



Meeting the Needs for Released Nanomaterials Required for Further Testing—The SUN Approach

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Meeting the needs for released nanomaterials required for further testing – the SUN approach

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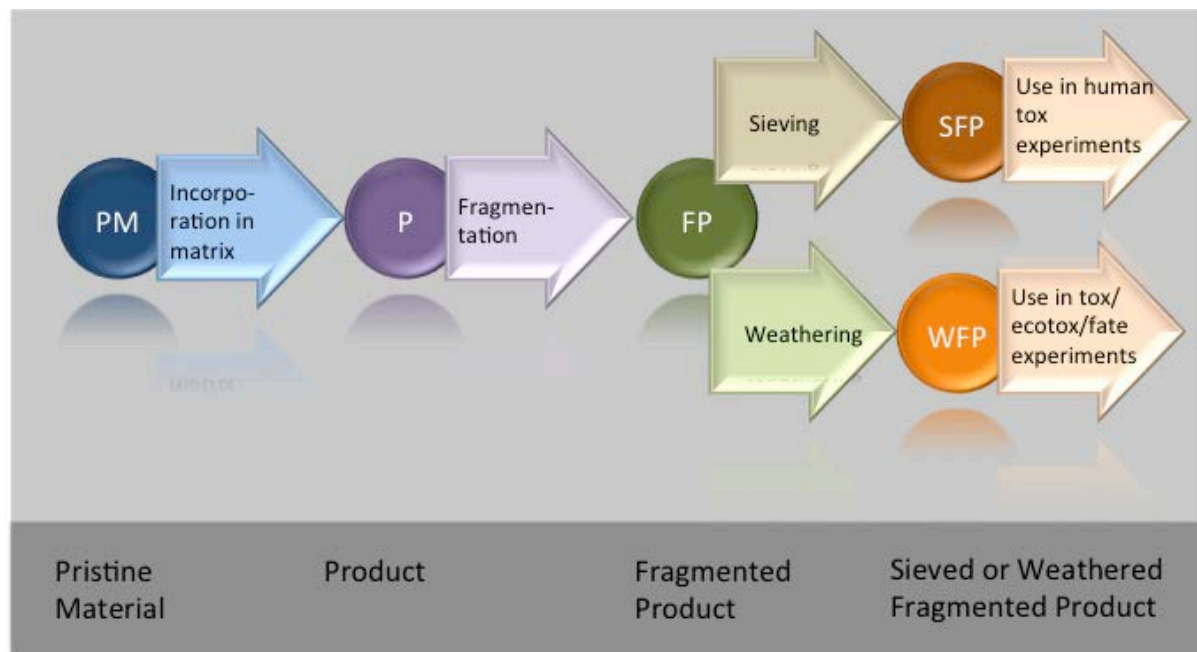
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33 **TOC Art**

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37 **Abstract**

38 The analysis of the potential risks of engineered nanomaterials (ENM) has so far been almost
39 exclusively focused on the pristine, as-produced particles. However, when considering a life-
40 cycle perspective, it is clear that ENM released from genuine products during manufacturing,
41 use, and disposal is far more relevant. Research on release of materials from nano-products
42 is growing and the next necessary step is to investigate the behavior and effects of these
43 released materials in the environment and on humans. Therefore, sufficient amounts of
44 released materials need to be available for further testing. In addition, ENM-free reference
45 materials are needed since many processes not only release ENM but also nano-sized
46 fragments from the ENM-containing matrix that may interfere with further tests. The SUN
47 consortium (Project on “Sustainable Nanotechnologies”, EU 7th Framework funding) uses
48 methods to characterize and quantify nanomaterials released from composite samples that
49 are exposed to environmental stressors. Here we describe an approach to provide materials
50 in hundreds of gram quantities mimicking actual released materials from coatings and
51 polymer nanocomposites by producing what is called “Fragmented Products” (FP). These FP
52 can further be exposed to environmental conditions (e.g. humidity, light) to produce
53 “Weathered Fragmented Products” (WFP) or can be subjected to a further size fractionation
54 to isolate “Sieved Fragmented Products” (SFP) that are representative for inhalation studies.
55 In this perspective we describe the approach, and the used methods to obtain released
56 materials in amounts large enough to be suitable for further fate and (eco)toxicity testing.
57 We present a case study (nanoparticulate organic pigment in polypropylene) to show
58 exemplarily the procedures used to produce the FP. We present some characterization data
59 of the FP and discuss critically the further potential and the usefulness of the approach we
60 developed.

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63 **Introduction**

64 An increasing number of products incorporating engineered nanomaterials (ENM) has
65 reached the market^{1, 2}. In these products, ENM are incorporated in different solid or liquid
66 matrices as for example CNT in plastics³, nano-TiO₂ in ceramics⁴, cement⁵, cosmetics⁶ and
67 paints⁷ or nano-SiO₂ in food⁸. ENM may be released from ENM-containing products during
68 the use or end-of-life phase. Gathering information on the release of ENM from products at
69 different stages of their value chain is therefore important for defining the hot spots of
70 release and for assessing the human and environmental exposure needed for a realistic risk
71 assessment⁹⁻¹².

72 The properties of the materials emitted from the nano-products during the manufacturing,
73 use or end-of-life phase are dominated by the matrix and the incorporation of the ENM into
74 the matrix (i.e. surface-bound or internally embedded)^{13, 14}. The materials released from
75 paints for example consist mainly of paint fragments with incorporated nanoparticles¹⁵⁻¹⁷.
76 Comparable observations were made for CNT-epoxy and CNT-polyurethane composites:
77 fragmentation or weathering resulted mainly in the release of composite fragments with
78 bound CNTs^{18, 19}. The methodology of aging-sampling-characterization of released materials
79 has undergone first interlaboratory comparisons with reproducible observations on silica
80 ENM in plastics²⁰.

81 Transformation reactions may affect the composition of the released materials. Whereas the
82 pristine materials may be released during production and manufacturing, the materials
83 liberated during use and disposal may be transformed to some extent. It has been shown
84 that sunscreen TiO₂ composite nanomaterials are significantly altered by exposure to water
85 and sunlight²¹⁻²³, changing their physico-chemical behavior. Consequently, the effects of
86 altered TiO₂ on organisms differ from the one of pristine nano-TiO₂ as well as from TiO₂
87 composites²⁴⁻²⁶. Al-Kattan et al.²⁷ have shown that TiO₂ released from paint has different
88 colloidal behavior than the pristine TiO₂ before incorporation into the paint, being much
89 more stable in the presence of Ca.

90 These studies show how important it is to evaluate the complete life-cycle of materials
91 containing ENM, as during the different stages materials may be imposed to very diverse
92 chemical and physical conditions that can strongly affect their properties²⁸.

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93 Whereas the definition of the pristine ENM is straightforward - the as-produced materials in
94 pure form - it is less clear what we mean by “aged”, “transformed”, “weathered” or
95 “released” ENM. A classification can be centered on the ENM itself and possible changes
96 during different life-cycle stages²⁹. After ENM are released from products and are changed
97 by environmental processes, they can be classified as environmentally transformed ENM.
98 “Weathered” and “aged” ENM are synonyms for ENM that were exposed to environmental
99 conditions with “transformed” ENM being the result of these reactions. These three
100 designations can be used interchangeably. Aging, transformation and weathering can be
101 carried out using pure pristine materials that are exposed to environmental stressors.
102 Examples include the transformation of TiO₂-nanomaterials used in sunscreens by UV light²¹
103 or the sulfidation of nano-Ag³⁰.

104 As we have stated above, most ENM in products are incorporated into a matrix or present
105 on the surface of a matrix. Thus, these ENM are not available as pure material. ENM
106 released by different mechanisms can be present in free form or bound to the matrix¹³.
107 Under environmental conditions these materials represent the most realistic form of the
108 ENM that enters the environment or that consumers are exposed to. Knowledge on released
109 materials is bound to knowledge on the use of the nano-enabled products during their life-
110 cycle (or different life-cycles)²⁸.

111 In the following we use “released materials” when all the particles released from a matrix
112 are referred to, and use “released ENM” when we only refer to the ENM that are released,
113 either in free or embedded form.

114

115 **Current state on production of aged and released materials**

116 While the need to use released materials in environmental and human fate and exposure
117 studies is clear, it is less straightforward how to obtain and handle released materials. The
118 released materials are not only containing ENM but also matrix fragments, often also in the
119 nano-range. Any (eco)toxicological experiment with released materials should therefore
120 always include the matrix without the ENM in order to relate any effect to the nanomaterial
121 and not to be confounded by possible matrix toxicity. This means that such experiments can
122 only be carried out with matrices specifically produced for experiments, as the nano-free
123 control sample needs to be prepared with the same matrix. Commercially available products

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124 intended for testing face the problem that exactly the same product without ENM (pure
125 matrix) would be needed, which is not possible in most cases. There might also be
126 synergistic effects of matrix and ENM, generating released materials different chemistry to
127 the pristine ENM. If the matrix that is used in actual products cannot be tested alone, then a
128 comparative understanding of the effects of matrix and ENM may not be achievable.

129 Having a matrix with ENM available then brings us to the next point: How to produce and
130 collect sufficiently high amounts of released materials? Ecotoxicological experiments often
131 require gram to even kilogram quantities of materials, e.g., in sediment or soil exposure or
132 mesocosm studies. When the ENM is contained in a solid matrix or in a surface coating,
133 collecting released materials becomes challenging. For example, a leachate from a
134 weathering experiment of a surface coating may only contain ENM in the $\mu\text{g/l}$ level and the
135 highest observed concentrations of Ag and TiO_2 in washing liquids from nano-textiles were in
136 the range of mg/l ^{31, 32}, meaning that any extraction and collection of these materials for
137 further experiments is not feasible. Standard tests that are used in many aging studies such
138 as the Taber abrasion test were developed to investigate the remaining material, not the
139 released fraction³³. Although procedures have been developed to collect materials released
140 in such tests and also perform toxicological tests on them³⁴, the amounts that can be
141 collected with reasonable effort are smaller than required by the SUN project partners.
142 Based on these facts, it is unrealistic to obtain a sufficiently high amount of the released
143 ENM fraction under conditions that are fully representative for real-world conditions.

144 Alternative approaches have to be applied to produce sufficient amounts for risk assessment
145 studies of “released” ENM. Released fragments of polymer nanocomposites were already
146 produced by abrasion in amounts sufficient for *in vitro* studies^{19, 34}. In the NanoHouse
147 project, fragments of a paint were produced by milling and these fragments were exposed to
148 UV light to further age them³⁵. The milling was intended to increase the available surface
149 area of the material for release and weathering. The particle size distribution of the milled
150 paint was in the μm range with a peak at $10 \mu\text{m}$ ¹⁵. These paint fragments represent to some
151 extent released paint fragments observed in field studies^{36, 37}. When the milled and aged
152 paint is extracted with water, a stable suspension with up to $500 \mu\text{g/l}$ Ti could be obtained
153 using 20 g/l of paint powder²⁷. The aged paint powder was then used in toxicological studies,
154 both *in vitro*³⁸ and *in vivo*³⁹. Another approach has also been published where paint was

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155 applied on boards and sanding dust was collected and then used in *in vitro* tests⁴⁰ as
156 released fraction.

157

158 **The SUN approach**

159 The SUN project on “Sustainable Nanotechnologies” aims to evaluate environmental, health
160 and safety risks during the entire life cycle of manufactured nanomaterials and to
161 incorporate the results into tools and guidelines for more sustainable manufacturing. To
162 achieve this goal, the focus of the project is not only on the pristine ENM but more on actual
163 nanoproducts in the form of case studies. It is therefore necessary to collect and
164 characterize ENM released from the materials used in the SUN case studies in different life
165 cycle stages for use in nano(eco)toxicological and behavior/fate studies. The formulated
166 materials are coatings, composites or powder mixtures and represent the actual forms in
167 which ENM are used in the real world. The key requirements identified by SUN partners for
168 producing released ENM are:

- 169 - To use formulated materials instead of just aging pristine particles;
- 170 - The process is reliable and quick;
- 171 - The samples are close to real-world exposure, such that assays can be prepared for
172 “released” materials;
- 173 - They have to be available in a sufficient amount (hundreds of grams to kg) for testing
174 in nano(eco)toxicity studies and with relevant size distribution;
- 175 - A nano-free formulated material is available as reference.

176 Using these criteria, the SUN partners used a consensus process to devise a method to
177 produce materials representing released and aged ENM considering the results from the
178 published release studies discussed above. Depending on the incorporation of the ENM into
179 the matrix and the properties of the matrix, different methods are necessary to produce
180 “fragmented products” (FP). The obtained FP represent a form of the matrix with a greatly
181 increased surface area, but constitute otherwise the identical material to the one in the real
182 products. Within SUN, the elasticity modulus of the matrix, a mechanical material property,
183 which can be identified by oscillatory stress-strain dynamical mechanical analysis, is
184 proposed as criterion for FP processing. For matrices with an elasticity modulus in the range
185 of 10^9 Pa, fragmentation of the matrix with or without ENM can successfully be performed

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186 by cryomilling to simulate the materials' life cycle. Due to the freezing the material gets very
187 brittle and therefore cryomilling can be regarded as a worse case scenario during the life
188 cycle of the material. In addition, cryomilling applies a much higher wear energy compared
189 to sanding which is known as a high mechanical stress inducing operation and therefore
190 releases more airborne particles compared to other handling or processing operations⁴¹. For
191 materials whose behavior under tensile load is comparable to a gel the such as in the case of
192 the CuO containing antimicrobial coating, cryomilling has proven to be not suitable to obtain
193 FP due to their viscoelastic behavior, which leads to a re-flow of the material. In the specific
194 case, this is attributed to the acrylic, non-crosslinked matrix. Therefore, viscoelastic
195 materials need another approach for FP production, which is realized within SUN by coating
196 the material onto a hard substrate. In this case the mechanical properties are dominated by
197 the hard support. FP production can be performed either by cryomilling of scraped-off flakes
198 or sanding of the dried, supported coating. In the latter case, the sanding dust could be
199 collected as FP and would represent a realistic life cycle released material consisting of
200 coating fragments, which coexist with or are attached to substrate fragments.

201 Within SUN eight case studies are carried out, representing important uses of ENM in
202 products. Table 1 shows the different procedures to obtain FP for these case studies as well
203 as resulting FP sizes. The diameter d_{10} represents the smallest 10% of the particles in volume
204 metrics.

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205 **Table 1: Overview of chosen fragmentation methods for each SUN case study, and of resulting fragment products (FP) and “Sieved Fragmented**
 206 **Products” (SFP) sizes and amounts.**

Case study	ENM @ matrix	Fragmentation method The sizes indicated are in volume metrics	Elasticity modulus of matrix (Pa)	Size of FP	FP produced	SFP produced
CNT in antifouling coating	a) CNT @ epoxy	a) Cryomilling of compounded plates	a) $2.8 \cdot 10^9$	d ₁₀ : 96 μm, d ₅₀ : 164 μm and d ₉₀ : 263 μm	180 g	3.1
	b) CNT @ silicone epoxy	b) Painting of compounded paint onto a hard substrate and cryomilling of scraped-off flakes	b) 10^8 (low viscous flow)	d ₁₀ : 77 μm, d ₅₀ : 182 μm and d ₉₀ : 386 μm	900 g	-
Inorganic pigment polymer	Fe ₂ O ₃ @ PE	Cryomilling of compounded granulates	$1.7 \cdot 10^9$	d ₁₀ : 101 μm, d ₅₀ : 154 μm and d ₉₀ : 314 μm	900 g	-
Organic pigment polymer	DPP @ PP	Cryomilling of compounded granulates	$9.7 \cdot 10^8$	d ₁₀ : 70 μm, d ₅₀ : 146 μm and d ₉₀ : 272 μm	900 g	2.8
CNT in lightweight conductive polymer	CNT @ PP	Cryomilling of compounded granulate	$9.7 \cdot 10^8$	d ₁₀ : 69 μm, d ₅₀ : 131 μm and d ₉₀ : 229 μm	900 g	5.4
CuO antimicrobial coating	CuO @ acrylic paint on wood	Preparation of paint films on PE foils. Mortar grinding of paint film (removed from PE) in liquid nitrogen. Transfer of FP to an aqueous phase before complete warm up to room temperature to prevent aggregation.	10^{-7} (viscoelastic)	d ₁₀ : 20 μm, d ₅₀ : 73 μm and d ₉₀ : 190 μm	10 g	-
Antifriction coating	WC @ Co on steel	Coating onto a substrate and fragmentation of scraped-off flakes by wet ball milling	-			
Self-cleaning coating for ceramic tiles	TiO ₂ @ ceramics	No process foreseen in SUN, due to thinness of TiO ₂ coating so that any mechanically generated FP would be completely dominated by ceramic support.	-			
Pancake mixture	SiO ₂ @ flour	No fragmentation needed, <i>in vitro</i> digestion considered	-			

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208 The obtained FP can undergo further processing, including size separation and/or
209 weathering. FP is sieved into “Sieved Fragmented Products” (SFP) either for characterization
210 purposes or to obtain FP samples of wanted size distribution for further testing.

211 In order to obtain samples close to exposure as expected in environmental settings,
212 weathering can be applied to the FP to generate “Weathered Fragmented Products” (WFP).
213 FP used in coatings or composites which are typically exposed to sunlight during the use-
214 phase are weathered in a Suntest apparatus (Atlas, US) according to an ISO guideline⁴². The
215 Suntest apparatus emits simulated solar radiation, which is comparable to natural sunlight
216 with regard to the emitted spectrum as well as to the applied irradiance.

217 When these WFP are then tested under environmentally relevant conditions in toxicological
218 or ecotoxicological test media, e.g. soil, sediments or water, they undergo further changes
219 resulting in “Aged Fragmented Products” (AFP). This aging reaction depends on the type and
220 composition of the medium and is specific for each test. The weathering is carried out after
221 fragmentation, so on FP and not the formulated materials. The FP provides a much higher
222 surface area for weathering whereas in actual products only a thin surface layer may
223 undergo some aging reactions. During this aging process, further release of ENM from the
224 matrix fragments can occur as shown for TiO₂ release from paint fragments.²⁷

225

226 **Example case study: Nanoparticulate organic pigment in polypropylene**

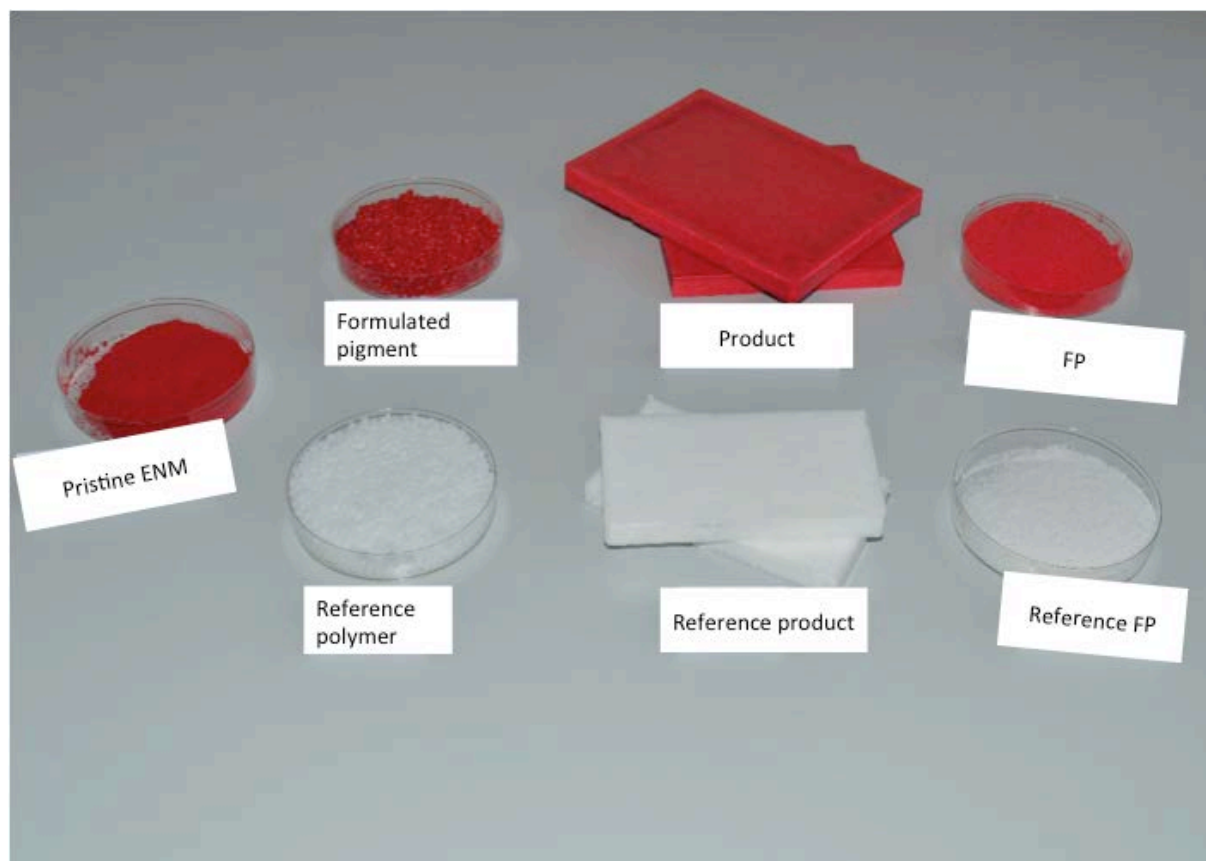
227 One of the SUN case studies is a nanoscale, transparent, solid organic pigment incorporated
228 into a polymer matrix of polypropylene (case study 3, Table 1). Figure 1 shows different life
229 cycle stages of both the nano-pigment and the nano-free reference matrix that are
230 simulated within SUN. The organic pigment nanoparticles were mixed with the plastic and
231 processed by hot melt extrusion and granulation. The resulting granulates had cylindrical
232 shapes with a length of approximately 4 mm and a diameter about 2 mm. Representative for
233 a final product such as car bumpers, sheets of the nano-composite were produced by melt
234 pressing of the granulates. To obtain FP, cryomilling of the compounded granulates was
235 performed. For this, granulates were frozen to cryogenic temperatures (at -193°C, liquid N₂)
236 to maximize their brittleness. In the mill (impeller breaker, Pallmann PPL 18), the impeller
237 rotated at 10,000 rpm (92 m/s circumferential speed). Within 40 minutes, the total amount
238 of 1 kg of the granulates passed through the 0.3 mm gap between the rotor/stator impeller.

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239 Granulates, sheets and FP were produced for the pure PP plastic as well which serve as a
240 reference during fate testing.

241



242
243 *Figure 1. The SUN nanoparticulate solid organic pigment in polymer case study (red, top)*
244 *with pure polymer reference (white, bottom): pristine ENM (on left), formulated product*
245 *after compounding, final nano-composite sheets and fragmented product (FP).*

246
247 To determine the size distributions of the FP, the material was suspended in water
248 containing SDS (sodiumdodecylsulfate) at 0.5 g/l and the sample was probe sonicated. The
249 samples were prepared at a concentration of 1 g/l. The apparatus used for laser diffraction
250 analysis was a Mastersizer 3000 (Malvern). The resulting mass-based size distributions
251 (Figure 2) show clearly that the vast majority of fragments resulting from cryomilling is far
252 from inhalable sizes⁴³. Therefore, the FP produced by cryomilling will be used within SUN for
253 ecotoxicological testing, but is not useful for inhalation studies.

254 To achieve a separation of the smallest possible fragments, dry sieving was performed with a
255 mesh size of 100 μm (sieve 14046173 by Retsch). The size distributions of the SFP were

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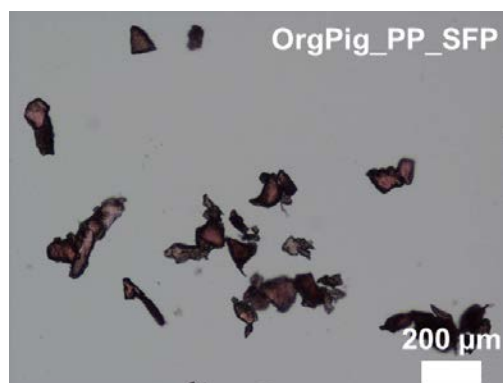
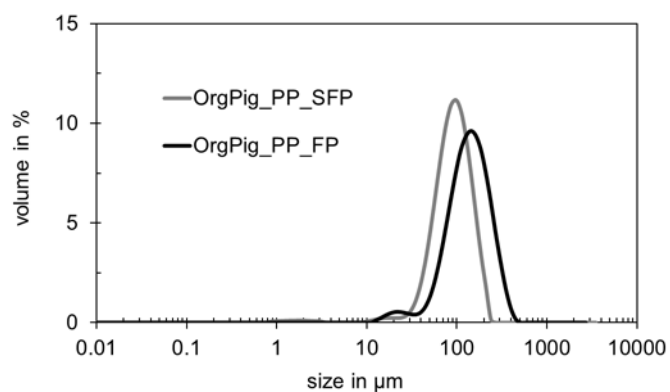
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256 obtained in the liquid by laser diffraction as described above (Figure 2). Obviously, the size
257 distributions of the SFP shifted towards smaller particle sizes with maximum sizes in the
258 range of 200 μm . The hydrophobic character of the PP matrix presumably lead to a shift of
259 the size distribution towards larger fragments due to agglomeration of the fragments in the
260 dispersion. However, the result from laser diffraction is valuable as it clearly demonstrates
261 that sieving successfully eliminated the coarse fraction of the FP. Additionally, images from
262 optical microscopy were taken (Figure 2) which visually confirmed the elimination of the
263 coarse fraction of the FP. In addition, Figure 2 demonstrates that the behavior during
264 cryomilling is dominated by the matrix. No obvious influence from the nano-filler on the
265 resulting size distributions can be observed.

266 On the determined mass-based scale, the amounts of the SFP were only 0.3-0.5% of the FP
267 resulting from cryomilling respectively. With this, the fraction of the smallest possible
268 fragments - which is, however, not in the inhalable size range - can be considered very low
269 on a mass-based scale but may represent a high particle number. Nevertheless, the SFP will
270 also be provided for ecotoxicological fate testing within SUN.

271 Studies within SUN about mechanical processing by sanding have already proven to deliver a
272 higher content of smaller sizes compared to cryomilling (data will be published separately).
273 These smaller sizes are detectable especially in the aerosol on a number-based metric, not
274 so easily on the entire collected dust in volume or mass metrics. Therefore it is planned
275 within SUN to use sanding dust for toxicological testing as well.

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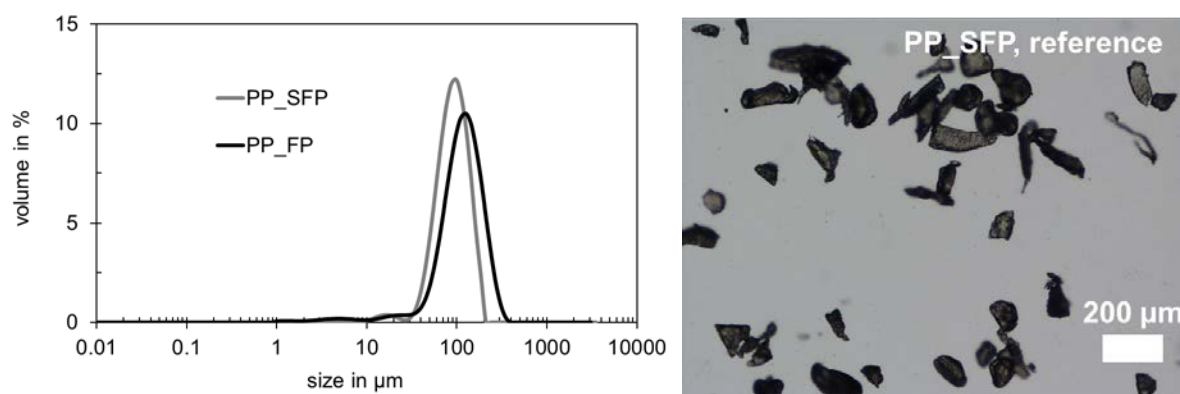


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282 *Figure 2. Size distributions of the fragmented product (FP) (black line) and the sieved*
283 *fragmented products (SFP) (grey line) determined by laser diffraction; on the right a*
284 *microscope image of the SFP is shown; the PP with organic pigment is shown on top, the*
285 *control without pigment on bottom.*

286

287 **Conclusions and Outlook**

288 The focus on aging and release of nanomaterials from products is relevant because of the
289 importance to holistically address the life cycle of nanoproducts. The form of ENM that
290 reaches the environment, or that consumers are exposed to, often depends on the nature of
291 the matrix in which the ENM were incorporated. Thus, it is necessary to investigate the
292 effects and fate of these released materials and compare them to pristine nanomaterials for
293 which a huge amount of data is already available. These investigations will need to elucidate
294 whether pristine and aged/released ENM have similar or different behavior and effects. The
295 progress in this field is currently hampered by methodological difficulties in how to obtain
296 released/aged materials in quantities sufficiently high for further testing. In this perspective,
297 besides characterizing and quantifying nanomaterials released from products exposed to
298 various environmental stressors we here show how to obtain a material fraction that is
299 comparable to the materials that were reported to be released under real-world
300 conditions¹³ and how to isolate nanomaterials in amounts needed for further testing.

301 We acknowledge that the FP and WFP obtained by our approach are not the materials
302 released from products, but they represent an approximation and allow the production of
303 hundred gram quantities of materials for testing (see Table 1). It may be necessary to use

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304 more than one process to obtain FP or especially WFP for each matrix as the same material
305 may be exposed to different stresses during the life cycle of the products.

306 A further size fractionation can be performed to isolate a SFP fraction that is more
307 representative for inhalation studies (PM10, PM2.5 for instance), however, only minute
308 amounts in terms of mass of the fragments obtained by our approach are actually small
309 enough for such studies. This finest fraction is clearly the most relevant for human toxicology
310 studies but also for ecotoxicological studies this fine fraction might be of interest.

311 The approach presented in this perspective is centered on the use phase of nano-products
312 and can equally be applied to the end-of-life stage. This is particularly important as
313 significant release could occur in the end-of-life stage (e.g. shredding, incineration,
314 landfilling, recycling), especially for products where the ENM are bound in a matrix^{11, 12}.

315

316

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323 **References**

324 1. Stark, W. J.; Stoessel, P. R.; Wohlleben, W.; Hafner, A., Industrial applications of
325 nanoparticles. *Chemical Society Reviews* **2015**, *44*, 5793-5805.

326 2. Nanodatabase The Nanodatabase <http://nanodb.dk/>. DTU Environment, Danish
327 Consumer Council and the Danish Ecological Council.

328 3. Kingston, C.; Zepp, R.; Andrady, A.; Boverhof, D.; Fehir, R.; Hawkins, D.; Roberts, J.;
329 Sayre, P.; Shelton, B.; Sultan, Y.; Vejins, V.; Wohlleben, W., Release characteristics of
330 selected carbon nanotube polymer composites. *Carbon* **2014**, *68*, 33-57.

331 4. Benedix, R.; Dehn, F.; Quaas, J.; Orgass, M., Application of titanium dioxide
332 photocatalysis to create self-cleaning building materials. *Lacer* **2000**, *5*, 157-168.

333 5. Ruot, B.; Plassais, A.; Olive, F.; Guillot, L.; Bonafous, L., TiO₂-containing cement pastes
334 and mortars: Measurements of the photocatalytic efficiency using a rhodamine B-based
335 colourimetric test. *Sol. Energy* **2009**, *83*, 1794-1801.

336 6. Nohynek, G. J.; Lademann, J.; Ribaud, C.; Roberts, M. S., Grey goo on the skin?
337 Nanotechnology, cosmetic and sunscreen safety. *Critical Reviews in Toxicology* **2007**, *37*,
338 251-277.

This is a post-print version of Nowack et al. (2016). Meeting the Needs for Released Nanomaterials Required for Further Testing—The SUN Approach. *Environmental Science and Technology*, 50(6), 2747-2753.

The printed version of the paper is available at: DOI: 10.1021/acs.est.5b04472

- 339 7. Khanna, A. S., Nanotechnology in High Performance Paint Coatings. *Asian J. Exp. Sci.*
340 **2008**, *21*, 25-32.
- 341 8. Peters, R.; ten Dam, G.; Bouwmeester, H.; Helsper, H.; Allmaier, G.; von der Kammer,
342 F.; Ramsch, R.; Solans, C.; Tomaniova, M.; Hajslova, J.; Weigel, S., Identification and
343 characterization of organic nanoparticles in food. *Trac-Trends in Analytical Chemistry* **2011**,
344 *30*, 100-112.
- 345 9. Som, C.; Berges, M.; Chaudhry, Q.; Dusinska, M.; Fernandes, T. F.; Olsen, S. I.;
346 Nowack, B., The Importance of Life Cycle Concepts for the Development of Safe
347 Nanoproducts. *Toxicol.* **2010**, *269*, 160–169.
- 348 10. Boldrin, A.; Hansen, S. F.; Baun, A.; Hartmann, N. I. B.; Astrup, T. F., Environmental
349 exposure assessment framework for nanoparticles in solid waste. *Journal of Nanoparticle*
350 *Research* **2014**, *16*, 2394.
- 351 11. Nowack, B.; David, R. M.; Fissan, H.; Morris, H.; Shatkin, J. A.; Stintz, M.; Zepp, R.;
352 Brouwer, D., Potential release scenarios for carbon nanotubes used in composites.
353 *Environment International* **2013**, *59*, 1-11.
- 354 12. Walser, T.; Gottschalk, F., Stochastic fate analysis of engineered nanoparticles in
355 incineration plants. *Journal of Cleaner Production* **2014**, *80*, 241-251.
- 356 13. Froggett, S. J.; Clancy, S. F.; Boverhof, D. R.; Canady, R. A., A review and perspective
357 of existing research on the release of nanomaterials from solid nanocomposites. *Particle and*
358 *Fibre Toxicology* **2014**, *11*, 17.
- 359 14. Duncan, T. V.; Pillai, K., Release of Engineered Nanomaterials from Polymer
360 Nanocomposites: Diffusion, Dissolution, and Desorption. *ACS Applied Materials & Interfaces*
361 **2015**, *7*, 2-19.
- 362 15. Al-Kattan, A.; Wichser, A.; Vonbank, R.; Brunner, S.; Ulrich, A.; Zuin, S.; Arroyo, Y.;
363 Golanski, L.; Nowack, B., Characterization of materials released into water from paint
364 containing nano-SiO₂. *Chemosphere* **2015**, *119*, 1314–1321.
- 365 16. Al-Kattan, A.; Wichser, A.; Vonbank, R.; Brunner, S.; Ulrich, A.; Zuin, S.; Nowack, B.,
366 Release of TiO₂ from paints containing pigment-TiO₂ or nano-TiO₂ by weathering.
367 *Environmental Science: Processes & Impacts* **2013**, *15*, 2186–2193.
- 368 17. Zuin, S.; Gaiani, M.; Ferrari, A.; Golanski, L., Leaching of nanoparticles from
369 experimental water-borne paints under laboratory test conditions. *J Nanopart Res* **2014**, *16*,
370 2185.
- 371 18. Cena, L. G.; Peters, T. M., Characterization and Control of Airborne Particles Emitted
372 During Production of Epoxy/Carbon Nanotube Nanocomposites. *Journal of Occupational and*
373 *Environmental Hygiene* **2011**, *8*, 86-92.
- 374 19. Wohlleben, W.; Meier, M. W.; Vogel, S.; Landsiedel, R.; Cox, G.; Hirth, S.; Tomovic, Z.,
375 Elastic CNT-polyurethane nanocomposite: synthesis, performance and assessment of
376 fragments released during use. *Nanoscale* **2013**, *5*, 369-380.
- 377 20. Wohlleben, W.; Vilar, G.; Fernández-Rosas, E.; González-Gálvez, D.; Gabriel, C.; Hirth,
378 S.; Frechen, T.; Stanley, D.; Gorham, J.; Sung, L.-P.; Hsueh, H.-C.; Chuang, Y.-F.; Nguyen, T.;
379 Vazquez-Campos, S., A pilot interlaboratory comparison of protocols that simulate aging of
380 nanocomposites and detect released fragments. *Environmental Chemistry* **2014**, *11*, 402-
381 418.
- 382 21. Auffan, M.; Pedeutour, M.; Rose, J.; Masion, A.; Ziarelli, F.; Borschneck, D.; Chaneac,
383 C.; Botta, C.; Chaurand, P.; Labille, J.; Bottero, J. Y., Structural Degradation at the Surface of a

This is a post-print version of Nowack et al. (2016). Meeting the Needs for Released Nanomaterials Required for Further Testing—The SUN Approach. *Environmental Science and Technology*, 50(6), 2747-2753.

The printed version of the paper is available at: DOI: 10.1021/acs.est.5b04472

- 384 TiO₂-Based Nanomaterial Used in Cosmetics. *Environmental Science & Technology* **2010**, *44*,
385 2689-2694.
- 386 22. Botta, C.; Labille, J.; Auffan, M.; Borschneck, D.; Miche, H.; Cabie, M.; Masion, A.;
387 Rose, J.; Bottero, J. Y., TiO₂-based nanoparticles released in water from commercialized
388 sunscreens in a life-cycle perspective: Structures and quantities. *Environmental Pollution*
389 **2011**, *159*, 1543-1548.
- 390 23. Labille, J.; Feng, J. H.; Botta, C.; Borschneck, D.; Sammut, M.; Cabie, M.; Auffan, M.;
391 Rose, J.; Bottero, J. Y., Aging of TiO₂ nanocomposites used in sunscreen. Dispersion and fate
392 of the degradation products in aqueous environment. *Environmental Pollution* **2010**, *158*,
393 3482-3489.
- 394 24. Bigorgne, E.; Foucaud, L.; Lapied, E.; Labille, J.; Botta, C.; Sirguey, C.; Falla, J.; Rose, J.;
395 Joner, E. J.; Rodius, F.; Nahmani, J., Ecotoxicological assessment of TiO₂ byproducts on the
396 earthworm *Eisenia fetida*. *Environmental Pollution* **2011**, *159*, 2698-2705.
- 397 25. Foltete, A. S.; Masfaraud, J. F.; Bigorgne, E.; Nahmani, J.; Chaurand, P.; Botta, C.;
398 Labille, J.; Rose, J.; Ferard, J. F.; Cotellet, S., Environmental impact of sunscreen
399 nanomaterials: Ecotoxicity and genotoxicity of altered TiO₂ nanocomposites on *Vicia faba*.
400 *Environmental Pollution* **2011**, *159*, 2515-2522.
- 401 26. Fouqueray, M.; Dufils, B.; Vollat, B.; Chaurand, P.; Botta, C.; Abacci, K.; Labille, J.;
402 Rose, J.; Garric, J., Effects of aged TiO₂ nanomaterial from sunscreen on *Daphnia magna*
403 exposed by dietary route. *Environmental Pollution* **2012**, *163*, 55-61.
- 404 27. Al-Kattan, A.; Wichser, A.; Zuin, S.; Arroyo, Y.; Golanski, L.; Ulrich, A.; Nowack, B.,
405 Behavior of TiO₂ released from nano-TiO₂-containing paint and comparison to pristine
406 nano-TiO₂. *Environmental Science & Technology* **2014**, *48*, 6710-6718.
- 407 28. Mitrano, D. M.; Motellier, S.; Clavaguera, S.; Nowack, B., Review of nanomaterial
408 aging and transformations through the life cycle of nano-enhanced products. *Environment*
409 *International* **2015**, *77*, 132-147.
- 410 29. Nowack, B.; Ranville, J. F.; Diamond, S.; Gallego-Urrea, J. A.; Metcalfe, C.; Rose, J.;
411 Horne, N.; Koelmans, A. A.; Klaine, S. J., Potential scenarios for nanomaterial release and
412 subsequent alteration in the environment. *Environ. Toxicol. Chem.* **2012**, *31*, 50-59.
- 413 30. Levard, C.; Reinsch, B. C.; Michel, F. M.; Oumahi, C.; Lowry, G. V.; Brown, G. E.,
414 Sulfidation Processes of PVP-Coated Silver Nanoparticles in Aqueous Solution: Impact on
415 Dissolution Rate. *Environmental Science & Technology* **2011**, *45*, 5260-5266.
- 416 31. Lorenz, C.; Windler, L.; Lehmann, R. P.; Schuppler, M.; Von Goetz, N.; Hungerbühler,
417 K.; Heuberger, M.; Nowack, B., Characterization of silver release from commercially available
418 functional (nano)textiles. *Chemosphere* **2012**, *89*, 817-824.
- 419 32. Windler, L.; Lorenz, C.; Von Goetz, N.; Hungerbühler, H.; Amberg, M.; Heuberger, M.;
420 Nowack, B., Release of titanium dioxide from textiles during washing. *Environ Sci Technol*
421 **2012**, *46*, 8181-8188.
- 422 33. ASTM, ASTM G195 - 13a. Standard Guide for Conducting Wear Tests Using a Rotary
423 Platform Abraser. In 2013.
- 424 34. Schlagenhauf, L.; Buerki-Thurnherr, T.; Kuo, Y.-Y.; Wichser, A.; Nüesch, F.; Wick, P.;
425 Wang, J., Carbon Nanotubes Released from an Epoxy-Based Nanocomposite: Quantification
426 and Particle Toxicity. *Environmental Science & Technology* **2015**.
- 427 35. Zuin, S.; Massari, A.; Ferrari, A.; Golanski, L., Formulation effects on the release of
428 silica dioxide nanoparticles from paint debris to water. *Science of the Total Environment*
429 **2014**, *476*, 298-307.

This is a post-print version of Nowack et al. (2016). Meeting the Needs for Released Nanomaterials Required for Further Testing—The SUN Approach. *Environmental Science and Technology*, 50(6), 2747-2753.

The printed version of the paper is available at: DOI: 10.1021/acs.est.5b04472

- 430 36. Kaegi, R.; Ulrich, A.; Sinnet, B.; Vonbank, R.; Wichser, A.; Zuleeg, S.; Simmler, H.;
431 Brunner, S.; Vonmont, H.; Burkhardt, M.; Boller, M., Synthetic TiO₂ nanoparticle emission
432 from exterior facades into the aquatic environment. *Environ. Pollut.* **2008**, *156*, 233-239.
- 433 37. Kaegi, R.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Mueller, E.; Vonbank, R.; Boller, M.;
434 Burkhardt, M., Release of silver nanoparticles from outdoor facades. *Environmental Pollution*
435 **2010**, *158*, 2900-2905.
- 436 38. Kaiser, J.-P.; Roesslein, M.; Diener, L.; Wick, P., Human Health Risk of Ingested
437 Nanoparticles That Are Added as Multifunctional Agents to Paints: an In Vitro Study. *PLoS*
438 *ONE* **2013**, *8*, e83215.
- 439 39. Smulders, S.; Luyts, K.; Brabants, G.; Landuyt, K. V.; Kirschhock, C.; Smolders, E.;
440 Golanski, L.; Vanoirbeek, J.; Hoet, P. H., Toxicity of Nanoparticles Embedded in Paints
441 Compared with Pristine Nanoparticles in Mice. *Toxicological Sciences* **2014**, *141*, 132-140.
- 442 40. Saber, A. T.; Koponen, I. K.; Jensen, K. A.; Jacobsen, N. R.; Mikkelsen, L.; Moller, P.;
443 Loft, S.; Vogel, U.; Wallin, H., Inflammatory and genotoxic effects of sanding dust generated
444 from nanoparticle-containing paints and lacquers. *Nanotoxicology* **2012**, *6*, 776-788.
- 445 41. Le Bihan, O.; Shandilya, N.; Gheerardyn, L.; Guillon, O.; Dore, E.; Morgeneyer, M.,
446 Investigation of the Release of Particles from a Nanocoated Product. *Advances in*
447 *Nanoparticles* **2013**, *2*, 39-44.
- 448 42. ISO, ISO 4892-1: Plastics - Methods of exposure to laboratory light sources - Part 1:
449 General guidance. ISO 4892-2: Plastics - Methods of exposure to laboratory light sources -
450 Part 2: Xenon-arc sources.
- 451 43. Heyder, J., Deposition of Inhaled Particles in the Human Respiratory Tract and
452 Consequences for Regional Targeting in Respiratory Drug Delivery. *Proceedings of the*
453 *American Thoracic Society* **2004**, *1*, 315-320.
- 454