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1	1 Nanoplastics are neither microplastics nor engineered		
2	nanoparticles		
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31	Abstract		
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33	Increasing concern and research on the subject of plastic pollution has engaged the community		
34	of scientists working on the environment, health, and safety (EHS) of nanomaterials. While		
35	many of the methods developed in nano EHS work have general applicability to the study of		
36	particulate plastics, the nanometric size range has important consequences for both the		
37	analytical challenges of studying nano-scale plastics and the environmental implications of		
20	these incidental nonometarials. Polated to their size, nononlastics are distinguished from		

these incidental nanomaterials. Related to their size, nanoplastics are distinguished from 38 microplastics with respect to their transport properties, interactions with light and natural 39 colloids, a high fraction of particle molecules on the surface, bioavailability, and diffusion times 40 for the release of plastic additives. Moreover, they are distinguished from engineered 41 42 nanomaterials because of their high particle heterogeneity and their potential for rapid further 43 fragmentation in the environment. These characteristics impact environmental fate, potential 44 effects on biota and human health, sampling, and analysis. Like microplastics, incidentally-45 produced nanoplastics exhibit a diversity of compositions, morphologies, and heterogeneity that is typically absent from engineered nanomaterials. Therefore, nano-scale plastics must be 46 considered as distinct from both microplastics and engineered nanomaterials.

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51 Introduction

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53 While the accumulation of microplastics and larger plastic debris in the world's oceans has 54 garnered much attention, colloidal plastic debris may represent a portion of released plastic that remains unaccounted for based on oceanic circulation models¹⁻⁴. Scientists also estimate that 55 56 plastic pollution on land and in freshwaters⁵ can be many times greater than the estimated 4.8 to 12.7 million metric tons³ of plastic annually emitted to the ocean, yet little is known regarding 57 58 the levels of colloidal plastics in these environmental compartments. Nanoplastics are the 59 smaller nano-scale fraction of these colloids and are most likely to be incidentally produced from the fragmentation of larger plastic debris. Although complete breakdown of larger plastic 60 debris can take up to hundreds of years, mechanical wear⁶, heat⁷, UV degradation⁸ and, in some 61 cases, biological factors⁹, lead to relatively rapid fragmentation of plastic debris down to the 62 63 nano-scale.

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Our understanding of the interactions between incidental nanoplastics and the environment is 65 in its infancy. Due to methodological challenges¹⁰, nanoplastics in environmental samples 66 remain largely unquantified, although recent work has reported the chemical signatures of 67 nanoplastic contamination in ocean waters¹¹. Due to their similar composition and origin, as 68 well as the nature of the research communities involved, nanoplastics have been largely treated 69 70 as an extension of microplastics. However, size-dependent properties of nanoplastics 71 distinguish them from microplastics with respect to their transport properties, interactions with 72 light and natural colloids, analytical challenges, bioavailability, potential toxicity, and leaching 73 times for additives. And, unlike engineered nanomaterials (ENMs) which can include polymer 74 formulations, incidentally-produced nanoplastics in the environment are essentially debris from 75 the environmental fragmentation of larger plastic objects. Chemists, biologists, physicists, 76 ecologists, engineers, toxicologists, and other scientific professionals in the community of 77 researchers examining the environmental, health, and safety (EHS) of ENMs have made 78 significant advances over the last twenty years in developing methodologies for studying nano-79 scale materials and elucidating the environmental behavior of nano-scale objects. ENMs have 80 been the focus of much of this nanoEHS research. The uniform size and composition that can 81 be achieved in making ENMs, has made them excellent tools for studying the behavior of nano-82 scale particles in complex environments. Conversely, the heterogeneity of most incidental 83 nanomaterials, including nanoplastics, presents numerous challenges to tracking and 84 quantifying these materials in complex environments. Consequently, nanoplastics should be 85 considered as a unique class of contaminants, distinct from both microplastics and ENMs. 86

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88 Nanoplastics are distinct from microplastics89

90 An increase in publications dealing with microplastics has coincided with a shift in terminology, and entry of new scientific communities in addressing the issue of plastic waste 91 as a growing environmental concern. For example, polystyrene spheres are increasingly 92 93 described in the recent scientific literature as either micro- or nano- "plastic" rather than simply 94 as particles or nanoparticles (Figure 1). The labeling of polystyrene spheres as microplastics (Fig 1a) or nanoplastics (Fig. 1b) introduces new terms for materials used in virtually identical 95 studies conducted with a different motivation many decades earlier¹². The re-casting of the 96 97 microplastic problem as one that distinctly entails nanoplastics (Figure 1b) may reflect a re-98 tooling of the nanoEHS community to apply methods to the problem of plastic debris. Indeed, there is a large body of knowledge gained from studying ENM behavior in the workplace. in 99 consumer products, and in natural and complex environmental systems that can be extended to 100

nanoplastics research. In the last two decades, significant analytical developments have been
 made to characterize, identify and quantify ENMs in aqueous media such as fullerenes¹³, silver
 nanoparticles¹⁴, and TiO₂ nanoparticles¹⁵ and to elucidate the environmental fate and impact of
 these materials. A key lesson learned from ENM research is that properly defining terms early
 in the trajectory of the research and establishing rigorous ontologies, enables data sharing across
 communities. It is therefore important to articulate what is meant by the terms nanoplastic,
 microplastic and their relationship to ENMs.

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Fig. 1. Number of publications obtained from Scopus that use the terms shown on the y-axis in the Title/Abstract/Keywords.
(a) particle* AND polystyrene* AND NOT microplastic*(green circle) versus microplastic* & polystyrene* (blue diamond) and (b) nanoparticle* AND polystyrene* AND NOT nanoplastic* (green circle) vs nanoplastic* & polystyrene (blue diamond). The light blue vertical line highlights the coincidence between the decrease in the number of publications and the exponential increase in publications that occurs by changing the terms from "particle" and "nanoparticle" to "microplastic" and "nanoplastic".

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While all plastics are polymer-based, not all polymers are plastic and nanoplastics are not 117 synonymous with nanopolymers¹⁶. Plastics will typically include material composed of 118 polymer as well as additives. The term "nanoplastic" has typically been used with reference to 119 120 solely size, and with some inconsistency as to what the exact size cut-off should be between a nanoplastic and a microplastic, with 100 nm and 1000 nm being the most common size cut-121 offs^{17–19}. The US National Nanotechnology Initiative (NNI) defines a nanomaterial as having 122 at least one dimension between 1 and 100 nm and exhibiting properties not found at larger sizes 123 124 of the same material. The definition of "nano" typically involves considerations that go beyond arbitrary size cut-offs²⁰. While conflicting scientific, commercial, and regulatory considerations 125 complicate the definition of nanomaterials²⁰, the distinction between nanomaterials and their 126 127 larger counterparts is generally held to be dependent on both size and the resulting properties. For example, semi-conductor quantum dots fluoresce at size-dependent wavelengths due to 128 129 quantum confinement of electrons at particle sizes well below electron wavelengths. Consequently, the properties and behavior of nanomaterials cannot be extrapolated from the 130 properties of their bulk counterparts. In our view, it is these characteristics, that separate "nano" 131 from "micro" regardless of a particular size range²⁰. Indeed, while a size cut off is attractive in 132 terms of simplicity in regulatory classification, we believe that a characteristic-based definition 133 will be consistent with the overall purpose of having regulations. Further, one may differentiate 134 between engineered, incidental, and natural nanoparticles, distinctions that may be blurred as 135 in the case of C₆₀ which can be produced as an ENM, but is also present in combustion products 136

ranging from forest fires to industrial combustion²¹. As incidental nanomaterials, nanoplastics
 are likely to exhibit size-emergent properties (Figure 2) that will depend on both the origin of
 the material and the pathway to its creation. Characteristics that distinguish nanoplastics from

- 140 microplastics are summarized in the box below.
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Nanoplastics versus Microplastics

- The dominance of Brownian motion over sedimentation and buoyancy characterized by random movements of the particles in a suspension medium.
 Departure from the geometric/ray approximation between light and matter. For microplastics, the interaction of light and microplastic can be adequately approximated by movimal in some analytical methods, including diffraction, are significant.
 A high proportion of molecules on the surface resulting in a higher relative importance of surface interactions compared to physical interactions.
 The thickness of the particle diffuse layer may be comparable to the size of environmental macromolecules (leading to adsorption/heteroaggregation), while it is small compared to the size of associated microorganisms (preventing biolinin formation).
 Sizes compatible with bio-uptake, translocation and transport across biological membranes.
 Short length scales that may speed the diffusive release of plastic additives and non-intentionally added substances in the original bulk plastic.
 High particle heterogeneity originating from variable sources and environmental conditions that result in nano-scale materials with a wide range of sizes, shapes, and overall composition even when the nanoplastics originate from a common source.
 Incidental nanoplastics may include the fragmentation products of engineered (primary) nanoplastics with the potential for further fragmentation in the environment at shorter time scales.
 - Text-box: Characteristics that distinguishing nanoplastics from microplastics and engineered nanomaterials.
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145 Nanoplastics have unique characteristics compared to engineered nanomaterials

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While nanoplastics may share many of the properties of ENMs, nanoplastics found in the 147 environment present unique considerations and challenges. To begin with, environmental 148 149 nanoplastics, largely incidental in origin, represent a vastly higher exposure potential compared to ENMs. Of the 6300 million metric tons of plastic waste generated between 1950 and 2015, 150 approximately 5000 million metric tons were emitted to the environment, where they can 151 eventually break down into nanoplastics². In 2018, plastic production was approximately 359 152 million metric tons worldwide²². In contrast, rough estimates for global production of ENMs of 153 all kinds range from thousands of metric tons per year to no more than 10^6 metric tons. 154 Compared to plastics production that emerged as early as the 1950s²³, ENM production is a 155 relatively recent activity that has expanded rapidly since the 1990s.

156 157 158 Furthermore, environmental nanoplastics as a class of contaminants are substantially more 159 heterogeneous compared to ENMs. ENMs are intentionally created to desired specifications, 160 typically with a uniform composition for a given material. The separation and characterization of ENMs in complex media are facilitated by knowing these specifications and their resulting 161 properties (i.e., optical, magnetic, conductor properties). Standardized techniques adapted to 162 ENMs are now widely used by the scientific community. Note that there exists ENMs 163 composed of polymer types that could be considered "plastic". For example, spherical and 164 165 monodisperse polystyrene nanoparticles, used in the ENM community, are used as a reference or model material for calibrating analytical tools such as field flow fractionation, size exclusion 166 chromatography, static light scattering, and other techniques, as summarized elsewhere²⁴. Due 167 to their high uniformity, these plastic spheres are easy to track during the analyses, but are not 168 169 representative of the diversity of incidental nanoplastics in the environment²⁵. Environmental

170 nanoplastics are generally not intentionally designed and vary greatly in shape, size, polydispersity, additives, adsorbed contaminants, surface properties, and composition as a 171 172 consequence of different source materials, fragmentation pathways and environmental exposure²⁶. The resulting physical and chemical heterogeneity of nanoplastics may influence 173 their reactivity and will certainly affect interactions with natural colloids and organisms. For 174 175 example, weathering induced by UV radiation enhances fragmentation of bulk plastic into 176 micro- and nanoplastics, in addition to modifying material chemical properties such as crystallinity and polarity which significantly influence the adsorption of substances^{27,28}. The 177 result is an extremely broad scope of materials to investigate. These incidental nanoplastics are 178 179 distinguished from ENMs (Figure 2) by the two main properties summarized in the text box.

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While some researchers are creating model materials intended to be used as nanoplastic 181 proxies^{25,29–31}, in contrast with ENMs, there are no "standard" materials that can be used to fully 182 represent environmental nanoplastics or track them. Nevertheless, many factors influence the 183 formation of environmental nanoplastics and since most of these processes are still poorly 184 understood, nanoplastic proxies used in the laboratory can include idealizations that are not 185 fully representative of environmental realities or that are not generalizable to other types of 186 nanoplastics^{32,33}. Therefore, as we should not overlook the fact that CuO nanoparticles can have 187 different effects from TiO₂ particles when interpreting experimental results, we should not 188 189 dismiss the varying impacts that different types of nanoplastics can have. The use of fieldcollected samples, and their subsequent fractionation using a "top-down" approach, followed 190 by their characterization, can provide a baseline comparison for results from experiments using 191 192 nanoplastic proxies.

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194 Environmental fate and behavior

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196 The distinct characteristics of nanoplastics will influence their environmental fate and behavior, 197 interactions with biological systems, sampling strategies, analytical methods, experimental and 198 computational modeling approaches that cannot be extrapolated from microplastics or ENMs. 199 Due to the colloidal nature and dominance of Brownian motion over sedimentation and buoyancy, vertical transport of individual nanoplastic particles will be small compared with 200 microplastics composed of the same material (property I in Figure 2). For example, 201 polypropylene and polyethylene have densities less than water, and are therefore expected to 202 203 float in water, while polyvinyl chloride would be expected to settle because of its higher density (~1.4 g/cm³). By Stokes' law, a 1 mm polyvinyl chloride microplastic is calculated to settle at 204 205 a rate of approximately 22 cm/sec in water compared with a 100 nm nanoplastic which would 206 be expected to settle at a rate of only some 7 cm per year. Thus nanoplastics, as with other colloidal species, are more likely to remain homogeneously dispersed in aqueous systems¹⁸ and 207 may be more likely to remain suspended in the atmosphere and form a portion of "ultra-fine" 208 (sub 100 nm) particulate matter³⁴. However, aggregation with other particles may increase the 209 210 effective settling rate of nanoplastics. 211

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Figure 2. Transformations and characteristics of plastic debris in the environment. The eight defining characteristics of 215 nanoplastics that distinguish them from microplastics and engineered nanomaterials. The time needed to leach one half of the 216 additives initially present in a plastic material is calculated for the representative additive tributyl phosphate ("leaching of 217 additives" curve, characteristic VI). Leaching of tributyl phosphate was calculated for different sized epoxy particles based on internal diffusion-limited transport 44. Bulk plastics break down into micro- and nanoplastics due to weathering action (UV 218 219 sunlight, mechanical abrasion, etc.), environmental (heteroaggregation) and biological (bacteria, krill, etc.) processes.

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222 Nanoplastics can be expected to heteroaggregate with natural colloids such as natural organic matter (polysaccharides, humic acids, leonardite, etc.), iron oxides, and clays, and/or 223 anthropogenic material 20,35 or with aerosols in the atmosphere (property IV in Figure 2). 224 Macromolecules that may associate with nanoplastics will have length scales similar to the 225 226 nanoplastic, while being much smaller than the size of a microplastic. This may affect the degrees of freedom of the macromolecule and therefore the attachment efficiency between 227 228 nanoplastics and heteroaggregates. Heteroaggregation and the conformation of charged macromolecules in water is in turn influenced by environmental conditions (i.e., porewater pH, 229 230 ionic strength, hardness, etc). These factors will disproportionately influence the attachment 231 efficiency of nanoplastics relative to microplastics due to the dominant role of surfaces at the 232 nanoscale. In addition, the collision rate kernel for nanoplastic heteroaggregation will be 233 dominated by Brownian diffusion rather than by settling, buoyancy, or fluid motion. 234 Consequently, nanoplastic distribution in the environment cannot be directly extrapolated from that of microplastics. 235

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237 The fact that nanoplastics are smaller than most microorganisms, implies differences in the microbial interactions and biofilm formation. Microplastics are large enough to host a 238 community of micro-organisms (*i.e.*, the "plastisphere")^{36,37} and accompanying development 239

of complex biofilms. These micro-organisms can form a significant portion of the overall mass 240 and can affect environmental distribution by altering the effective density of microplastics, but 241 the overall particle is still primarily the microplastic²¹. In contrast, nanoplastics may evolve to 242 be minor components of a larger nanoplastic-microbial complex and, like ENMs²², may make 243 up a small percentage of the mass of heteroaggregates. The environmental fate of these 244 245 heteroaggregates may not have a strong dependence on the properties of nanoplastics (property 246 IV in Figure 2). Figure 3 illustrates the contribution of natural organic matter (NOM) relative 247 to a volume of plastic debris. It is interesting to note the shift in the slope of the curve which 248 occurs at the transition between nanoplastics and microplastics. The NOM is a minor 249 component compared to the host plastic from the millimeter to the micrometer scales. As the 250 plastic size decreases, the contribution of the NOM increases and it becomes analytically challenging to discriminate the plastic component, especially given similar carbon-based 251 252 structures. Unlike with microplastics, the transport, uptake and accumulation pathways of nanoplastics will be highly influenced by NOM relative to the intrinsic properties of the plastic. 253 254 The outsized influence of NOM on environmental fate can lead to inappropriate strategies 255 during sampling, analysis and lifecycle assessments if only the plastic component of 256 heteroaggregates is considered.

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Figure 3. Relative proportion of natural organic matter compared to plastic according to the size (radius) of plastic
 debris. The relative proportion is represented by the ratio of the volume of the natural organic matter to the volume of plastic
 for a given particle.

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Plastics often contain a wide variety of chemical additives as well as non-intentionally added
 substances such as degradation products, reaction by-products and/or impurities^{26,38,39}. Since
 these other chemicals are not generally covalently bound to the polymer matrix, they may leach
 out of the plastic^{26,40}. These leached chemicals include bisphenol A, phthalates, nonylphenols,

brominated flame retardants, to name a few ^{26,40}. We have used analytical solutions describing 269 leaching of sorbed materials from a homogeneous particle matrix (e.g., Crank, 1975)⁴¹ in 270 conjunction with literature data describing the leaching of additives from plastic pipe materials 271 to obtain estimates of additive leaching from particles of various sizes⁴². We estimate the rates 272 that additives leach from nanoplastics to be many orders of magnitude greater than the leaching 273 274 rate from microplastics due to the smaller distances for diffusive transport (property VI in 275 Figure 2). These rates, predicted in many cases to be comparable to the inverse residence time 276 of digestion, depend on both the type of additive and the plastic and may vary by two or more 277 orders of magnitude. Thus, additive release can be anticipated in many cases to occur in 278 organisms as well as in the environment. Combined with increased bioavailability and the 279 enhanced accessibility to tissues, nanoplastics may be expected to deliver locally high doses of 280 leached compounds compared with microplastics and therefore exhibit differences in apparent 281 dose-response. The specificity of releases to tissues and the consequences of these localized 282 releases have not been studied.

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285 Biological Consequences

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287 Size has been established as a key factor in the ability of nano-sized particles to translocate in organisms⁴³. Bio-uptake, biomagnification and maternal transfer have all been observed for 288 ENMs^{26,35,44,45}. Approaching the size of natural proteins, nanoplastics may be small enough to 289 travel across biological membranes via passive diffusion and access certain endocvtosis 290 pathways^{46,47} (property V in Figure 2). Johnston et al. found that fresh fumes containing 291 nanoscale polytetrafluorethylene were more toxic to rodents than aged fumes which contained 292 larger, coagulated aggregates of polytetrafluorethylene⁴⁸. Bioavailability of ENMs to plants 293 appears to increase as particle diameter decreases below 20 nm⁴⁹. Increased bioavailability is 294 295 consistent with the higher toxicities often found in ENMs compared to their larger counterparts. 296 For example, in plants exposed to CuO, nanoscale particles resulted in greater toxicity compared to micron-sized particles⁴⁴. Rist et al. found that nanoscale polystyrene caused 297 298 decreased feeding rates and were egested to a lesser degree in contrast to microscale 299 polystyrene⁵⁰. Moreover, nanomaterials can interact with subcellular components and trigger 300 responses including reactive oxygen species production.

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302 A considerable portion of the molecules in nanoplastics are exposed to the surface resulting in 303 greatly enhanced surface reactivity compared to their micro- and macroscale counterparts 304 leading to heightened importance of surface chemistries on interactions with biological systems (property III in Figure 2). For example, Miao et al. (2019) showed clear effects on the biological 305 306 activity of biofilms for nanoscale polystyrene beads (100 nm) as opposed to larger polystyrene particles and for positively charged nanoscale polystyrene compared to their carboxyl-307 functionalized counterparts⁵¹. Interactions with proteins and changes in protein conformation, 308 production of reaction oxygen species, and acting as a vector for other contaminants (Trojan 309 horse effects) are among the phenomena that have been observed for ENMs that may also come 310 into play with nanoplastics. These effects could potentially enhance the toxicity of the 311 312 nanoplastics.

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315 Sampling and analysis of nanoplastics

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Table 1 summarizes how the characteristics of nanoplastics that distinguish them from microplastics (I to VI) and ENMs (VII-VIII) impact the analytical approaches used to study their physicochemical properties and environmental fate and behavior. Brownian motion distinguishes separation and analytical techniques used for nanoplastics studies in contrast to microplastics. While the small size of nanoplastics generally makes dead-end filtration (typically used for microplastic separation) impractical, the resulting dominance of Brownian motion allows separation and analytical techniques typically applied to ENMs^{52–55}. These techniques include: crossflow ultrafiltration, asymmetric flow field flow fractionation^{56,57}, nanoparticle tracking analysis and dynamic light scattering.

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327 The nanoscale size of nanoplastics limits far-field geometric optics-based imaging and chemical 328 analysis techniques. As nanoscale sizes are smaller than the wavelength of visible or infrared 329 light, optical diffraction becomes significant, resulting in an Abbe diffraction resolution limit of ~200 nm for analysis techniques that rely on laser spot sizes (micro-Raman) or 330 331 transmitted/reflected light (micro-FTIR, brightfield microscopy). These techniques are popular in microplastics analysis as physical and chemical information can be obtained with a single 332 333 instrument. Consequently, as with ENMs, electron microscopy or diffraction unlimited light 334 microscopy (e.g., stimulated emission depletion microscopy, photoactivated localization microscopy) is required to image nanoplastics. These far field light techniques are typically not 335 compatible with full chemical characterization of nanoplastics. Near field techniques avoid 336 337 diffraction limitations on resolution by confining light to an evanescent field. Recent 338 developments of these techniques show promising results for chemical and topographic characterizations of nanoplastics (i.e., spatial resolution limit of 10-20 nm for AFM-IR and 339 AFM-Raman)⁵⁸. However, near field techniques require samples to be scanned by the 340 evanescent field emitted from probes which limits their throughput and ability to analyze 341 342 morphologically complex samples.

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Overall, as with ENMs, a multi-parameter analytical approach is required to fully characterize 344 nanoplastics in environmental samples ^{59,60}. This includes determining physical and chemical 345 346 characteristics while retaining information about the initial dispersion state and nanomaterial-347 matrix interactions. Multiple techniques can be combined to elucidate multiple parameters from 348 environmental nanoplastics. Consequently, selection of sampling and analytical techniques 349 should consider compatibility with other complementary techniques. For example, field flow fractionation, recently applied for nanoplastics⁵⁶, itself allows size separation and 350 351 characterization of materials in the colloidal size range but can also be coupled to other 352 techniques including light scattering (provides information on size and shape) and mass 353 spectrometry (provides information on composition and quantity). Depending on the detectors 354 used (on-line or off-line), different particle properties can be characterized, such as: inorganic 355 element concentration with ICP-MS, polymer identification with pyrolysis-GC-MS and shape 356 information with the combined use of DLS and MALS.

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358 Coupling of techniques is commonplace for analysis of natural colloids and inorganic nanomaterials in environmental media where so called "hyphenated" analysis techniques are 359 the norm. Inductively coupled plasma-mass spectrometry (ICP-MS) coupled to flow field 360 fractionation is one such combination that allows size discrimination and chemical 361 362 characterization of inorganic environmental nanomaterials at exceptionally high sensitivity. Similarly, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), an established 363 characterization method for organic material, coupled to flow field flow fractionation is gaining 364 use to study nanoplastics⁶¹. Moreover, there is increasing interest in taking advantage of the 365 366 sensitivity of ICP-MS in the nanoplastics context. Recently, Mitrano et al. synthesized palladium-doped nanoplastics compatible with ICP-MS detection³¹. However, ICP-MS has yet 367 368 to be applied to environmental samples as an inorganic tracer for plastic. Nevertheless, potential exists for applying ICP-MS to analyzing nanoplastics in environmental samples, particularly those originating from plastic using metal-based dopants, or nanofillers or by using adsorbed metals⁶² as a proxy. Moreover, ICP-MS, or other mass spectrometry techniques, could be coupled to techniques established for ENM analysis such as the electrospray ionizationdifferential mobility analyzer to obtain size information.

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375 Outlook

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377 Incidental nanoparticles produced from the fragmentation of plastic waste are an important 378 element in the life cycle of plastic wastes. Lessons learned from 20 years of nanoEHS work should be applied to understanding the dimensions of the problem of plastic wastes. One such 379 380 lesson is that international, interdisciplinary teams are able to tap specific areas of expertise that may be sparsely distributed across the world. Researchers studying nanoplastics in the 381 382 environment can help facilitate this needed knowledge transfer by bringing in personnel, in the 383 form of hiring and visiting researchers, with nanoEHS expertise within nanoplastics research 384 groups as well as directly reaching out to nanoEHS research groups for collaborative 385 opportunities.

The establishment of clear terminology and methods that can be harmonized across groups is critical in facilitating such collaborations, data sharing and data interpretation. Size is integral to the definition of nanoplastic; however, we cannot, as for ENMs, be limited by arbitrary size cut-offs (e.g., 100 nm or 1000 nm). It is more meaningful to rely on the particle characteristics to define a nanoplastic. The defining characteristics of incidental nanoplastics which distinguish them from microplastics are described in Figure 2.

- 392 These characteristics guide the development and application of analytical methods, sampling 393 procedures, modeling approaches, and data curation that are directly relevant to the study of nanoplastics in the environment some of which carries over from the nanoEHS field. The 394 395 experience gained from nanoEHS work has underscored important differences in the 396 environmental behavior of nano-scale materials compared to larger particles of identical 397 composition, a distinction that will be important in investigating the prevalence, fate, and 398 impacts of nanoplastics. However, the instability of incidental nanoplastics in the environment 399 and their particle heterogeneity present additional challenges distinct from the ENMs typically 400 studied in the nanoEHS field. Consequently, studying environmental nanoplastics must also integrate lessons learned in dealing with the ubiquity and diversity of environmental 401 402 microplastics.
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Table 1. Analytical consequences of the nanoplastic defining characteristics.

Nanonlastic defining	Analytical Possibilities	Complications
characteristics	Anarytical I ossibilities	Compleations
I. Brownian motion	Enables sizing by SLS, DLS, NTA, and chromatography-based separation (AF4, SEC, HDC) and electrophoretic mobility measurement	More limited density separation (i.e., sedimentation, centrifugation)
	(CE, DMA).	
II. Below geometric	Can use SLS, DLS, AFM, electron	Prevents resolution by diffraction-
optical resolution	microscopy, PALM, STORM, STED, and hyperspectral imaging with dark field microscopy.	limited optical methods (conventional light microscopy) and characterization by Infrared/Raman diffraction-limited laser spot.
III. High specific	Enhances surface interactions.	Faster surface chemistry changes (e.g.,
surface area	Facilitates dispersion fixation on a substrate (<i>e.g.</i> sample drying on a SEM or TEM grid).	chemical oxidation). Risk of sample alteration and loss (attachment) during analysis
IV. Adsorption- heteroaggregation	Can be tracked by fluorescence microscopy, using adsorbed fluorophores, ICP-MS using adsorbed metal, hyperspectral imaging. Enables the use of adsorption-based samplers.	Interference from background material, C in particular.
V. Bio-uptake and translocation	Relatively lower variability in body burden	Extraction/purification due to lower sizes and concentrations, difficulties differentiating between C-based particles and tissues.
VI. Rapid release of additives and co- contaminants	Simplifies characterization of additives and co-contaminants by ICP-MS, LC-MS, and Py-GC-MS.	Complicates ecotoxicity assessment.
VII. Heterogeneous particle properties	Enables detection and quantification by co-localization of target components by single particle analysis (e.g., a metal additive in a polymer matrix)	Global characterization difficult - multiple sampling points required. Difficult to acquire environmentally- relevant material for ecotoxicity and fate assessments.
VIII. Rapid fragmentation due to environmental stressors	Bulk plastics and microplastics can be fragmented to obtain representative nanoplastics	Unstable samples (e.g., size distribution, disaggregation). Increases potential for sample damage during extraction (e.g., by digestion)

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