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Nanoplastics are neither microplastics nor engineered nanoparticles

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Abstract

Increasing concern and research on the subject of plastic pollution has engaged the community of scientists working on the environment, health, and safety (EHS) of nanomaterials. While many of the methods developed in nano EHS work have general applicability to the study of particulate plastics, the nanometric size range has important consequences for both the analytical challenges of studying nano-scale plastics and the environmental implications of these incidental nanomaterials. Related to their size, nanoplastics are distinguished from microplastics with respect to their transport properties, interactions with light and natural colloids, a high fraction of particle molecules on the surface, bioavailability, and diffusion times for the release of plastic additives. Moreover, they are distinguished from engineered nanomaterials because of their high particle heterogeneity and their potential for rapid further fragmentation in the environment. These characteristics impact environmental fate, potential effects on biota and human health, sampling, and analysis. Like microplastics, incidentally-produced nanoplastics exhibit a diversity of compositions, morphologies, and heterogeneity that is typically absent from engineered nanomaterials. Therefore, nano-scale plastics must be considered as distinct from both microplastics and engineered nanomaterials.

Introduction

While the accumulation of microplastics and larger plastic debris in the world's oceans has 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 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 the levels of colloidal plastics in these environmental compartments. Nanoplastics are the 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 debris can take up to hundreds of years, mechanical wear⁶, heat⁷, UV degradation⁸ and, in some cases, biological factors⁹, lead to relatively rapid fragmentation of plastic debris down to the nano-scale.

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

Nanoplastics are distinct from microplastics

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 as a growing environmental concern. For example, polystyrene spheres are increasingly described in the recent scientific literature as either micro- or nano- "plastic" rather than simply 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 studies conducted with a different motivation many decades earlier¹². The re-casting of the microplastic problem as one that distinctly entails nanoplastics (Figure 1b) may reflect a re-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 consumer products, and in natural and complex environmental systems that can be extended to

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.

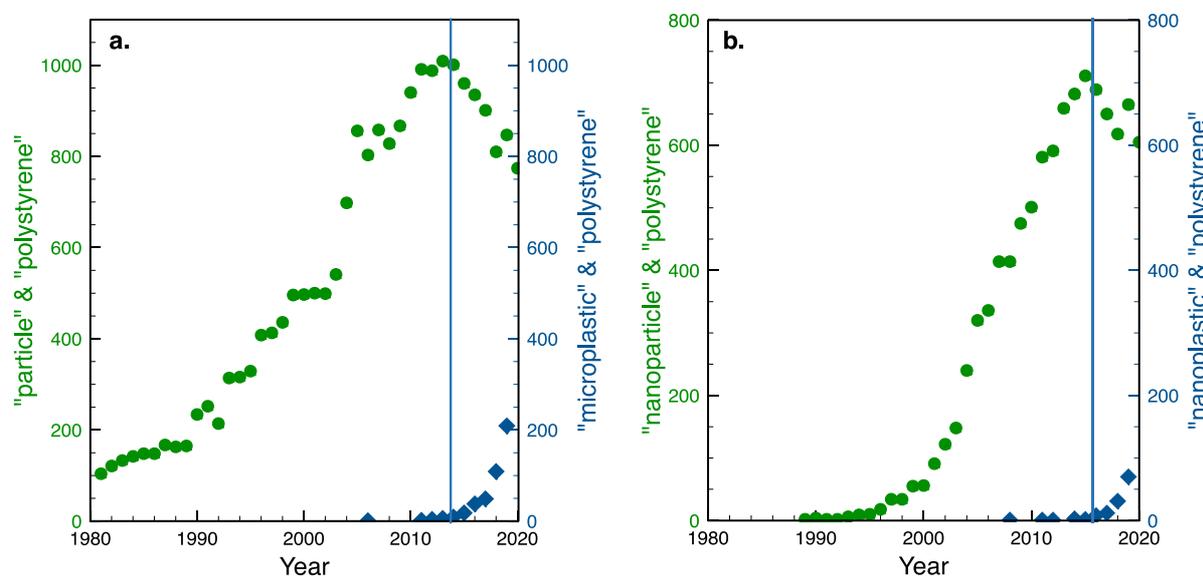


Fig. 1. Number of publications obtained from Scopus that use the terms shown on the y-axis in the Title/Abstract/Keywords. (a) particle* AND polystyrene* AND NOT microplastic* (green circle) versus microplastic* & polystyrene* (blue diamond) and (b) nanoparticle* AND polystyrene* AND NOT nanoplastic* (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”.

While all plastics are polymer-based, not all polymers are plastic and nanoplastics are not synonymous with nanopolymers¹⁶. Plastics will typically include material composed of polymer as well as additives. The term “nanoplastic” has typically been used with reference to 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-offs^{17–19}. The US National Nanotechnology Initiative (NNI) defines a nanomaterial as having at least one dimension between 1 and 100 nm and exhibiting properties not found at larger sizes 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 complicate the definition of nanomaterials²⁰, the distinction between nanomaterials and their 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 quantum confinement of electrons at particle sizes well below electron wavelengths. Consequently, the properties and behavior of nanomaterials cannot be extrapolated from the properties of their bulk counterparts. In our view, it is these characteristics, that separate “nano” from “micro” regardless of a particular size range²⁰. Further, one may differentiate between engineered, incidental, and natural nanoparticles, distinctions that may be blurred as in the case of C₆₀ which can be produced as an ENM, but is also present in combustion products 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 microplastics include:

- I. The dominance of Brownian motion over sedimentation and buoyancy characterized by random movements of the particles in a suspension medium;
- II. Departure from the geometric/ray approximation between light and matter. For microplastics, the interaction of light and microplastic can be adequately approximated by modeling light as a straight ray. In contrast, with nanoplastics, the wave-nature of light and associated phenomena important in some analytical methods, including diffraction, are significant;
- III. A high proportion of molecules on the surface resulting in a higher relative importance of surface interactions compared to physical interactions;
- IV. 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 biofilm formation);
- V. Sizes compatible with bio-uptake, translocation and transport across biological membranes and;
- VI. Short length scales that may speed the diffusive release of plastic additives and non-intentionally added substances in the original bulk plastic.

Nanoplastics have unique characteristics compared to engineered nanomaterials

While nanoplastics may share many of the properties of ENMs, nanoplastics found in the environment present unique considerations and challenges. To begin with, environmental 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, approximately 5000 million metric tons were emitted to the environment, where they can eventually break down into nanoplastics². In 2018, plastic production was approximately 359 million metric tons worldwide²². In contrast, rough estimates for global production of ENMs of all kinds range from thousands of metric tons per year to no more than 10⁶ metric tons. Compared to plastics production that emerged as early as the 1950s²³, ENM production is a relatively recent activity that has expanded rapidly since the 1990s.

Furthermore, environmental nanoplastics as a class of contaminants are substantially more heterogeneous compared to ENMs. ENMs are intentionally created to desired specifications, 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 properties (i.e., optical, magnetic, conductor properties). Standardized techniques adapted to ENMs are now widely used by the scientific community. Note that there exists ENMs composed of polymer types that could be considered “plastic”. For example, spherical and 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 chromatography, static light scattering, and other techniques, as summarized elsewhere²⁴. Due to their high uniformity, these plastic spheres are easy to track during the analyses, but are not representative of the diversity of incidental nanoplastics in the environment²⁵. Environmental nanoplastics are generally not intentionally designed and vary greatly in shape, size, polydispersity, additives, adsorbed contaminants, surface properties, and composition as a consequence of different source materials, fragmentation pathways and environmental exposure²⁶. The resulting physical and chemical heterogeneity of nanoplastics may influence their reactivity and will certainly affect interactions with natural colloids and organisms. For

example, weathering induced by UV radiation enhances fragmentation of bulk plastic into 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 result is an extremely broad scope of materials to investigate.

These incidental nanoplastics are distinguished from ENMs (Figure 2) by:

- VII. 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;
- VIII. Incidental nanoplastics may include the fragmentation products of engineered (primary) nanoplastics with the potential for further fragmentation in the environment at shorter time scales.

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

Environmental fate and behavior

The distinct characteristics of nanoplastics will influence their environmental fate and behavior, interactions with biological systems, sampling strategies, analytical methods, experimental and computational modeling approaches that cannot be extrapolated from microplastics or ENMs. 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 microplastics composed of the same material (property I in Figure 2). For example, polypropylene and polyethylene have densities less than water, and are therefore expected to float in water, while polyvinyl chloride would be expected to settle because of its higher density (~1.4 g/cm³). By Stoke’s law, a 1 mm polyvinyl chloride microplastic is calculated to settle at a rate of approximately 22 cm/sec in water compared with a 100 nm nanoplastic which would 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 may be more likely to remain suspended in the atmosphere and form a portion of “ultra-fine” (sub 100 nm) particulate matter³⁴. However, aggregation with other particles may increase the effective settling rate of nanoplastics.

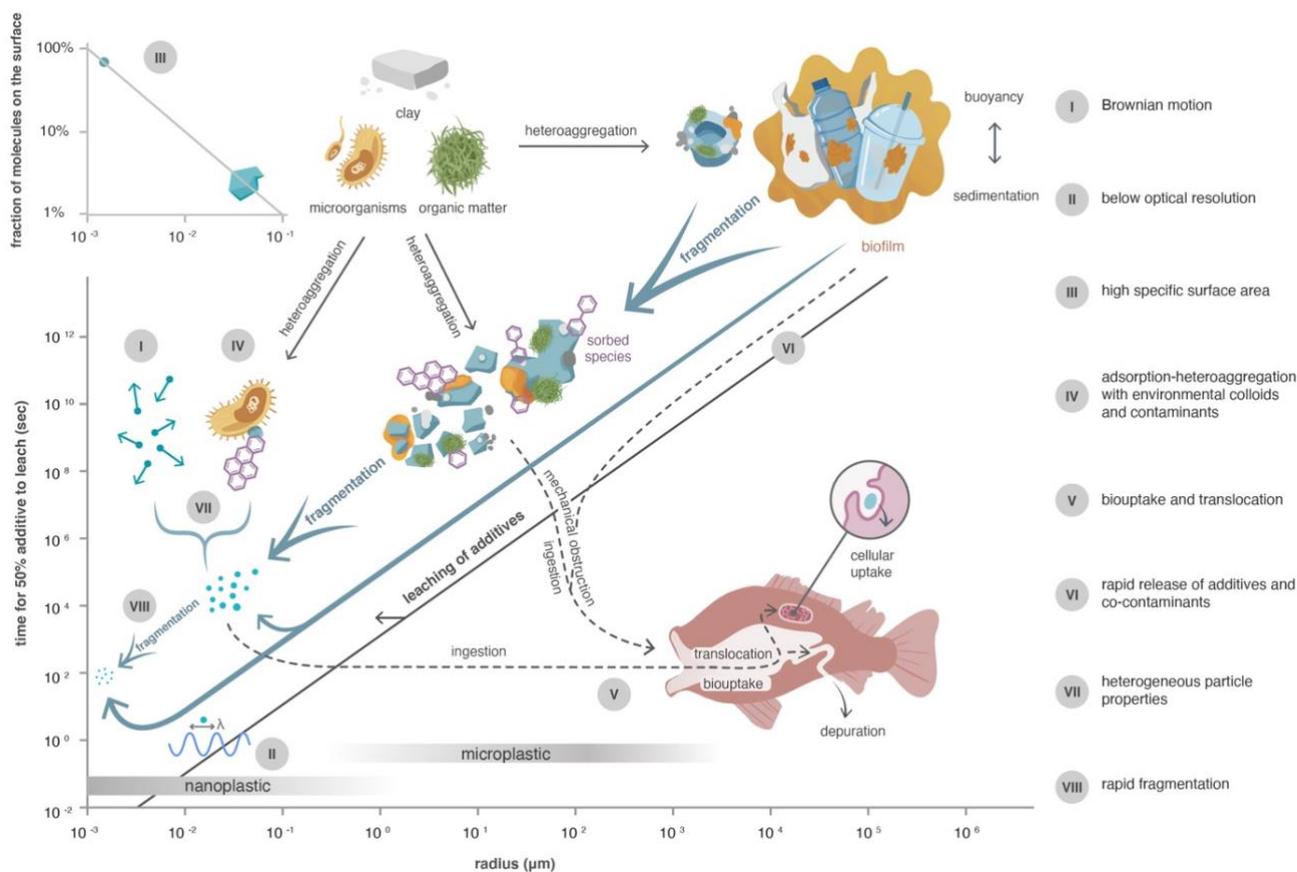


Figure 2. Transformations and characteristics of plastic debris in the environment. The eight defining characteristics of nanoplastics that distinguish them from microplastics and engineered nanomaterials. The time needed to leach one half of the additives initially present in a plastic material is calculated for the representative additive tributyl phosphate (“leaching of additives” curve, characteristic VI). Leaching of tributyl phosphate was calculated for different sized epoxy particles based on internal diffusion-limited transport⁴⁴. Bulk plastics break down into micro- and nanoplastics due to weathering action (UV sunlight, mechanical abrasion, etc.), environmental (heteroaggregation) and biological (bacteria, krill, etc.) processes.

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 anthropogenic material^{20,35} or with aerosols in the atmosphere (property IV in Figure 2). Macromolecules that may associate with nanoplastics will have length scales similar to the 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 nanoplastics and heteroaggregates. Heteroaggregation and the conformation of charged macromolecules in water is in turn influenced by environmental conditions (i.e., porewater pH, ionic strength, hardness, etc). These factors will disproportionately influence the attachment efficiency of nanoplastics relative to microplastics due to the dominant role of surfaces at the nanoscale. In addition, the collision rate kernel for nanoplastic heteroaggregation will be dominated by Brownian diffusion rather than by settling, buoyancy, or fluid motion. Consequently, nanoplastic distribution in the environment cannot be directly extrapolated from that of microplastics.

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 community of micro-organisms (i.e., the “plastisphere”)^{36,37} and accompanying development

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

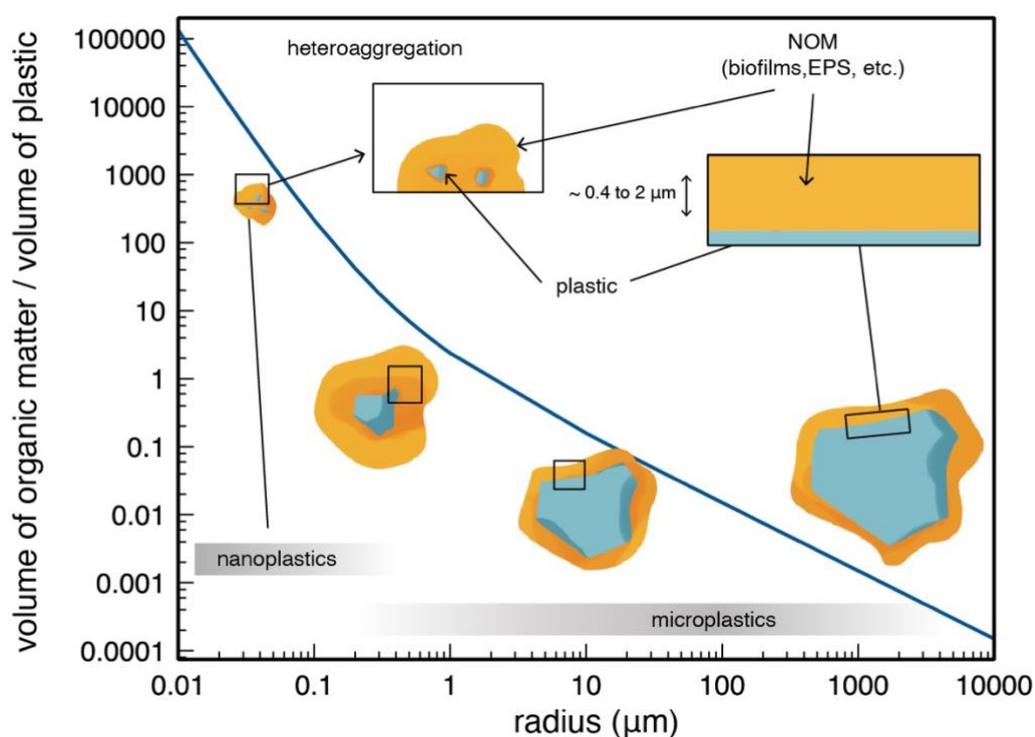


Figure 3. Relative proportion of natural organic matter compared to plastic according to the size (radius) of plastic debris. The relative proportion is represented by the ratio of the volume of the natural organic matter to the volume of plastic for a given particle.

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

Biological Consequences

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 ENMs^{26,35,44,45}. Approaching the size of natural proteins, nanoplastics may be small enough to travel across biological membranes via passive diffusion and access certain endocytosis pathways^{46,47} (property V in Figure 2). Johnston et al. found that fresh fumes containing nanoscale polytetrafluorethylene were more toxic to rodents than aged fumes which contained larger, coagulated aggregates of polytetrafluorethylene⁴⁸. Bioavailability of ENMs to plants appears to increase as particle diameter decreases below 20 nm⁴⁹. Increased bioavailability is consistent with the higher toxicities often found in ENMs compared to their larger counterparts. 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 decreased feeding rates and were egested to a lesser degree in contrast to microscale polystyrene⁵⁰. Moreover, nanomaterials can interact with subcellular components and trigger responses including reactive oxygen species production.

A considerable portion of the molecules in nanoplastics are exposed to the surface resulting in greatly enhanced surface reactivity compared to their micro- and macroscale counterparts 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 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-functionalized counterparts⁵¹. Interactions with proteins and changes in protein conformation, production of reaction oxygen species, and acting as a vector for other contaminants (Trojan horse effects) are among the phenomena that have been observed for ENMs that may also come into play with nanoplastics. These effects could potentially enhance the toxicity of the nanoplastics.

Sampling and analysis of nanoplastics

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 ENMs⁵²⁻⁵⁵. These techniques include: crossflow ultrafiltration, asymmetric flow field flow fractionation^{56,57}, nanoparticle tracking analysis and dynamic light scattering.

The nanoscale size of nanoplastics limits far-field geometric optics-based imaging and chemical analysis techniques. As nanoscale sizes are smaller than the wavelength of visible or infrared 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 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 instrument. Consequently, as with ENMs, electron microscopy or diffraction unlimited light microscopy (e.g., stimulated emission depletion microscopy, photoactivated localization microscopy) is required to image nanoplastics. These far field light techniques are typically not compatible with full chemical characterization of nanoplastics. Near field techniques avoid diffraction limitations on resolution by confining light to an evanescent field. Recent 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 AFM-Raman)⁵⁸. However, near field techniques require samples to be scanned by the evanescent field emitted from probes which limits their throughput and ability to analyze morphologically complex samples.

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

Coupling of techniques is commonplace for analysis of natural colloids and inorganic nanomaterials in environmental media where so called “hyphenated” analysis techniques are the norm. Inductively coupled plasma-mass spectrometry (ICP-MS) coupled to flow field fractionation is one such combination that allows size discrimination and chemical characterization of inorganic environmental nanomaterials at exceptionally high sensitivity. Similarly, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), an established characterization method for organic material, coupled to flow field flow fractionation is gaining use to study nanoplastics⁶¹. Moreover, there is increasing interest in taking advantage of the 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 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 ionization-differential mobility analyzer to obtain size information.

Outlook

Incidental nanoparticles produced from the fragmentation of plastic waste are an important 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 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 environment can help facilitate this needed knowledge transfer by bringing in personnel, in the form of hiring and visiting researchers, with nanoEHS expertise within nanoplastics research groups as well as directly reaching out to nanoEHS research groups for collaborative 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.

These characteristics guide the development and application of analytical methods, sampling 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 experience gained from nanoEHS work has underscored important differences in the environmental behavior of nano-scale materials compared to larger particles of identical composition, a distinction that will be important in investigating the prevalence, fate, and impacts of nanoplastics. However, the instability of incidental nanoplastics in the environment and their particle heterogeneity present additional challenges distinct from the ENMs typically studied in the nanoEHS field. Consequently, studying environmental nanoplastics must also integrate lessons learned in dealing with the ubiquity and diversity of environmental microplastics.

Table 1. Analytical consequences of the nanoplastic defining characteristics.

Nanoplastic defining characteristics	Analytical Possibilities	Complications
I. Brownian motion	Enables sizing by SLS, DLS, NTA, and chromatography-based separation (AF4, SEC, HDC) and electrophoretic mobility measurement (CE, DMA).	More limited density separation (i.e., sedimentation, centrifugation)
II. Below geometric optical resolution	Can use SLS, DLS, AFM, electron microscopy, PALM, STORM, STED, and hyperspectral imaging with dark field microscopy.	Prevents resolution by diffraction-limited optical methods (conventional light microscopy) and characterization by Infrared/Raman diffraction-limited laser spot.
III. High specific surface area	Enhances surface interactions. Facilitates dispersion fixation on a substrate (e.g. sample drying on a SEM or TEM grid).	Faster surface chemistry changes (e.g., 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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