produce
375 small amounts of material. For a variety of reasons, very few studies reporting methods for the
376 production of sMP and NP directly assess (or attempt to assess) the environmental relevance of
377 the particles from a physical and chemical perspective (especially the latter). In line with
378 previous assessments, we also see that most reported methods (ten papers) have focused on
379 production of PS (**Fig. 1**), although this is not the most abundant polymer in the environment
380 (Pradel *et al.* 2023). A similar number of studies have produced PET particles, while only a
381 handful have produced PP and/or PE particles. This probably reflects the applicability of the

382 methods to the various polymers, but also the interest in their production. We believe that it is
383 important for methods to be able to produce comparable particles of several/most polymer
384 types found in environmental plastic.

385

386 **4.2 Size and shape of sMPs and NPs**

387 Top-down methods generally produce irregularly shaped particles, whereas particles formed
388 through dissolution and re-precipitation have a tendency to be more spherical in morphology
389 (Johnson *et al.* 2021). The studies reviewed here show that weathering process that plastics
390 undergo natural environmental can be reproduced relatively well in the laboratory and that the
391 secondary MP and NP formed share many similarities with particles isolated from environmental
392 matrices. The applied techniques and combinations of techniques typically resulted in a mixture
393 of highly irregular particle morphologies, a critical property of naturally weathered plastics. The
394 majority of the top-down studies, (mechanical-, UV- and dissolution-based approaches) were
395 able to produce a continuum of particle sizes, including a proportion of particles in the ranges
396 required for a hypothetical sMP and NP test/reference material (i.e., just over 1 μm and $<1 \mu\text{m}$,
397 respectively; **Fig. 1**). Particles $< 100 \text{ nm}$ are only reported in a few studies, but this can be caused
398 both by the lack of formation and the lack of appropriate characterization methods for such
399 small sizes. As such there is potential for further processing of the products to isolate and
400 characterize specific particle size ranges for use as test materials. Across the reported studies,
401 such top-down approaches have been applied to a variety of polymer types, with varying
402 degrees of success being reported. However, it is important to highlight that such approaches
403 are typically limited by very low yields, low reproducibility, the need for costly instrumentation
404 and a significant time investment.

405

406 All studies employed some form of particle size and/or size distribution characterisation, for
407 which a range of techniques was applied. In the reviewed studies, nine used DLS and five used
408 SEM, with other reported techniques including nano tracking analysis (NTA), asymmetric flow
409 field flow fractionation (AF4) fitted with either (i) a multi-angle light scattering (MALS) detector,
410 (ii) static light scattering (SLS), or (iii) inductively coupled plasma-mass spectrometry (ICP-MS),
411 laser diffraction or granulometry, transmission electron microscopy (TEM) and Raman
412 microscopy. Several studies employed a combination of particle size characterisation methods.
413 It should be noted that the different techniques are based on different principles for assuming
414 size and so the final determined values are not always directly comparable. For example, DLS
415 generally provides larger sizes than optical techniques such as SEM, likely due to a combination
416 of (i) presence of surfactants on the particle surface, (ii) calculation of hydrodynamic radii
417 assuming spheres when actual sMP and NP are not spherical, and (iii) particle aggregation
418 (Caldwell *et al.* 2021; Ji *et al.* 2020; Johnson *et al.* 2021). Conversely, NTA provides lower,
419 possibly more accurate, average particle sizes than DLS (Ekvall *et al.* 2019).

420

421 ***4.3 Influence of fragmentation methods on particle and polymer physicochemical*** 422 ***properties***

423 Both naturally occurring environmental degradation mechanisms and those degradation
424 processes simulated in the laboratory have the potential to impact or modify multiple
425 physicochemical properties of plastic materials, including crystallinity, glass transition and phase
426 transition temperatures (Ainali *et al.* 2021). From the perspective of environmental relevance of
427 test and reference materials, it is important to have a robust characterisation relative to the
428 start materials in order to ensure they reflect the properties of sMP and NP found in the
429 environment. It is only recently, however, that a few studies have started to document the
430 effects of degradation methods on particle properties beyond their size and shape. For example,

431 Lionetto *et al.* (2021) investigated if there were changes in the polymer crystallinity when
432 producing PET NPs through a combination of ultra-centrifugal milling and ball milling. The milling
433 process was found to lead to a progressive reduction in the degree of crystallinity and an
434 increase in the proportion of amorphous material, resulting in an overall change in material
435 properties. Considering that the amorphous regions of a polymeric material are often more
436 susceptible to different degradation mechanisms (e.g., UV degradation and microbial
437 degradation) than the crystalline regions, it is likely that some differences are observed between
438 the parent material and the resulting sMP and NP test materials. For the production of
439 environmentally relevant materials in terms of behaviour, this needs to be considered further
440 and compared to eventual changes of the same properties under environmental or simulated
441 environmental degradation (Ainali *et al.* 2021; Conradie *et al.* 2022). It is also worth highlighting
442 that top-down test/reference material production methods have an increased likelihood of
443 preserving the additive chemical profile of the start material, thus increasing its environmental
444 relevance.

445

446 ***4.4 Stability of particles in aqueous suspension***

447 Beyond the initial preparation of the sMP and NP test and reference materials, their dispersion
448 stabilisation and longer-term stability in aqueous media is another key consideration that must
449 be addressed. It is well established that common surfactants can be used to maintain small
450 particles in aqueous dispersion, avoiding aggregation at the higher than environmentally
451 realistic concentrations typically used for test and reference material stock solutions (Ji *et al.*
452 2020; Johnson *et al.* 2021). However, there are some critical drawbacks to using surfactants
453 when the particles are to be utilised in environmental fate and effects assessment. Any particles
454 previously exposed to surfactants are unlikely to behave in a 'natural' way in environmental fate
455 studies, while the surfactants may influence the outcomes of toxicity studies. Despite these

456 drawbacks, there has been an unfortunate overuse of stabilized PS spheres for environmental
457 fate and effects studies conducted to date (Pradel *et al.* 2023) and it is critical that research
458 moves towards more realistic assessments.

459

460 It is possible to look towards the natural world for inspiration when it comes to dispersion
461 stabilisation of sMP and NP test/reference materials. Most small particles present in natural
462 aquatic environments, including sMP and NP, can be naturally 'stabilised' by the sorption of
463 natural organic matter or through biofilm formation (Junaid and Wang 2021; Pradel *et al.* 2021).
464 While several of the studies reviewed herein have used synthetic surfactants to stabilize sMP
465 and NP suspensions (Caldwell *et al.* 2021; Rodríguez-Hernández *et al.* 2019), success in using
466 biosurfactants has also been reported (Balakrishnan *et al.* 2019). Interestingly, Ji *et al.* (2020)
467 found that bovine serum albumin provided better suspension and less agglomeration than SDS,
468 suggesting that natural surfactants are perfectly suitable for this purpose. It is therefore possible
469 to consider producing test and reference sMP and NP materials that are stabilised using different
470 natural surfactants depending on the intended use of the particles in experimental studies. We
471 therefore propose that future work on development of sMP and NP reference materials should
472 include a focus on using natural surfactants to ensure dispersion and dispersion stability.

473

474 **5. From test materials to reference materials – what are the next steps?**

475 To date, the vast majority of environmental fate and effects studies with MP and NP have used
476 either spherical, monodispersed, single-polymer particles from commercial suppliers or single-
477 batch test materials produced using bespoke methods developed by the researchers
478 themselves. Only the first approach realises the possibility for interlaboratory comparison and
479 validation of methods. Despite some success in recent years to produce more environmentally
480 relevant certified MP reference materials (e.g., the Hawaii Pacific University Center for Marine

481 Debris Research (HPU CMDR) Polymer Kit 1.0 (The Center for Marine Debris Research 2020),
482 there remains a lack of certified reference materials representing sMP and NP particles
483 (Balakrishnan *et al.* 2019; Eitzen *et al.* 2019; Seghers *et al.* 2022). The current review has
484 evaluated recent efforts to produce relevant sMP and NP test materials, with some promising
485 techniques already documented and available for further development and optimisation.
486 However, there remains a large gap to cover in terms of reaching availability of reference
487 materials for these size classes. Some studies have taken initial steps towards documenting the
488 reproducibility of their protocols, as well as the homogeneity of produced samples (von der Esch
489 *et al.* 2020), but most protocols presented have been developed and applied only on a case
490 study basis.

491

492 Importantly, we recommend that there is a focus on considering the additive chemical profiles
493 of test and reference sMP and NP materials, where these chemicals should be incorporated and
494 robustly characterised. Given the potential impact such chemicals could play in the toxicity of
495 MP and NP emissions, this is a critical aspect that needs to be incorporated into the design and
496 production of test/reference particles. In addition to the highest production volume
497 thermoplastics (e.g., PE, PP, PS, PET, PA), synthetic rubbers (elastomers) are expected to be
498 significant contributors to sMP and NP pollution world-wide. For example, tyre wear particles
499 (TWPs) have been shown to be a major constituent of road dust (PM₁₀ and PM_{2.5}) and
500 represent a priority form of sMP emissions globally (European Commission 2023). Elastomers
501 are emerging as one of the more toxic groups of polymeric materials, suggesting there is a need
502 for increased focus on understanding the impacts of their release (Sørensen *et al.* 2023).
503 However, none of the examined papers have investigated the production of elastomer test and
504 reference materials, which are inherently more challenging to produce due to their elasticity
505 reducing the potential for fragmentation. Methods for production of TWP test materials in the

506 MP size range have been proposed, including abrasion of rubber vulcanizate and combination
507 with mineral particles and surface solvent treatment for enhancing "stickiness" (Son and Choi
508 2022), but no reports of methods for sMP and NP size ranges are found. We are aware of several
509 ongoing EU and JPI Oceans funded projects (e.g., POLYRISK, PlasticsFate, ANDROMEDA,
510 EUROqCHARM and PlasticTrace) that are currently dealing with harmonisation and
511 standardisation of sMP and NP test and reference material production. Furthermore, this is an
512 ongoing focus for well-established producers and suppliers of certified reference materials, such
513 as NIST (US), the EU Joint Research Centre (JRC) and The Bundesanstalt für Materialforschung
514 und -prüfung (BAM, Germany). The development of standardised methods for the reproducible
515 production of microplastic test and reference materials for use by the scientific community could
516 be of relevance to well-established standards organisations such as the International
517 Organization for Standardization (ISO), the Organisation for Economic Co-operation and
518 Development (OCED) and the European Committee for Standardization (CEN).

519

520 **6. Conclusions and future needs**

521 This review has summarised and evaluated some of the most promising methods currently
522 available for producing more environmentally relevant sMP and NP test and reference materials,
523 particularly for use in environmental fate and effects assessment studies. Most methods have
524 focused on conventional thermoplastics, while a few papers have addressed biodegradable
525 plastics, reflecting their increasing importance and focus as part of the solution to addressing
526 global plastic pollution. However, elastomers do not currently appear to be a focus, which
527 represents an important gap. A combination of wet and dry grinding/milling appears to be the
528 most promising technique to produce of reasonable quantities of sMP, although yields need to
529 be determined and reported more frequently and the approach can be costly and time
530 consuming. All reported methods appear to need further optimisation to maximise yields and

531 reproducibility, while robust and reproducible fractionation techniques will be needed for
532 isolating specific sMP and NP size fractions. Based on the current state-of-the-art, it remains a
533 question as to whether environmentally realistic NPs can be obtained in desired quantities, but
534 combination protocols incorporating milling, chemical degradation (e.g., oxidation), sonication
535 followed by filtration may offer the best chance. Furthermore, test/reference sMP and NP
536 stabilisation issues during storage and in aqueous matrices is another challenge that needs to
537 be addressed in the future. It is also important to consider that it may never be possible to
538 produce test/reference sMP and NP materials that truly reflect the complex mixture of partially
539 degraded plastic particles that is present in the environment. However, there needs to be a
540 move towards finding a middle ground where the environmental relevance of test and reference
541 materials used in fate and effects assessment is improved to the point that more reliable risk
542 assessment for MP and NP pollution can be conducted. Given the global economic and societal
543 importance of plastics, combined with the problems of mis-managed waste and plastic
544 pollution, it is important that future policy and regulations are based upon robust science and
545 data. To this end, the ongoing global plastic treaty negotiations should play an important role in
546 catalysing the development and use of standardised reference materials in micro- and
547 nanoplastic risk assessment.

548

549 Author contribution statement

550 LS: conception and design of the work; methodology; acquisition, analysis and interpretation of
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552 MG: methodology, acquisition and analysis of data.

553 AMB: conception and design of the work; methodology; interpretation of data; drafting and
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555

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566

567 **Conflict of interest statement**

568 The authors declare that they have no known competing financial interests or personal
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570

571 **Data availability statement**

572 All data and information used in the development of this work was taken from published
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574

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828 TABLES

829 **Table 1. Overview of top-down and re-precipitation sMP and NP test material production methods available in literature. *Yield is quoted as "Not reported" if produced**
 830 **amounts cannot be directly compared to the amount of starting material on a mass- or volume-basis. Polymer types studied were polystyrene (PS), polyethylene**
 831 **terephthalate (PET), polyethylene (PE), polypropylene (PP), polylactic acid (PLA), polyhydroxybutyrate (PHB), polybutylene adipate terephthalate (PBAT), polyether ether**
 832 **ketone (PEEK) and polycarbonate (PC).**

Reference	Polymer(s)	Method	Size range produced	Yield*
Top-down methods				
(Lionetto <i>et al.</i> 2021)	PET	Ultra centrifugal milling followed by annealing and wet ball milling: Three stages of very fast ultra-centrifugal dry milling with three different sieves (500 μm , 250 μm , 80 μm) to reduce pellets (~4 mm) to micrometric powders (100-120 μm). Annealing treatment at 160 °C for 4 h, followed by a slow cooling to room temperature and wet ball milling (0.5 mm ZrO ₂ balls)	70–400 nm (laser diffraction)	~35% (volume) < 1 μm
(Schmidt <i>et al.</i> 2012)	PS, PEEK	Wet grinding of pre-milled powders in organic solvents (6.5-7% ethanol, hexane) at temperatures down to –80 °C.	1-5 μm (DLS)	Not reported
(Ji <i>et al.</i> 2020)	PET	Wet grinding using a hand blender in aqueous dispersant (20 g to 300 mL 0.01% SDS or 0.05%BSA), 1 min of blending alternating with 5 min of resting for a total of 6 h, followed by differential centrifugation to isolate nanosized fractions.	20-800 nm (DLS, SEM)	~0.01% (mass) < 20 nm, ~0.1% < 800 nm
(Ekvall <i>et al.</i> 2019)	PS	Wet grinding using an immersion blender (2 g to 115 mL water) for 5 min, followed by filtration (1.2 and 0.45 μm).	100-200 nm (NTA, DLS, TEM)	Not reported
(El Hadri <i>et al.</i> 2020)	Pristine PE and PS, environmental PE and PP	Combination of dry and wet milling: Pre-grinding into powder <1 mm. Ball milling using a planetary ball mill (ZrO ₂ balls, 0.5, 1, 5 and 10 mm): 4 steps with 10 cycles: 3 min grinding, 6 min pause. First step dry, other steps dispersed in EtOH. The optimized grinding duration and speed was 120 min and 450 rpm, respectively. The powder is dried, dispersed in water by bath sonication and filtrated (5-6 μm) to obtain submicronic particles.	20-500 nm (DLS, AF4)	Not reported

(Ciobotaru <i>et al.</i> 2020)	PET, HDPE	Combination of dry and wet milling: Shredded material (5-15 g) with 50 g NaCl milled 10 min (550 rpm). Direction reversal every 3 min, with 10 s pause. Added 10 mL of H ₂ O, then further milling for 50 min. Filtered (1 mm, 0.4 μm porosity) with 5% SLS. Clogging of the filter was prevented by adding EtOH. Final wash of the filter with distilled water. The samples were dried at 20 °C.	10-200 μm (laser granulometry)	Not reported
(Blanco <i>et al.</i> 2021b)	PS, PP	Ball milling: Pellets were fragmented using a planetary ball mill (5 or 10 mm ZrO ₂ balls) applying the method by (El Hadri <i>et al.</i> 2020), followed by freeze-drying, redispersion in water and filtration (3 μm).	100-1000 nm (DLS)	Not reported
(Eitzen <i>et al.</i> 2019)	PS	Cryogenic ball milling (30 Hz, 5 cycles of 10 minutes) with pre-cooled (12 minutes, liquid N ₂) particles.	1-200 μm (SEM)	Not reported
(Astner <i>et al.</i> 2019)	LDPE, PBAT	Cryomilling of pre-cut materials followed by wet grinding . PBAT films were pre-treated by soaking in water followed by cryogenic treatment. Cryogenic-treated PBAT fragments or untreated LDPE pellets (~1.0 g) were fed to a rotary mill by sieve sizes of 840 μm for the first pass and 250 μm for the second pass. The residence time for milling was 20 min per pass. The particles recovered from milling (~1.0 g of PBAT or LDPE) were fractionated via a cascade of four sieves (840 μm, 250 μm, 106 μm, and 45 μm). An aqueous slurry (4.0 L) containing 1.00 wt.% of the 106 μm MP fraction was prepared, which underwent stirring for 24 h. After stirring, slurries were subjected to the wet-grinding process using a “supermass colloidier” at a speed of 1500 rpm and 27 subsequent passes. The slurry from wet-grinding was stirred for 4 h (300 rpm at 25 °C). The resultant particles were dried at 40 °C for 48 h.	30-1400 nm (DLS)	Not reported
(Caldwell <i>et al.</i> 2021)	PET, PP, PS	Cryomilling followed by wet ball milling: Liquid nitrogen cooling, 2 cycles of 3 min milling (steel milling rod, steel chamber plugs, polycarbonate chamber) at 12 CPS, 2 min cooling cycle and 15 min pre-cooling. Cryomilled particles <0.3 mm (8 g in MilliQ) milled with 500 μm ZrO ₂ beads with	75-222 nm (DLS, SEM)	Not reported

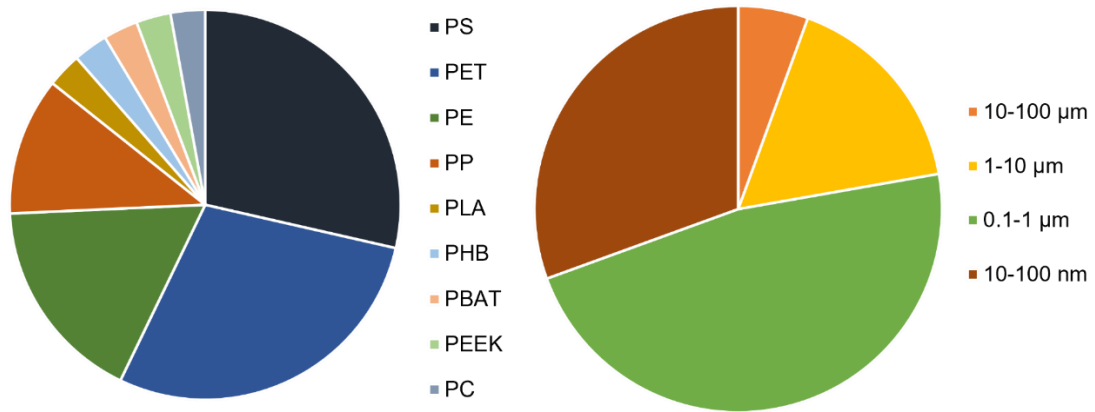
		a milling bead ratio of 60% v/v in 10% w/w SDS at a 4.7 m/s agitator speed and at 15 °C for 3 hours. The suspensions were filtered (0.2 µm) and placed into dialysis membranes with a 14 kDa cutoff. The dialysis membranes were submerged in 2 L of MilliQ water, which was changed daily. Dialysis was considered complete once the conductivity remained constant, typically after 3–4 days.		
(von der Esch <i>et al.</i> 2020)	PET, PLA, PS (PE, PP, PVC, PA)	Ultrasonication: Polymer pieces (30 mg) sonicated (15 h at 35 kHz) in 15 mL 0.25 M KOH (pH 13). Smaller fragments (< 1 mm) were collected from the alkaline suspension through centrifugation (3,000 rpm 20 °C, 30 min) removal of the supernatant and resuspension in MilliQ (pH = 7).	1-100 µm (Raman)	Not reported
(Baudrimont <i>et al.</i> 2020)	PE, pristine and environmental	Ultrasonication of MP (350 µm virgin PE, environmentally collected MP, 100 mg/L) 1 h with pulses of 5 µs (50 % of active cycles) in an ice bath. Filtered through 0.45 µm filter.	<450 nm (nominal based on filter pore size)	17 (pristine)-31 (environmental) % (measured as TOC compared to starting amount).
(González-Pleiter <i>et al.</i> 2019)	PHB	UV-degradation: 25-100 mg/L MPs in MilliQ buffered with 2 mM of phosphate (pH 7) irradiated (65 µmol photons/m ² s) for up to 20 days under constant shaking followed by ultra centrifugal filtration (50 kDa).	75-200 nm (DLS, NTA, SEM)	~0.4% by mass measured as TOC.
(Blanco <i>et al.</i> 2021a)	PE, PP	Ultrasonication followed by UV-degradation: Suspension agitated for 2 days (250 rpm), then 1 hour of sonication. Filter (40 µm). Add 1 % H ₂ O ₂ , then 5 h UVC.	200-500 nm (DLS)	Not reported
(Sarkar <i>et al.</i> 2021)	PS	UV-degradation followed by ultrasonication: Ground plastic (<100 µm, 100 mg) heated (12 h, 70 °C), then UVC-Ozone oxidation (3 h, dry), then sonication (10 min in 20% EtOH).	1-3 µm (FT-IR, SEM)	Not reported
(Davranche <i>et al.</i> 2019)	Not specified, environmental MP	Sonication of 58 g of environmental microplastics (5 mm–2 cm) in 300 mL of ultrapure water. Sonication was performed over 5 days. The suspension was filtrated at 0.8 µm.	150–450 nm (DLS, AF4-SLS)	0.03% (measured as resulting DOC mass concentration)
(Magri <i>et al.</i> 2018)	PET	Laser ablation: 50 irradiation pulses (λ = 248 nm and 4.5 J/cm ² fluence) shot at a solid PET target	10-100 nm (DLS, NTA, AF4)	Not reported

Solubilization and precipitation methods				
(Peller <i>et al.</i> 2022)	PE, PET, PS, PP, PC	Plastic pieces (3-20 mg) in deionized water were partially solubilized through the addition of small volumes (10-30 μ L) of dodecane or kerosene and agitation. The sMP were formed through shaking (1 min), while NP were formed through sonication (30 minutes).	0.9-5.6 μ m, 300-700 nm (DLS)	Not reported
(Elhady <i>et al.</i> 2022)	PET	Pellets (25 g) were completely dissolved in 250 mL concentrated trifluoroacetic acid (TFA) solution (90% v/v) at 60 °C. A To precipitate the nanoparticles, 100 mL of a diluted aqueous solution of TFA (20% v/v) was added under vigorous stirring (2 h). The precipitate was centrifuged and washed with distilled water several times and dried at 80 °C (3 h).	46-93 nm (TEM), 50-550 nm (DLS)	Not reported
(Rodríguez-Hernández <i>et al.</i> 2019)	PET	Particles (1 g, < 0.2 mm) were completely dissolved in concentrated TFA (10 mL, 90% v/v) at 50 °C (~2 h). To precipitate the NP/sMP, 10 mL of a diluted aqueous solution of TFA (20% v/v) was added to the initial mixture under vigorous stirring (2 h) The suspension was centrifuged (2500g, 1 h) and the supernatant discharged. The pellet was resuspended in 100 mL 0.5% SDS, vigorously stirred and ultrasonicated. MPs were allowed to settle (1 h) and the top 50 mL containing a suspension of nanosized particles was recovered.	50-200 nm (DLS, TEM)	Not reported
(Johnson <i>et al.</i> 2021)	PET	Dissolution of fibres (0.58 g) in hexafluoroisopropanol (35 mL) followed by precipitation in deionized water (75 mL) and removal of solvent by evaporation, centrifugation and resuspension in water.	170 \pm 3 nm (DLS), 80-110 nm (SEM)	Not reported
(Balakrishnan <i>et al.</i> 2019)	PE	Dissolution in toluene, followed by addition of biosurfactant and emulsification in water and final evaporation of water and solvent.	200-800 nm (Confocal laser scanning microscopy)	Not reported

834 **FIGURES**

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838 **Figure 1. Overview of the number of peer-reviewed manuscripts reporting methods for the production**
 839 **of sMP and NP comprised of various polymer types (left) and the resulting particle sizes (right). Polymer**
 840 **types studied were polystyrene (PS), polyethylene terephthalate (PET), polyethylene (PE), polypropylene**
 841 **(PP), polylactic acid (PLA), polyhydroxybutyrate (PHB), polybutylene adipate terephthalate (PBAT),**
 842 **polyether ether ketone (PEEK) and polycarbonate (PC).**

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