Harmonizing Across Environmental Nanomaterial Testing Media for Increased Comparability of Nanomaterial Datasets

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Harmonizing Across Environmental Nanomaterial Testing Media for Increased Comparability of Nanomaterial Datasets

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Abstract

The chemical composition and properties of environmental media determine nanomaterial (NM) transport, fate, biouptake, and organism response. To compare and interpret experimental data, it is essential that sufficient context be provided for describing the physical and chemical characteristics of the setting in which a nanomaterial may be present. While the nanomaterial environmental, health and safety (NanoEHS) field has begun harmonization to allow data comparison and re-use (e.g. using standardized materials, defining a minimum set of required material characterizations), there is limited guidance for standardizing test media. Since most of the NM properties driving environmental beaviour and toxicity are medium-dependent, harmonization of media is critical. A workshop in March 2016 at Duke University identified five categories of test media: Aquatic Testing Media, Soil and Sediment Testing Media, Biological Testing Media, Engineered Systems Testing Media and Product Matrix Testing Media. For each category of test media, a minimum set of medium characteristics to report in all NM tests is recommended. Definitions and detail level of the recommendations for specific standardized media vary across these media categories. This reflects the variation in the maturity of their use as a test medium and associated measurement techniques, variation in utility and relevance of standardizing medium properties, ability to simplify standardizing reporting requirements, and in the availability of established standard reference media. Adoption of these media harmonization recommendations will facilitate the generation of integrated comparable datasets on NM fate and effects. This will in turn allow testing of the predictive utility of functional assay measurements on NMs in relevant media, support investigation of first principles approaches to understand behavioral mechanisms, and support categorization strategies to guide research, commercial development, and policy.

Environmental significance statement

To support regulatory and other environmental evaluation of nanomaterials, efficient generation of large arrays of standardized and comparable data are vital. To fulfill these requirements, the selected set of parameters must allow investigation of possible effects of both the surrounding medium and the nanomaterial properties on various endpoints. The authors propose minimum sets of parameters needed to provide such context in five system categories, providing rationale for their designation. Adherence to adequate media characterization provides a basis for comparison even while allowing researchers to employ preferred media of choice in terms of specific parameter values. In some cases standard media are also proposed, calling for specific parameter values that allow studies to be directly compared and benchmarked.

List of abbreviations

ALI - air-liquid interfaces; ASTM - American Society for Testing and Materials; ASW - Artificial Seawater; AVS - acid volatile sulfide; BOD – Biological Oxygen Demand; CNTs – carbon nanotubes; COD – Chemical Oxygen Demand; DIW - deionized water; DMEM – Dulbecco's Modified Eagle's medium; DOC - dissolved organic carbon; EHS - Environmental Health and Safety; ENTM - Environmental Nano Testing Media; EU – European Union; FAs - Functional assays; FBS – foetal bovine serum; ISA-TAB-nano - Investigation-Study-Assay data capture approach using delimited tabs; ISO – International Standardization Organization; MBL - Marine Biological Laboratory; MHW - Moderately Hard Water; MHRW - Moderately Hard Reconstituted Water; MSW – Municipal solid waste; NCI NanoWG - National Cancer Institute Nanotechnology Working Group; NM – nanomaterial; NOM - natural organic matter; NZVI - nano zero-valent iron; OECD – Organization for Economic Cooperation and Development; SF - serum-free; SRMs - standard reference materials; SVI - volume index; TOC - total organic carbon; TSS - Total Suspended Solids; US EPA – United States Environmental Protection Agency; VSW - Very Soft Water; WWTP - wastewater treatment plant.

1. INTRODUCTION

The chemical composition and properties of environmental media play defining roles in determining nanomaterial (NM) transport, fate, biouptake, and even organismal response. To compare and interpret experimental data, it is therefore essential that sufficient context be provided describing the chemical dimensions of the setting in which a nanomaterial may be present. The Nanomaterial Environment, Health and Safety (nanoEHS) field has agreed upon the need for standardized NMs[1], that a minimum set of NM characterization is required (although the specifics are still debated)[1], and has even agreed upon specific assays for key parameters.[2-4] Similarly, standard tests in a set of consistent media are also needed since most of the NM properties driving environmental behavior and toxicity are context-dependent. Here we use NM to refer to nanomaterials generally, since the advancement of nanoEHS has brought increasing understanding of the relevance of nanoscale materials of engineered, incidental, and natural origin, and the testing of all categories of NMs benefits from efforts to harmonize datasets.

Consistency across testing is already appreciated within nanoEHS communities. For example, it has been proposed that aggregated semi-empirical parameters called functional assays (FAs) be investigated for their utility in predicting nanomaterial behavior in complex systems.[5] Functional assays to test dissolution rate,[6] attachment efficiencies,[7] and reactivities of NM would help to categorize these materials at various stages of their lifecycles in a way that is meaningfully predictive of their potential subsequent transport and impact. Besides capturing the functional behaviors most useful for forecasting impacts, the parameters generated by FAs, describing where NMs go and what they do, are inherently inclusive of phenomena that derive from initial nanomaterial characteristics (e.g., primary particle size) as well as those controlled by surrounding media (e.g. zeta potential, aggregation state). As a next step, the nanomaterial testing community must adopt a suite of relevant media in which to conduct functional assays and other studies. Establishing a set of consistently defined media will not only serve to build in a measure of quality assurance and interoperability to the resulting data but will make resulting datasets more conducive to modeling and cross-study analyses.

To address this need for media harmonization, an expert workshop was convened in February 29 through March 2 of 2016 entitled "Environmental Nano Testing Media (ENTM) Harmonization". This Perspective presents the resulting recommendations for relevant and consistently characterized medium types in which nanomaterial characterization and testing should be carried out, along with proposed media characterization parameters that should be consistently measured and reported. The intent is to collectively endorse a handful of high priority media and matrices. Where appropriate, we also propose the adoption of some defined values for those parameters in common systems to support maximum comparability in emerging datasets. Consistent characterization allows researchers to employ any medium of choice, whereas media standards allow studies to be directly compared and benchmarked. It should be clearly stated that these media, though proposed for NM testing, are defined here without any NMs in them.

On community-generated harmonization guidance. These recommendations represent a user-community consensus on approaches for harmonization on behalf of a collaborative sub-set of the nanoEHS field. This is distinct from products of official standards organizations and processes (e.g., ASTM, ISO, or OECD). This framework and the recommended media, if adopted into future experimental plans, will support downstream data integration and provide helpful community-based input to discussions in the US and EU regarding more formal standardization processes for generating and storing integrated data. In the broader arena of nanomaterial testing and data integration, there have been early successes with the model of community- initiated or -facilitated harmonization that supports cross-disciplinary translation and education on appropriate method applications and standards adoption. Two examples include the development of the ISA-TAB-nano data sharing file format extension by the open National Cancer Institute Nanotechnology Working Group (NCI NanoWG)[8] and the community-developed guidance on the proper execution and interpretation of zeta potential measurements[9]. Such harmonization is an acknowledgment of a philosophical and cultural shift required to address interlinked problems with the size and complexity of forecasting the behaviors and impacts of NMs. Community convergence on minimum reporting requirements for relevant media in which to test nanomaterials can foster shared, integrated research strategies. Adoption of such requirements will provide guidance and feedback to official standardization processes concerning current research consensus that can feed into standard test guidelines and guidance documents, and highlight research needs and emerging datasets to facilitate building of weight of evidence arguments through enhanced inter-comparability.

Workshop Methods. A working group of forty-one experts representing different disciplines, geographies and sectors was convened at Duke University. Attendees were asked to provide input prior to the workshop regarding classes of media that were important to address, any specific defined media they recommended for consideration, and critical parameters necessary to describe these media types. Breakout groups were organized to address five categories of communally-defined priority systems, and workshop activities were designed to move each breakout group through the process of: A) reaching consensus on media types within the category, B) arrive at consistent parameters that must be measured for each media type, and C) draw upon existing standard systems wherever appropriate to define values for the consistently defined parameters. Workshop process and outputs are depicted in Figure 1 generally; more detail on the workshop method is available in the Supplementary Information (SI) section of this Perspective. Figure S1 provides additional detail on the workshop decision-making process.

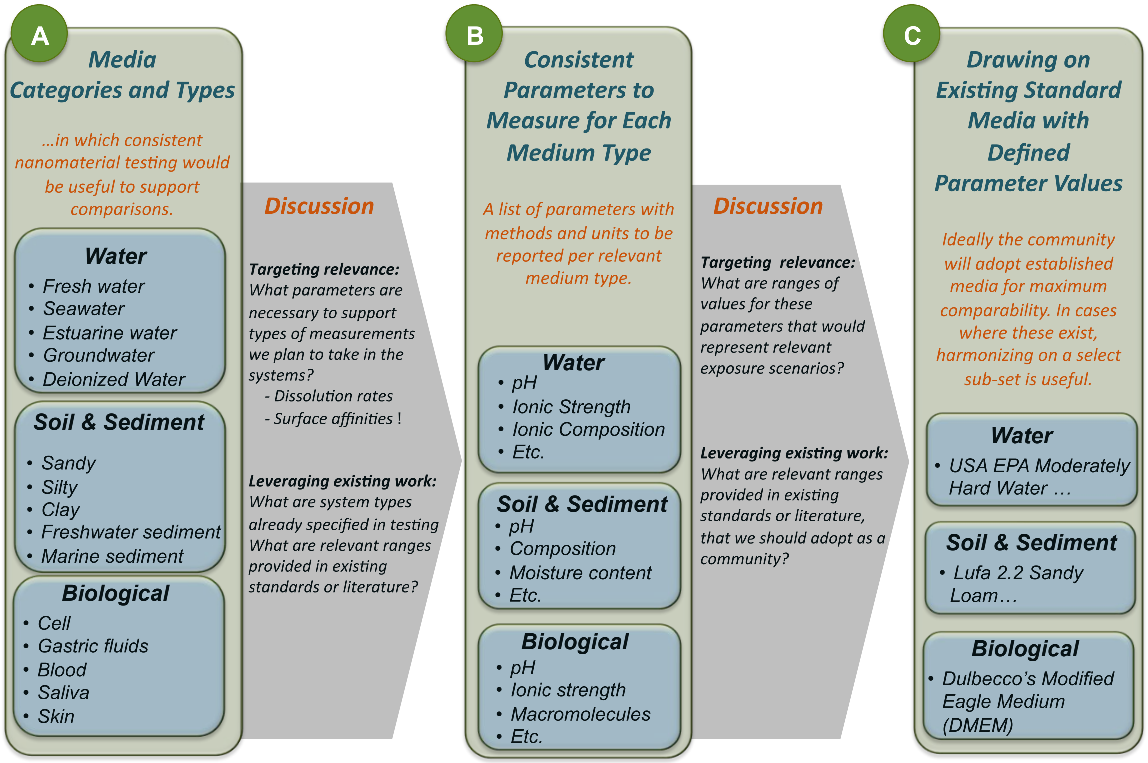

Figure 1: Workshop method overview; corresponds to resulting recommendations for a subset of media

2. HARMONIZED MEDIA RECOMMENDATIONS

Recommendations are presented here for harmonizing across five broad categories of testing media, all identified as important for testing of nanomaterial behavior in terms of both exposure and hazard endpoints. The media considered are aqueous and solid phase in nature; atmospheric media are not considered in this Perspective.

The broad categories presented here include:

- Aquatic Testing Media (Section 2.1)
- Soil and Sediment Testing Media (Section 2.2)
- Biological Testing Media (Section 2.3)
- Engineered Systems Testing Media (Section 2.4)
- Product Matrix Testing Media (Section 2.5)

Different medium categories present different challenges. Some (e.g., soils) have an enormous number of competing standards from which to select the most critical representative systems and parameters. Others do not have any competing standards (e.g., product testing media) yet are nevertheless essential for key measurements across material life-cycles. Therefore, the content and detail level of the harmonization recommendations vary accordingly among categories.

For each of the systems, we propose two tiers of key characteristics in terms of primary (absolutely required) and secondary (required in cases where these characteristics are available and/or pertinent to the specific measurement being taken). The hypothesis for encouraging measurement of primary, and when possible secondary, characteristics is that knowing these attributes will enable comparability among studies when different media are used.

2.1. Aquatic Testing Media

2.1.1. Context and background

Aquatic systems are of particular relevance to nanomaterial testing, because engineered nanomaterials have been predicted to arrive in natural waters via multiple routes of release such as wastewater treatment plant effluent, run-off from agricultural and urban areas, and deposition by rain.[10, 11]

Aquatic systems can be broadly differentiated based on levels of salinity, ranging from marine to soft freshwater ecosystems. Low salinity freshwater includes lakes, rivers and streams, urban runoff, groundwater, rain water, ephemeral pools, and tap water; all of which have a wide range of possible water quality characteristics. Representative parameters are given in Table S1. The characteristics of estuarine systems are also highly variable, as well as dynamic, depending on tidal cycles, freshwater discharge and Coriolis effects. The characteristics of marine systems close to land are similarly dynamic. Open ocean ecosystems are less variable, yet are influenced by the degree of biological productivity, which in turn is governed by nutrient inputs, temperature, and other factors. Therefore, there is a wide range of test systems that could be developed to represent the range of scenarios where nanomaterials may be present in an aquatic system.

Other factors may well be strong determinants of NM behavior outcomes depending on the scenario, including light, temperature, and well-defined natural organic matter (NOM). For a streamlined first step toward harmonization, we focus discussion on the chemical composition of aquatic media rather than the entirety of parameters required to define aquatic systems for experimental design that would additionally include conditions of energy flux (e.g., light, mixing, heat) and biotic composition.

Dissolution and agglomeration are perhaps the most important transformation processes affecting the fate and toxicity of NMs within aquatic systems, although many others exist.[12] Understanding the extent of NM dissolution and agglomeration in aquatic test systems is therefore essential for estimating environmental risks. The extent of these transformations in a test system will depend on physicochemical conditions, such as concentrations of dissolved organic carbon (DOC), pH, ionic strength and redox conditions (*i.e.*, oxidizing or reducing environment), in addition to NM specific properties and concentration.[5, 13, 14]

2.1.2. Recommended media and parameters

Table 1 provides the primary and secondary parameters for the three generic environmental aquatic ecosystems considered: Freshwater, Estuarine, and Marine, and for deionized water (DIW), which is also commonly used for testing and characterization of NMs because it alleviates any interference from ions on the NM . The list of characteristics here are intended to apply laboratory-simulated waters and waters obtained from the environment. Definitions of primary and secondary parameters follow.

Table 1: Minimum set of parameters recommended for inter-study comparison of the fate and effects of NMs in aqueous media

Media type	Primary Parameters	Secondary Parameters
Freshwater	• pH • Ionic strength • Major cations (e.g., hardness) • Major anions (e.g., alkalinity)	• Specific anionic ligands (e.g., sulfate, chloride) • Key nutrients (e.g., nitrogen & phosphorus species)

	• Dissolved organic matter (e.g., DOC)	• Redox potential • Particulate matter: o Organic o Inorganic
Estuarine	• pH • Ionic strength • Dissolved organic matter (e.g., DOC)	• Major anions (e.g., alkalinity) • Key nutrients (e.g., nitrogen & phosphorus species) • Dissolved organic matter (e.g., DOC) • Particulate matter: o Organic o Inorganic
Marine	• pH • Ionic strength • Dissolved organic matter (e.g., DOC)	• Key nutrients (e.g., nitrogen & phosphorus species) • Particulate matter: o Organic o Inorganic
Deionized Water	• pH • Resistivity	

Discussion of primary parameters. The minimum specifications considered to be adequate for evaluation of NM data in all aquatic systems were quantification of pH, ionic strength, and a measure of DOC (typically Total Organic Carbon). Additional characterization of the composition of ionic components, such as hardness and alkalinity, was also considered to be essential for freshwater systems. Hardness and alkalinity are important as indicators of ionic composition beyond ionic strength; differences in ionic composition, such as the presence of divalent cations, are known to have significant impact on nanomaterial stability. The relative uniformity of seawater composition and their low DOC concentrations suggest that characterization of DOC may be a secondary consideration in marine systems. However, they should still be reported when possible for comparability with freshwater systems.

Discussion of secondary parameters. In addition to the parameters considered to be essential for minimum characterization of aqueous media, several additional parameters were identified as being highly desirable. In some cases, synthetic laboratory media include detailed analysis or complete specification of the medium content. In these cases, a reference for these media should be provided that will allow readers to obtain the full details of media composition. However, in many cases, such as where experiments are performed in field or mesocosm conditions, the complete composition of the medium will be unknown. In these cases, measurement of specific divalent ions (Ca^{2+} and Mg^{2+}) are among the most important secondary measures for characterizing ionic content beyond total hardness and conductivity. Measurement of nitrogen and phosphorus species are highly desirable from the perspective of their potential impact on biotic components in a given experiment. These species, while likely to be present in low concentrations, are also potentially important in determining surface properties of NMs due to their propensity for inner sphere coordination with NM surfaces resulting in effects on NM surface charge. Alkalinity was also deemed to be an important parameter as a partial characterization of the anionic composition of aqueous solutions.

Most aquatic solutions include complex mixtures of dissolved organic matter and naturally occurring particulates that may merit additional characterization that, in the simplest instance, include measurements of Total Organic Carbon (TOC) in unfiltered samples and in samples that have been membrane filtered (typically , 0.2 µm or 0.45 µm membranes). The selection of filter cut-off size may be influenced by what specific information is sought about the system; if it is desirable to capture agglomerates of nanoscale particles, 0.45 µm may be preferable. The materials retained by such membranes are commonly used to characterize the mass concentration of total suspended solids, which may give a very approximate estimate of the potential for NM heteroaggregation. Measures of organic carbon in the unfiltered (TOC) and filtered (DOC) samples additionally yield estimates of the percent organic carbon averaged over all suspended particulates, which may play a role in determining NM stability and affinity of these background particulates for heteroaggregation with engineered NMs.

Measurement of the UV absorbance at 254 nm in filtered samples provides additional information regarding the quality of the organic matter in terms of aromaticity, often normalized by the DOC concentration and reported as the specific UV absorbance.[15] More detailed analysis of suspended particulates in terms of particle size distributions and the electrophoretic mobility of suspended particulates are also among the secondary parameters that may be important in specific experimental settings. Though measurements of naturally occurring background particulate size distributions and concentrations would be considered required input for modeling NM heteroagglomeration, persistence and transport, it is recognized that many tests seek to evaluate NM toxicty or dissolution in the absence of heteroagglomeration. Therefore, characterization of the naturally occurring background particulate phases present in these systems was considered to be a secondary consideration in these instances. Table 2 provides stardard units and references for standard methods to measure each of the parameters listed in Table 1.

Table 2. Units, rationale and references for recommended parameters to characterize aqueous media

Parameters	Units	Rationale	Reference
pH	-logM	Assay independent indication of water pH, good for comparability	16, 17
Ionic strength	mmol L^{-1}	Calculate from either reagent addition or from conductivity measurements	18
Major cations	mmol L^{-1}	Importance in NM stability and properties of organic matter	19
Major anions	mmol L^{-1}	importance in NM stability and properties of organic matter	19
Dissolved organic carbon (DOC)	mg L^{-1}	Importance in cation binding and NM stability	20
Specific anionic ligands (e.g., sulfate, chloride)	mmol L^{-1}	Importance in NM stability and properties of organic matter	19, 21
Redox potential	mV	Possible electron sources/ sinks	22
Particulate matter: Organic Inorganic	mg L^{-1}	Importance in heteroaggregation	23, 24

2.1.3. Considerations of benchmark media for specific parameter values

Three reconsituted waters are recommended as a basis for simulating the three broad categories of aquatic systems indicated above for laboratory tests. For freshwater with low hardness, a medium conforming to the specifications for the U.S. Environmental Protection Agency (US EPA) Very Soft Water (VSW) is proposed (Table SI 1)[25]. Where experimental conditions are meant to simulate fresh waters with a significant concentration of monovalent and divalent ions, a medium conforming to the specifications for the EPA Moderately Hard Water (MHW) is proposed. The detailed benchmark parameters for these media are listed in Table 3. Commercially available products are proposed for simulating seawater or, at various degrees of dilution, estuarine waters. The rationale for recommending each of these media formulations is given in the following sections.

In addition to the three environmental categories of aqueous systems, deionized water (DIW) is one of the media options that many NM research laboratories have already converged on. Although not representative of a natural water system, DIW is a medium that is frequently and easily implemented for comparison across laboratories. It is used for the synthesis of many NMs as well as the dilution of media.

Table 3. Recommended benchmarks for Very Soft Water (VSW) and Moderately Hard Water (MHW)

Parameters	VSW	MHW	Units
pH	6.4	7.4-7.8	-
Hardness	10-13	80-100	mg $CaCO_3$ L^{-1}
Alkalinity	10-13	60-70	mg $CaCO_3$ L^{-1}
Deionized Water (DIW)			
$NaHCO_3$	12.0	96.0	mg L^{-1}
$MgSO_4$	7.5	60.0	mg L^{-1}
KCl	0.5	4.0	mg L^{-1}
$CaSO_4.2H_20$	7.5	60.0	mg L^{-1}

Rationale for Very Soft Water (VSW) recommendation. As discussed above, the USEPA reconstituted freshwaters may be formulated to have a range of hardness values. For functional assays[5] and toxicity bioassays, lower Ca^{2+} concentration/hardness may be advantagous for maintaining more stable dispersions in the case of charge-stabilized NMs, although the impacts on animal health in this diluted media must be considered.[26-28] The lowest hardness standard water (10 to 13 mg L^{-1} as $CaCO_3$), which has relevance to alpine streams and e.g. Canadian Shield lakes, is Very Soft Water (VSW). This test water has the lowest Ca^{2+} content (0.044 mmol/L) of any available test standardized test water.[29] Relative to higher Ca^{2+} concentration/hardness media, VSW and comparable low hardness media have been shown to enable higher stability of carbon

NMs,[30, 31] nanoAg[27, 32-34] and other metal NMs [35] during fate and toxicity testing. Other coatings using steric stabilizaton methods show less sensitivity to ion concentration and composition.[36, 37]

Rationale for Moderately Hard Water (MHW) recommendation. The USEPA has standardized a series of reconstituted freshwaters for toxicity (hazard) testing. Regulatory testing programs in the USA, under the umbrella of the Clean Water Act, that require use of these test waters include industrial effluent testing[38] and open water dredged material placement operations. In addition, regulatory program testing for superfund toxic substances and pesticides[39] use USEPA test waters. These standard test waters range in hardness from 10 mg L^{-1} to 320 mg/L as $CaCO_3$, (10^{-2} mmol L^{-1} to 3.2 mmol L^{-1} of Ca^{2+}) to allow selection based on site-specific relevance.

Moderately Hard Water (MHW), with an acceptable hardness range of 80 mg/L to 100 mg/L as $CaCO_3$ (or 0.8 mmol L^{-1} to 1 mmol L^{-1}) is the most commonly applied test water for hazard assessment. MHW is a reconstituted water, designed generically to satisfy the basic requirements of taxonomically diverse freshwater organisms (algae, invertebrates, fish) that are used in standardized acute and chronic toxicity test methods [40, 41]. The recipe for MHW is freely available and consists of four simple salts ($NaHCO_3$, $CaSO_4$, $MgSO_4$, KCl) dissolved in DI water. There is a very large database of standardized toxicity tests using this test system for both traditional substances and NMs;[26, 42] because of this coalescence around MHW use for toxicity tests, it makes sense to adopt similar media for exposure research as well.

Limitations are also recognized in the MHW recommendation. While MHW was the standardized test media that most closely represented the ideal parameters for NM hazard testing, it must be recognized that it is not ideal for NM dispersion and stability investigations. It is a USA-centric test water that may be used less frequently relative to OECD and ISO test media in laboratories outside of the USA and is not used in OECD testing standards (which are internationally recognized). Further, its Ca-Mg ratio is not representative of surface waters under certain conditions.[29] However, this is balanced by the reproducibility and ease of synthesis of the water with appropriate hardness, pH, and ionic strength.

Rationale for Artificial Seawater (ASW) recommendation. The primary parameters of concern when attempting to model seawater are ionic composition, and to a lesser extent trace elements. As is the case for most of the media categories and types covered in this paper, multiple competing seawaters have been defined without consensus adoption to date. Many studies have been conducted with filtered natural seawater[43] or a mixture of natural and artificial seawater [44], which can vary temporally and spatially, necessitating extensive characterization of each batch collected for site specific accuracy. However, compared to terrestrial waters, seawaters are relatively homogenous, and for the purposes of enabling cross-comparison of data we propose agreement on a harmonized recipe. There are numerous commercially available ASW alternatives, several of which have been used extensively in scientific research (e.g. Instant Ocean and Crystal Sea Marine Mix).[45] The ionic and elemental compositions of these are quite similar to actual seawater,[46] but quality control and batch variability are a significant concern. Alternatively, several artificial seawater standards are commonly used and can be made with reagent-grade chemicals. EPA synthetic seawater,[41] ASTM D1141-98,[47] and Marine Biological Laboratory (MBL)[48] are a few examples.

Given the need for quality control and reproducibility, there are significant concerns about recommending commercially available ASW mixtures, or filtered natural seawater. Based primarily on batch-to-batch variability of commercially available artificial seawaters, we recommend using EPA artificial seawater (EPA ASW), made with reagent-grade chemicals, at 35 ‰ salinity in accordance with the EPA protocol. Ionic composition and the trace element complexity of EPA ASW are similar to ASTM D1141-98 Substitute Ocean Water. We are recommending EPA ASW as the recipe is freely available. The inclusion of trace elements along with the major ionic components allows EPA ASW to be used for both acute and chronic experiments,[40] whereas standard MBL does not contain trace elements (although several variants exist for MBL with increased complexity).[49]

For estuarine subsystems, we recommend using 3.5‰ salinity EPA ASW, although species' physiological requirements may prevent the usage of this salinity. Alternative dilutions of EPA ASW may be used where necessary to accommodate differing species' requirements. In all cases, full documentation is needed. Further recommendations for experiment-specific amendments to these media, including natural organic matter and inorganic colloids, may be found in the SI.

2.2. Soils, sediments and other important solid phases

2.2.1. Context and background

NMs will become associated with a variety of solids (e.g. soil, subaquatic sediment, heat-treated sludges, plant residues) in built and natural environments. Note that only treated biosolids will be considered in this section, whereas other wastewater treatment products are discussed in section 3.4 (Engineered waste systems) due to the special considerations below specifically for solid-dominated systems. Deposition, when defined as the attachment and detachment processes of NMs and stationary surfaces, is an important interaction of NMs in solid phases, because it determines the extent of NM transport and most likely also their bio-availability[50] and toxicity.[51]

2.2.2. Recommended media and parameters

Considering the current state of knowledge about sinks for NMs, exposure routes, and sensitive ecosystems, the following recommended minimum characterization and categories of solid phases was created (Table 4). [50, 52-54] The use of standard porous media (e.g. soils or sludges) that hold these parameters roughly constant will be the simplest mechanism to readily compare across studies and across NM types. Further, these parameters will drive key NM processes in these media, including surface attachment, dissolution, core material transformation, and bioaccumulation. Tests in porous media collected at a selected site should be similarly characterized. A list of these standard methods, associated units and definitions, rational for including the parameter, and references on their performance are included in Table 5.

Table 4: Minimum set of parameters recommended for inter-study comparison of the fate and effects of nanomaterials in soil and sediment media

Media type	Primary Measurements	Secondary Measurements

Agriculture and forestry soils	• Soil pH • Total organic carbon (TOC) • Texture • Acid extractable Si, Ca, Mg, Fe, Al, Mn • Redox potential • Specific conductance	• Dissolved organic carbon (DOC) • Porewater pH
Biosolids	• All agriculture soil parameters except texture • Acid extractable C, S, P, K, N and potentially toxic metals • Acid volatile sulfide (AVS)	• All of the above
Subaquatic sediments	• All agriculture soil parameters • AVS	• All of the above
Unconsolidated aquifers	• All agriculture soil parameters • Effective porosity • Grain size • Dispersivity	• All of the above
Consolidated aquifers	• All agriculture soil parameters • Equivalent aperture of fractures • Coefficient of variation of fractures	• All of the above

Table 5. Units, rationale and references for recommended parameters to characterize solid media

Parameters	Units	Rationale	Reference
pH in 1 M KCl or 0.01 M $CaCl_2$ extract		Assay independent indication of pore water pH good for comparability	55
Total organic carbon (TOC)	mg kg^{-1}	Immobile OM to which NM can attach, contributes to structure, indicator of biological activity	56, 57
Texture (clay, silt, sand)	%	Indicator of % mineralogical clays and or oxides and thus of quantity of favorable deposition sites for NMs, contributes to structure, indicator of straining potential and permeability, nutrient retention capacity, Water Holding Capactiy, dispersable clays	58
redox potential	V to SHE	master variable for speciation, indicator of microbial activity, controls extent of	59

		dissolution of redox-sensitive NPs (Ag, Fe, Al, …), especially in sludges	
water extractable Ca, Al, P, Mn, Fe, Si, SO_4, Mg, K, Na, NO_3^-, Cl^-	$mg\ L^{-1}$	Calculate ionic strength/divalent versus monovalent to determine colloidal stability, specific adsorption to NM and soil surfaces (PO_4, Ca), nutrient availability, metal speciation	60
Dissolved organic carbon (DOC)	$mg\ C\ L^{-1}$	Sterically stabilizes NMs, usually increases pore water residence times	20
porewater pH		Master variable for dissolution, surface charge, speciation, ….	61, 62
Specific conductance	µS/cm	Affects NM (homo- and hetero-) aggregation and deposition	63
Total C, S, P, K, N	$mg\ kg^{-1}$	Nutrient status of sludge, potential for sulfidisation and phospatization of NMs	64
Total toxic metals	$mg\ kg^{-1}$	Accounting for toxic effects of metals	64
Acid volatile sulfide	$mg\ kg^{-1}$	Sulfidation potential of NMs	65
Effective porosity		Important transport parameter in aquifers	66
Dispersivity	m	Important transport parameter in aquifers	67
Grain size	µm	Important transport parameter in aquifers	58
Equivalent aperture of fractures	µm	Accounting for preferential flow in consolidated aquifers	68
Coefficient of variation of fractures	µm	Important transport parameter in consolidated aquifers	68
Tortuosity		Important transport parameter in consolidated aquifers	69

2.2.3. Considerations of benchmark media for specific parameter values

Benchmark porous media must be relatively simple in structure, readily accessible, inexpensive, and consistent in their properties over time. Potential choices of standard soils and sediments, and the rationale for those choices, are provided in this section.

Soils

A large variety of soils have been used as reference materials in environmental studies. Some are too simplistic for use in studies of NM transport, fate and effects, including the OECD standard soil[70] as outlined below. Several well-characterized reference materials soils are being or have been sold by the National Institute of Science and Technology (NIST) (e.g., San Joaquin Soil, NIST 2709a) in quantities of up to 50 g per unit. However, these soils are intended for use as standard reference materials for chemical analysis and as such they are characterized with respect to composition of certain chemical analytes, not parameters like texture, cation exchange capacity, and organic matter content. Most soils are not readily available in sufficient quantities to support widespread adoption as reference soils for some functional assays of NM fate and effects (up to 1 kg), although the required quantity of soil would depend upon the assay. For the purpose of harmonizing studies of NM transport, a commercially available natural sandy soil may be a good choice. One example of a commercially available natural sandy soil that is widely available is Lufa 2.1 (Speyer).[71] However, restrictions on importing soils may make this a challenge for some researchers. The choice of a sandy soil was driven by the need for a soil allowing measurable NM transport parameters in column studies. For NM transformation, bioaccumulation, and toxicity studies, a natural sandy loam soil may be a better choice, because it represents a textural class common among agricultural soils. One example of a commercially available soil that provides consistent soil characteristics, has been used extensively in studies of NMs and other soil contaminants,[72, 73] and can support the organisms used in bioaccumulation and toxicity studies (e.g., plants, earthworms) is Lufa 2.2 (Speyer). The properties of these soils are provided in the Supporting Information. Note that not all the recommended parameters mentioned are supplied by the provider, so additional characterization will be required for NM exposure, fate and behavior studies.

Benchmark Sediments

In contrast to soil, few examples of reference subaquatic sediments are available. Natural freshwater sediment from West Bearskin Lake, MN, USA has been used as a control sediment by the USEPA for the development of benthic invertebrate toxicity assays[74] and subsequently used by other researchers.[75] However, the physico-chemical properties for West Bearskin Lake sediment reported by different authors vary considerably so its immediate use as a benchmark sediment may not be advisable.[76, 77] NIST sells estuarine sediment[78] and river sediment[79] at up to 70 g quantities per unit; however, like the NIST soil SRMs, these SRMs are intended for use in chemical analysis. Identification of a suitable reference material for sediments should be based on finding a material in sufficiently large supply that can be considered relevant in terms of the properties outlined in Table 4. This Table highlights that redox-sensitive properties of porous materials are relevant for the fate of NM, particularly for dissolution and transformation, imposing additional requirements on the anoxic storage and testing of any reference material.

Sludge

Treated sludges, also called biosolids, are an important sink for a large proportion of NMs entering wastewater streams. Sludges are often applied to agricultural lands as a soil amendment. Reference sludges only exist as analytical chemistry standard reference materials (SRMs), such as SRM 2781 (NIST, Gaithersburg, Maryland, USA). This material is neither intended for, nor suitable for, use as a medium in fate, transport or toxicity studies due to its complexity and variability. A number of recent studies have operated pilot scale wastewater treatment systems, utilizing local primary sludge as a feed.[80, 81] This approach will not be feasible for routine studies and local primary sludge and wastewater treatment processes vary considerably from location to location. Municipal biosolids are commercially available and could possibly be used as reference media. For example, Milorganite®, which has been produced for over 90 years by heat treatment of sewage sludge from the municipal sewerage district of Milwaukee, Wisconsin, USA, [82] has been used in a variety of studies concerning behavior of contaminants, such as metals, after land application of sludges.[83-86]

2.3. Biological Media

2.3.1. Context and background

Guidelines for the selection of appropriate biological media are needed for testing of toxicological and ecotoxicological impacts of NMs. We also provide recommendations for some specific biofluids where sufficient confidence exists as to their applicability and broad relevance. Where possible and appropriate, the media recommendations for ecotoxicity testing are aligned with the recommended media from the aquatic and solid compartments defined above, as this streamlines experimental approaches and facilitates maximum integration of the exposure and hazard data for risk assessment.

2.3.2. Recommended media and parameters

Given the very broad scope of the topic biological testing media and the range of organisms each with distinct physiologies, biological media in this Perspective are further broken down into categories. The first categorization distinguishes between submerged exposure scenarios (i.e. NMs in a liquid medium exposed to an organism surface), versus an air-surface exposure scenario such as mimicking the lung-air (or gill for some aquatic organisms) barrier (the so called Air-liquid interfaces) as well as NMs in contact with skin, eyes, plant leaves etc. as shown in Table 6. The recommended characterization measurements for media within these categories are shown in Table 7. Note that only those characteristics identified as inherent to the media, and not explicitly a function of the assay in question (see Figure 1), are included.

Table 6: Minimum set of parameters recommended for inter-study comparison of the fate and effects of nanomaterials in biological media

Media type		Primary Measurements	Secondary Measurements
Liquid-surface exposure scenarios	Mammalian culture medium	• pH • Ionic Strength • Ionic content • % serum • Source of serum (Bovine calf, mouse, rabbit, human etc.) • Whether serum was heat inactivated or not	• Relative Humidity • % CO_2
	Aquatic organism culture media	• pH • Ionic strength • Major cations (e.g. hardness) • Major anions (e.g. alkalinity) • Dissolved oxygen	• Ionic content (e.g. monovalent, divalent, Cl-, Sulfate, etc.) • Food type and amount • Natural organic matter (NOM) or other biomolecules added – concentration, source
	Microbial medium	• pH • Ionic Strength	• Major cations (e.g. hardness) • Major anions (e.g. alkalinity)
	Rhizosomal system (roots) – see soil pore extracts in Section 3.2	• Soil pH • Total organic carbon (TOC) • Texture • Extractable Si, Ca, Mg, Fe, Al, Mn • Redox potential • Specific conductance	• Dissolved organic carbon (DOC) • Porewater pH

	Simulated digestive fluids Humans Organisms Insects	• pH • Ionic strength • Enzymes or other additives	• Ion content (towards speciation)
	Artificial Lysosomal Fluid	• pH • Ionic strength • Enzymes or other additives	• Ion content (towards speciation)
	Plant transport mimics	• pH • Ionic strength	• Ion content (towards speciation)
Air-surface exposure scenarios	Simulated surface contact media Dermal Lung Ocular	• pH • Ionic strength • Biological molecules (proteins)	• viscosity
	Leaves e.g. epicuticular wax	• pH • Alkane chain length • fluidity	• Hydrophobicity • Viscosity

Discussion of primary parameters.

pH: Most normal mammalian cell lines grow well at pH 7.4, with very little variability among different cell strains. However, some transformed cell lines have been shown to grow better in slightly more acidic environments (pH 7.0 to 7.4), and some normal fibroblast cell lines prefer a slightly more basic environments (pH 7.4 to 7.7). Insect cell lines such as Sf9 and Sf21 grow optimally at pH 6.2.[87] Bacterial testing is also routinely performed at pH 7.4, with OCED 301 stating an optimal pH of 7.4 ± 0.2. Similarly, most organisms have an optimal pH range, and media derived for these usually sit in physiological ranges. For example, Daphnia have been found to have optimal survival, growth and reproduction at pHs in the range 7.9 to 8.3.[88] Thus, pH is an essential parameter for all liquid media types, as physiological changes related to environmental stress will arise when organisms are outside of their optimal pH range, which will compromise any subsequent exposure or hazard assessments. In the case of simulated fluids, such as digestive fluids, or Artificial lysosomal fluids (ALF), these are specific fluids where an unusual pH is required to achieve a specific function, and thus assessment of the effectiveness of this function can only be done at the relevant pH.

Ionic Strength and Ionic Content: The physiological ionic strength is between 100 mmol/L to 200 mmol/L KCl or NaCl. The growth medium controls the pH of the culture and buffers the cells in culture against changes in the pH. Usually, this buffering is achieved by including an organic (e.g., HEPES) or CO_2-bicarbonate based buffer. Note that ionic strength and pH may both impact nanomaterial properties, and are therefore essential parameters to report. Biomolecule protonation and deprotonation further depend on the ionic composition of the surrounding medium. At pH 7.5, phosphate buffers add approximately 7 × more ions to the medium than zwitterionic Tricine (nitrogen containing) buffers.[89]

Serum percentage, source of serum, and whether serum was heat inactivated: This consideration is quite specific to mammalian cell culture, where serum is routinely utilized as a food source for cells. Depending on the cell type, the amount of serum ranges between 2 % (e.g. for blood-brain barrier cells) to 20 % often recommended to speed up the growth of Caco-2 cells, with 10 % being a common standard. However, with NMs, the ratio of the NM surface to the amount of proteins present can have an important role in terms of proteins bound in the corona: for some NMs more proteins available results in thicker coronas of the same composition, while for others quite different coronas occur at different surface area: FBS ratios.[90, 91]

The source of the serum (fetal bovine, fetal calf, or other animal sera such as mouse, rat, rabbit or horse, as well as human) is an important consideration, as the different sera have been shown to result in quite different corona compositions and differential cellular uptake of the particles and thus differential toxicity induction in cells.[91, 92] For example, lower rates of uptake of the same NMs by the same cells were observed with the NMs/cells incubated in human serum compared to bovine serum (for 50 nm amine-modified polystyrene NMs in A549 cells).[91] Heat inactivation of the FBS has also been shown to affect the NM corona composition,[93] and thus should be reported.

Discussion of secondary parameters.

CO_2 Levels: Because the pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO_2) and bicarbonate (HCO_3^-), changes in the atmospheric CO_2 can alter the pH of the medium. Therefore, it is necessary to use exogenous CO_2 when using media buffered with a CO_2-bicarbonate based buffer, especially if the cells are cultured in open dishes or transformed

cell lines are cultured at high concentrations. Most researchers use 5 % to 7% CO_2 in air, however, each medium has a recommended CO_2 tension and bicarbonate concentration to achieve the correct pH.

Relative Humidity: Using an incubator humidity of 85 % to 95% limits evaporation of water from cell culture media. Evaporation can substantially raise the media concentrations of salts, minerals, etc., potentially resulting in toxicity and cell death. High humidity is the most difficult condition to maintain but is critically important, as evaporation is 4 times faster at 80% humidity than at > 93 %.[94]

Table 7. Units, rationale and references for recommended parameters to characterize biological media

Parameters	Units	Rationale	Media Types	Reference
pH		Many biomolecules are pH sensitive; different biological compartments have specific pHs for optimal functionality	All buffers and liquid media for toxicity & NM fate assessment	17
Ionic strength	mmol L^{-1}	Calculate from either reagent addition or from conductivity measurements	All liquid media	18
CO_2 concentration	%		Tissue / cell culture	95
Dissolved oxygen (DO) concentration	%	Important for both biological growth and NM behavior	All liquid media	20
Relevant minimum biomolecular components & relative concentration	%	Relative concentration (e.g. concentration to surface area)	Tissue / Cell Culture	
Major cations	mmol L^{-1}	Hardness – needs to be suitable for the organism being tested, affects NM stability and size distribution	Aquatic & soil organism test media	19
Major anions	mmol L^{-1}	Alkalinity – needs to be suitable for the organism being tested, affects NM stability and size distribution	Aquatic & soil organism test media	19
Dissolved organic carbon (DOC)	mg L^{-1}	Important for both biological growth and NM behavior	All liquid media	20
Growth factors	mg L^{-1}	Modulators and antibiotics / antimiotics	Cell culture	

2.3.3. Considerations of benchmark media for specific parameter values

Tissue medium (submerged culture)

Mammalian culture medium. FBS is a ubiquitously used essential supplement in cell culture media, for both human and mammalian cells. However, there are serious scientific and ethical concerns about the use of FBS regarding its harvest and production.[96] Efforts are underway to develop serum-free cell cultures in other fields, mostly as means to harmonize or reduce the inherent variability from animal-derived compounds. There are various degrees of chemical definition, e.g. serum-free (SF), animal-derived component free or chemically defined, and the type of medium, e.g. basal media, medium supplements, or full replacement media.[96] However, these are far from being standardized, and are thus not discussed further here.

The large reactive surface area of NMs makes the addition of biomolecules to the medium essential in order to avoid physical damage of membranes. Thus, SF conditions may not be feasible for use with NMs, unless other synthetic macromolecules can be substituted instead to bind to the NMs and passivate their surface. Further potential complications with biomolecule addition include the potential for non-self immune recognition. [97]

In light of these and other considerations, our recommendation for cell culture medium is a commonly applied medium, Dulbecco's Modified Eagle's Medium (DMEM), which is a modification of Basal Medium Eagle (BME) that contains a four-fold higher concentration of amino acids and vitamins, as well as additional supplementary components. DMEM requires supplementation with 1 % to 5 % FBS and 4 mmol L^{-1} L-glutamine supplement. The FBS concentration must be optimized for each cell line to obtain maximum serum reduction. DMEM uses a sodium bicarbonate buffer system (3.7 g L^{-1}), and therefore requires a 5 % to 10 % CO_2 environment to maintain physiological pH. See Table 8 for full details.

Table 8: Recommended benchmark mammalian cell culture medium (DMEM)

Parameters	Value (Range)	Units
Chemical		
Sodium bicarbonate buffer	3.7	mg L^{-1}

Phenol Red	15.0	mg L^{-1}
D-Glucose (Dextrose) 180.0	4500.0	mg L^{-1}
Inorganic salts: **CACL$_2$ (ANHYD.)** **FE(NO$_3$)$_3$.9H$_2$O** **MGSO$_4$ (ANHYD.)** **KCL** **NAHCO$_3$** **NACL** **NAH$_2$PO$_4$-H$_2$O**	200.0 0.1 97.67 400.0 3700.0 6400.0 125.0	mg L^{-1}
Physical		
Requires CO_2 to maintain physiological pH	5–10	%
Biological		
L-glutamine	584.0	mg L^{-1}
Amino acids (14 in addition to the L-glutamine)	30-584	mg L^{-1}
Vitamins (8 different)	0.4 – 7.2	mg L^{-1}
Requires supplementation with e.g. 10% Fetal Bovine Serum (FBS)	5-10	%

Simulated human saliva. Recent studies that have simulated saliva fluid lack cohesiveness of background media constituents. It is challenging to duplicate human saliva because (a) it is excreted from several different glands at different volumes, (b) its contents can vary at different times during the day, and (c) it can be influenced by the diet.[98] Based on a critical review of reported synthetic saliva used for *in vitro* studies between 1931 and 1996 (n = 60)[99], a standard saliva gastric fluid medium has been proposed. We endorse this as a model simulated saliva (Table 9). Depending upon the assay purpose, it may relevant to also test the saliva with slight modifications such as additional biomolecules (i.e. mucin, amylase) or an altered pH.

Table 9: Recommended benchmark for simulated saliva

Parameters	Value (Range)	Units
Chemical		
pH	6.4 - 6.8	-
Ionic strength	Media Specific	Total mmol/L

Ion content (towards speciation)	Media Specific	Composition of each component in mmol/L
Deionized Water (DIW)		
Urea (CH_4N_2O)	0.200	g L^{-1}
Sodium chloride (NaCl)	0.126	g L^{-1}
Ammonium chloride (NH_4Cl)	0.178	g L^{-1}
Potassium chloride (KCl)	0.964	g L^{-1}
Potassium thiocyanate (KSCN)	0.189	g L^{-1}
Monopotassium phosphate (KH_2PO_4)	0.654	g L^{-1}
Sodium sulfate (Na_2SO_4, 10 H_2O)	0.763	g L^{-1}
Calcium chloride ($CaCl_2$, 2 H_2O)	0.228	g L^{-1}
Sodium bicarbonate ($NaHCO_3$)	0.631	g L^{-1}
Physical		
Biological		
Appropriate Sterility	-	-
Enzymes / proteins		

Simulated human gastric fluids. Here we define a specific model simulated gastric fluid (Table 10), closely resembling the fluids found in the stomach of mammals.[100] In the development of a model gastric fluid, careful consideration must be given to the behavior of NMs in such a medium. For that reason, we decided that the frequently used 0.07 mol L^{-1} HCl solution is insufficient to accurately capture NM behaviors such as aggregation state, deposition kinetics, and transformation among others, all of which will strongly impact nearly any nanoparticle assay run in the medium.[75] Therefore, the current proposed standard medium includes a small number of gastric proteins in addition to the proper ionic strength expected in a gastric fluid. This has been adapted from model gastric fluids reported previously with some adaptation in order to include a more complete picture of relevant biomolecules in a fasted state. Adjustments for fed states include higher pH and ionic strength.[101]

Table 10: Recommended benchmark for simulated gastric fluid

Parameters	Value (Range)	Units
Chemical		
pH	1.6	-

Ionic strength	100	mmol L^{-1}
Ion content: NaCl KCl	 70 50	mmol L^{-1}
Deionized Water (DIW)		
Physical		
Biological		
Pepsin	0.2	g L^{-1}
Mucin	1.5	g L^{-1}
Lecithin	0.02	g L^{-1}

Simulated digestive fluids for fish and other organisms. Simulated gut or digestive fluids are significantly less widely used in environmental organisms. Only a small number of recipes for simulated fish (carp) digestive fluid have been reported. [102-104] However, closer inspection of these recipes revealed that they were intended to simulate human digestive fluids. Non-human digestive fluids would thus seem like an area where more research is needed, and as such as we are not making any recommendations regarding simulated digestive fluids for non-human organisms at this time.

Simulated surface contact media

Simulated biological fluids have been used traditionally in the pharmaceutical and biomedical industries for testing and defining the dosage of drugs.[101] We recommend the use of these established media to investigate the physico-chemical behavior of NMs at the point of contact with biological systems and for quantifying the potential absorption of NMs by those systems.

Pulmonary fluids. Here we endorse an existing simulated pulmonary surfactant fluid[105] for use as a model medium in NM inhalation exposure scenarios (Table 11). As with the other proposed simulated biological fluids, the composition was chosen for its ability to capture NM behavior in complex biological systems, which will depend on not just the quantity of surfactant in the fluid but on its composition and relative concentrations. This has been observed in several studies, in which protein or natural amphiphiles exhibited differential binding for NM surfaces.[106, 107] While the entire spectrum of possible pulmonary proteins and surfactants is not included here for the sake of simplicity in preparation and analysis, we believe it to be a representative minimum set of relevant biomolecules.

Table 11: Recommended benchmark for Simulated Pulmonary surfactant fluid

Parameters	**Value (Range)**	**Units**
Chemical		
pH	7.4	-
Ionic strength	122	mmol L^{-1}
Ion content (towards speciation) NaCl KCl $CaCl_2$ Na_2HPO_4 $NaHCO_3$ $MgCl_2$	103 4 2.3 1 32 2.1	mmol L^{-1}
Physical		
Biological		
Relevant minimum biomolecular components: Albumin Ascorbic Acid Cysteine DPPC Glutathione Glycine Mucin Uric Acid	260 18 122 100 30 376 500 8	mg L^{-1}

Artificial Lysosomal Fluid (ALF). When considering the interactions of NMs with mammalian and other living cells, lysosomes are frequent endpoints of interest. This environment has a particular impact on the dissolution and speciation of NMs within cells.[108, 109] Our recommended ALF composition is given in Table 12.

Table12: Recommended benchmark for Artificial Lysosomal Fluid (ALF)

Parameters	**Value (Range)**	**Units**
Chemical		
pH	4.5	-
Ionic strength	Media Specific	Total mmol L^{-1}
Ion content (towards speciation)	Media Specific	Composition of each component in mmol L^{-1}
Deionized Water (DIW)		

Sodium chloride (NaCl)	3.21	gL^{-1}
Sodium hydroxide	6	gL^{-1}
Citric acid	20.8	gL^{-1}
Calcium chloride ($CaCl.2H_20$)	0.128	gL^{-1}
$NaHPO_4$ dibasic	0.071	g L^{-1}
$MgCl_2$ hexahydrate	0.106	g L^{-1}
Sodium sulfate (Na_2SO_4)	0.039	g L^{-1}
Glycerol or Glycerine ($C_3H_8O_3$)	0.059	g L^{-1}
Glycine (NH_2CH_2COOH)	30.3	g L^{-1}
$C_6H_5Na_3O_7 \cdot 2H_2O$ (Na_3 citrate $\cdot$ $2H_2O$)	0.077	g L^{-1}
$C_4H_4O_6Na_2 \cdot 2H_2O$ (Na_2 tartrate $\cdot$ $2H_2O$)	0.09	g L^{-1}
$C_3H_5NaO_3$ (Na lactate, 60% in water)	0.065	mL
$C_3H_5O_3Na$ (Na pyruvate)	0.086	g L^{-1}
Formaldehyde	1	mL
Physical		
N/A		
Biological		
N/A		

Simulated leaf surfaces (e.g. for nanopesticide formulations^{110}*).* This is an emerging area for NMs with no studies found to date, however simulated leaf surfaces have been prepared for chemical testing. For example, hydrocarbon wax and beeswax were compared by forming surfaces on stainless steel plates, with a target wax coverage of 1 mg cm^{-2}.[110] Some additional work is needed in order to assess the suitability of this approach for assessing NM interactions, and thus no recommendation is made at this point.

2.4. Engineered waste systems

2.4.1. Context and background

There are a number of engineered systems, such as waste treatment and holding systems, that NMs will enter and potentially accumulate in. These media types are addressed separately here because they are not naturally occurring, and their properties and inputs are in many cases controlled by human decisions.

2.4.2. Recommended media and parameters

The OECD[111] has identified four waste treatment systems as being most relevant for examining the possible impacts of nanowastes: wastewater treatment, incineration, landfilling and recycling. The overall goal of all waste treatment systems is to remove / separate unwanted components from waste streams, but individual facilities operate according to very different principles. High temperatures during waste / sludge incineration result in the combustion of organic materials contained in the waste material and an enrichment of inorganic compounds in the bottom and fly ash. During activated sludge processing, organic materials are biologically degraded and inorganic materials are separated from the wastewater by sedimentation in the secondary clarifier. Considerable variability also exists within the same type of waste treatment system, such as the water chemical parameters of landfill leachates vary strongly depending on the landfill type and age. It is thus challenging to balance the variability between systems and the variability within systems. Based on the life cycle perspective of NMs and in accordance with the OECD report on nanowaste,[111] we identified four systems (activated sludge, treated wastewater, sewage sludge ash, and landfill leachate) as the focus of this Perspective.

Incineration is a very important treatment process, which affects the form and availability of NMs. Werther and Ogada[112] defined three categories of thermal sludge treatment: i) mono-incineration ii) co-combustion and iii) alternative thermal processes (pyrolysis, gasification). Each thermal process will produce ash with different chemical and physical properties. Thermal processes are prone to modify the physical state of NMs as a function of the temperature and the thermal stability of the NMs. Carbon NMs are of particular concern as the temperature reached during incineration may lead to only partial combustion and transformation of carbon-based NMs.[113-116] Due to the relatively small variations in sewage sludge ash, these ashes may be appropriate for standardization purpose, but no standardized sewage sludge ash yet exists. However, using the sewage sludge ash as a medium to conduct experiments addressing the fate of NM (e.g., released in column experiments) is not particularly meaningful, as directly adding the NMs to sewage sludge ash omits the high temperature process leading to a fundamentally different incorporation of NMs into the ash matrix. We therefore did not consider sewage sludge ash as a useful medium in the context of this Perspective. Nevertheless, the use of ashes should include characterization of both the source material (Tables 7, 8) and the process by which it was produced. Relevant media associated with wastewater treatment and landfilling are defined in Table 13. Definitions, rationale, and references for the measurement of each parameter are shown in Table 14.

Table 13: Minimum set of parameters recommended for inter-study comparison of the fate and effects of nanomaterials in engineered waste media

Media type	**Primary Measurements**	**Secondary Measurements**
Activated sludge	• pH • Total Suspended Solids (TSS) • Dissolved organic carbon (DOC) • Electrical conductivity • Sludge volume index (SVI)	• Total organic carbon (TOC) • O_2 • Major cations (e.g. K^+, Na^+, Ca^{2+}, Mg^{2+}) • Major anions (e.g., Cl^-, SO_4^{2-}) • Nitrate (NO_3^-) • Ammonium (NH_4^+)
Treated wastewater	• pH • TSS • DOC • Electrical conductivity	• TOC • Major cations (e.g. K^+, Na^+, Ca^{2+}, Mg^{2+}) • Major anions (e.g., Cl^-, SO_4^{2-}) • Nitrate (NO_3^-)
Landfill leachate	• pH • TSS • DOC • Electrical conductivity • Redox potential	• O_2 • TOC • Major cations (Ca^{2+}, Mg^{2+},) • Major Anions (SO_4^{2-}, Cl^-, NO_3^-)

Discussion of primary parameters:

For all three media, pH, TSS, DOC and the electrical conductivity were considered key parameters which should be reported. In addition, the redox potential should be reported in landfill leachates. From the measurement of the electrical conductivity, the ionic strength and the total dissolved solids can be estimated based on well-established correlations between these parameters.[117] The redox potential is a crucial parameter for predicting the speciation of metals in aqueous environments (see Soils and Sediments section discussion). Therefore, the redox potential will be particularly important to assess the fate Cu, Zn, and Ag NMs. It should be noted that speciation calculations are only applicable under thermodynamic equilibrium conditions, which may not be reached in the media described in this section or elsewhere in this Perspective.

Discussion of secondary parameters:

Apart from the primary parameters listed above, we have identified a set of secondary parameters which should be reported if possible. Nitrate and ammonia concentrations are of key importance for activated sludge media and provide information about the performance of a wastewater treatment plant. Furthermore, the oxygen concentration informs about processes (nitrification / denitrification) that are occurring within the sludge. Although it is possible to calculate the ionic strength based on empirical correlations with the electrical conductivity, measurements of major cations and anions will provide more reliable data on the ionic strength of the respective media. TOC content provides information about the condition of the sewage sludge, and when measured in the treated water can be used as a measure of the performance of the wastewater treatment plant. Oxygen concentrations measured in landfill leachates are useful to assess the conditions in the landfill (oxic vs. anoxic), which will have a direct impact on NM transformations including dissolution, oxidation, and redox activity.[118] For example, anoxic conditions will limit dissolution of some NMs (e.g., AgNPs), while the redox activity will also impact the extent to which the speciation of other NMs such as cerium oxide NMs occurs .[12]

Table 14. Units, rationale and references for recommended parameters to characterize engineered waste media

Parameters	Units	Rationale	Media Types	Reference
pH		Indication of the state of the associated wastewater treatment plant[1,2]. It is important to assess conditions in the landfill and the stability of inorganic compounds (e.g. dissolution or precipitation of mineral phases)[3]	1,2,3	55, 62
Total suspended solids (TSS)	mg L^{-1}	Indication of the state of the associated wastewater treatment plant[1,2]. Useful for comparability between different field sites / synthetic mixtures [3]	1,2,3	24
Dissolved organic carbon (DOC)	mg L^{-1}	Influence on colloidal stabilities of NMs [1-3]	1,2,3	20, 57

Electrical conductivity	µS cm^{-1}	Allows estimation of total dissolved solids and ionic strength (key parameter for stability calculations) [1-3]	1,2,3	[18]
Redox potential	mV	Important for speciation calculations of mineral phases (and selected MNs) [3]	3	[22, 59]
Sludge volume index	mg g^{-1}	Informs about the ‘quality’ of the sewage sludge[1]	1	[119]
Major cations	mg L^{-1}	Required for the accurate calculation of the ionic strength (key parameter for stability calculations) [1-3]	(1,2,3)	[19]
Major anions	mg L^{-1}	Required for the accurate calculation of the ionic strength (key parameter for stability calculations) [1-3]	(1,2,3)	[19]
Total organic carbon (TOC)	mg L^{-1}	Informs about the state / performance of the wastewater treatment process [1,2], gives an indication of the biological activity in the landfill.	(1,2,3)	[20, 56, 57]
O_2	mg L^{-1}	Provides information about the wastewater treatment process (nitrification, denitrification) and characterizes the respective sludge (oxic / anoxic) [1], informs about the conditions and processes in the landfill (influences mineral / NM stability) [3].	(1,3)	[120]
Nitrate	mg L^{-1}	Key parameter used to evaluate the performance of the wastewater treatment process [1,2]	(1,2)	[121]
Ammonium	mg L^{-1}	Important to assess the performance of the wastewater treatment process (nitrification) [1]	(1)	[122]

[1]: activated sludge, [2]: treated wastewater, [3]: landfill leachate. Values in bracket refer to secondary measurements for the different media types.

2.4.3. Considerations of benchmark media for specific parameter values

Sludge. Depending on the process design of a wastewater treatment plant and on the specific requirements, different types of sludge are used / produced. Examples include primary sludge, activated sludge, granular sludge and digested sludge. A typical sludge, as summarized from various sources[123] is the primary solid-containing residual produced from the separation of water and solids in the primary, secondary and tertiary wastewater treatment processes. Activated sludge mainly consists of bacteria and protozoa that form biological flocs. Activated sludge must be biologically active to allow degradation of wastes and could be affected by NMs. The partitioning of NMs to sludge indicates the amounts of NMs that may pass through sewage treatment processes and enter receiving soils, sediments, and surface waters. Sewage sludge is expected to represent a major sink for many NMs [124, 125] and waste water treatment plants will therefore be central to decipher the fate of NMs after their use.

A standardized activated sludge is not available. For analytical purposes, a powered sludge is available as standard reference material (e.g. SRM 2781, NIST, Gaithersburg, Maryland, USA), but powdered sludge cannot be used as an analog for activated sludge for the purpose outlined in this Perspective. Several authors have used sewage sludge collected from field- or pilot – scale wastewater treatment plants to study the behavior of NMs in sludge medium.[80, 81, 126] We argue that the general properties of sewage sludge resulting from the activated sludge process are broadly comparable. Therefore, we recommend use of activated sludge collected from local sewage treatment plants as sludge media. By reporting the key parameters described above (Table 8), differences in the general properties of the sludge are revealed and can be compared.

Treated wastewater. Engineered NMs are released into municipal wastewater streams towards the end of the lifetime of the materials, where the NM will partition into the solid phase (sludge, or biosolids) or aqueous phase (effluent). A majority (> 95 %) of NMs tend to be attached to the heterogeneous, dense bacterial communities found in biological wastewater treatment processes.[127-129] Nevertheless, despite the efficient removal of NMs during the wastewater treatment, a small fraction still escapes the treatment and is discharged into surface waters. Therefore, we consider treated wastewater as an important medium to assess potential exposure routes for NMs.

A standardized treated wastewater does not exist; however, wastewater effluents need to fulfill certain quality criteria before being discharged into surface waters. Although these criteria can vary from country to country, they set a general baseline for the quality of treated wastewater. In addition to variations caused by different influent waters, the contents of dissolved components are further influenced by the local geological environment. Thus, the natural variability caused by the geological settings may lead to considerable differences of dissolved components in treated wastewater. We suggest the use of 'Moderately Hard Reconstituted Water (MHRW)' described in the aqueous media section (section 3.1.3), with a few modifications as an analog for treated wastewater. The most important modification recommendations are strongly elevated concentrations of Na^+ and Cl^-, and possibly HCO_3^-, affecting the ionic strength of the treated wastewater and thereby the colloidal stability of NMs. Furthermore, increased concentrations of DOC in treated wastewater are conceivable, which may stabilize NMs against agglomeration. Thus, we suggest modifying the MHRW by increasing the Na^+ and Cl^- concentrations to 1000 mg L^{-1} each. The DOC can be adjusted to 10 mg L^{-1}, representing effluent values of proper operating WWTPs, by adding humic acid. In agreement with recommendations for freshwater and estuarian systems, we recommend the use of Suwannee River Humic Acid.

Landfill effluent. Four different types of landfills (sanitary, municipal solid waste (MSW), construction and demolition, and industrial waste landfills) are generally distinguished, each of which receive different kinds of wastes. Increasingly important categories of landfill materials include MSW, which are either directly landfilled or are incinerated and landfilled mainly as bottom ash. In developed counties, incineration of municipal waste is most popular, but the disposal of MSW in landfill remains the most important waste management strategy worldwide.[130] The properties of the landfill effluents strongly vary with: i) the type of landfill (and thus the kinds of materials that are deposited); ii) the operation principles; and iii) the age of the landfill.[131] The most important parameters determining the composition of the landfill leachates (MSW) is the age of the landfill which is related to the respective landfill fermentation stage.[132] The following four phases are typically described: aerobic, acid, initial methanogenic, stable methanogenic. Over extended periods of time (hundreds to thousands of years), other phases have been postulated, however, the composition of the respective leachates are still very speculative as no experimental data are available for such systems.[131]

We therefore identified the leachate originating from a landfill under the stable methanogenic phase, which lasts longest (of the three phases for which experimental data are available) and extends over several decades, as the most relevant and suitable for harmonization purposes. As no reference or standardized landfill leachate compositions are available, we recommend average values reported in the literature[131] to define an average landfill leachate which can be used for NM testing purposes. Cl^- and Na^+ (both up to 1 to 2 gL^{-1}) are much higher than in treated wastewater, but considerably lower compared to ASW (see water media) and also K^+ concentration can reach 1 g L^{-1}. Furthermore, Ca and sulfate concentrations can be as high as a few hundred mg L^{-1}. The considerably high ionic strength may strongly affect the agglomeration behavior of NMs in landfill effluents. Furthermore, DOC (extrapolated from BOD and COD values reported by Kjeldsen[131]) can range from a few tens to a few thousands mgL^{-1}. This large variation makes a selection of one specific value rather arbitrary. However, for a worst-case scenario, we recommend using elevated DOC concentrations added in the form of Suwannee River or other appropriately characterized humic acid. Following this reasoning, we recommend modifying the MHRW medium by adjusting the Cl^- and Na^+ concentrations to 1000 mg L^{-1} and a Ca concentration of 100 mgL^{-1}. Furthermore, DOC concentrations of 1000 mgL^{-1} should be adjusted by adding respective amounts of humic acid.

2.5. Product matrix media

2.5.1. Context and background

Previous sections focused on environmental testing media in which NMs are investigated. There is one critical additional medium that the ENM are in contact with, namely the product matrix they may be embedded in or deposited on through their use in nano-enabled products. When NM are released from polymer nanocomposites, they still are to a large extent still embedded in or associated with the matrix from the product.[133]

2.5.2. Recommended media and parameters

The harmonization of the product matrix characterization poses challenges that are different to those encountered for environmental media, due to enormous variability in product types and matrices. However, harmonizing reporting of product medium characteristics remains critical for comparability of studies. As a starting point to categorize product matrices, the NM categorization scheme from Hansen *et al*[134] can be used. These authors define the materials depending on the location of the nanostructure in the system. For our purposes, their third category "*Materials that contain nanostructured particles*" is relevant. Its four subcategories are:

- surface bound,
- suspended in liquids,
- suspended in solids,
- free (the scope of this Perspective which excludes consideration of air, "free" includes intentionally directly released nanomaterial products into media other than air).

For each category, a large number of potential matrices with very different chemical-physical behavior could be chosen. In the solid medium for example, the range of matrices could go from polyethylene to concrete and it has been shown that the potential for release of NMs from these matrices varies by five orders of magnitude.[135] Moreover, for a given matrix, different additives/surface coatings have to be used depending on the NM to be incorporated in order to allow NM dispersion and facilitate NM/Matrix compatibility.

Most published studies on release of NMs from a product matrix have used commercially available products with only limited description of the matrix and only in a few studies has a more defined matrix been used.[136] To study NM release from paints, standard paint formulations have been described in the NanoHouse project.[137, 138] For polymer nanocomposites, standard materials have been used in inter-laboratory comparisons.[139] Also for NM release from textiles, materials with full characterization of the fabrics and the methods to produce them have been described.[140] A number of case studies have emerged including methods to generate and characterize releases from matrix-embedded materials.[141] These few studies with materials that are relatively well described are clearly not sufficient to allow the proposition of standard testing materials for product matrices.

The recommended product matrix types are shown in Table 15. Two are solid matrices with NMs embedded within or with surface-bound NMs, and one is a colloidal suspension of NMs (e.g., a cream). The primary measurements that are required are the NM concentration inside the matrix and the composition of the matrix. In the case of nano-enabled products, NM concentrations may be significant due to potential impacts on the matrix structure itself in addition to a direct impact on release rates.[142] Depending on whether the NM/matrix is obtained from a commercial source or is produced in-house, more or less information is available about the matrix and the embedded NMs. The characterization of the identity of the NM and the chemical composition of the matrix is more challenging in the case of a commercial product and often relies on manufacturer's information only. It is therefore recommended to produce the matrix in-house to allow a full control over its composition and the type of NM added. A very close collaboration with manufacturers and a full disclosure of all ingredients of the matrix is also a preferred option because it ensures that the matrix is relevant from a real-world perspective but still allows full knowledge about the matrix composition. The use of "generic" formulations that combine the requirement of both scientists and industry has been shown to be a good compromise.[143]

A secondary set of measurements should deal with the changes in product matrix over time when added to an environmental medium. The product matrix ages over time when present in an environmental medium and these changes drive the behavior of the NM. The characterization of the "matrix in the matrix", i.e. the product matrix that is present in the environmental matrix, is also necessary. These environmental matrices should follow the recommendations in the previous sections of this Perspective.

Table 15: Minimum set of parameters recommended for product matrix media

Media type	**Primary Measurements (matrix)**	**Secondary Measurements (material release)**
Solid matrix with nanomaterials embedded	• nanomaterial concentration • composition of product matrix	○ changes in product matrix over time
Solid matrix with surface bound nanomaterials	• composition of product matrix	○ changes in product matrix over time
Colloidal suspension of nanomaterials	• composition of product suspension	○ changes in product matrix over time

The recommendations for harmonizing across this medium are therefore not equivalent to selecting a particular representative matrix in the way that aqueous, soils, biological or even engineered matrices may present. In this case, the recommendations are limited to conditional lists of parameters that are appropriate to report for comparison across the broadly varying set of product matrices into which NMs will be incorporated.

2.5.3. Considerations of benchmark media for specific parameter values

Solid matrix with NMs embedded: This type of matrix is one of the most frequently used product matrices[135, 136] and also constitutes the major type of matrix reported in release studies.[133] Not all NM/matrix combinations make sense from the point of view of actual product use and therefore for each combination another test material might be needed. The chemical 'compatibility' between matrix and NM must be taken into account. This means that different to the environmental testing media, where all NMs can enter the same system, in the case of the product matrix, different test media need to be prepared for each NM and each product type (e.g., polymer nanocomposite) that exists. In that specific case, the application domain, what NMs are used in which types of solid matrices, needs to be taken into account. The matrix composition needs to be known or has to be determined analytically regardless if a commercially available matrix or one produced in-house is used. It has been shown that it is mainly the type of matrix that determined the release of materials.[135]

Solid matrix with surface bound NMs: The general issues discussed for NMs embedded in a solid matrix also apply to surface-bound NMs. The choice of this matrix is justified by the much higher release potential of NMs when bound onto the surface than when incorporated into / embedded in the matrix.[144] The technology used to bind the NMs onto the surface is a crucial determinant of the system and needs to be known so that the behavior can be linked to composition.

Colloidal suspension of NMs: This matrix represents the simplest form of a product matrix because the NMs are present suspended in a liquid (or gel) matrix, therefore being similar to the pristine NMs that are usually used in experiments. Nevertheless, the additional presence of matrix materials influences the behavior of the NMs in the system and therefore the detailed characterization of the matrix and knowledge about the major constituents is necessary. While there have been a number of studies, there is not convergence on a reference matrix that can span across groupings or even phases. It may be that more specialized groupings can be developed based in part on use and in part on phase (e.g. liquid foods require a particular set of characteristics to harmonize reporting).

3. DISCUSSION AND CONCLUSIONS

A number of consistently observed tensions were encountered in selecting and compromising on harmonized media, including the desire to propose a minimum set of characteristics that would be required for comparison without overburdening researchers. The separation of recommended measurements into primary and secondary parameters addresses that tension. Another significant difficulty was encountered in separating media conditions from broader assay conditions; the scope of this effort was limited to characterizing the media in which NMs are tested. Integrated data and cross-study comparison will be enabled by a combination of the harmonized media parameters proposed here, together with standard material characterizations and assays. This was especially challenging for the biological fluids, waste water treatment and product categories, where several iterations were required to tease out the boundaries between medium and assay. Figure 2 shows the split that was agreed for the biological fluids parameters as a representative example.

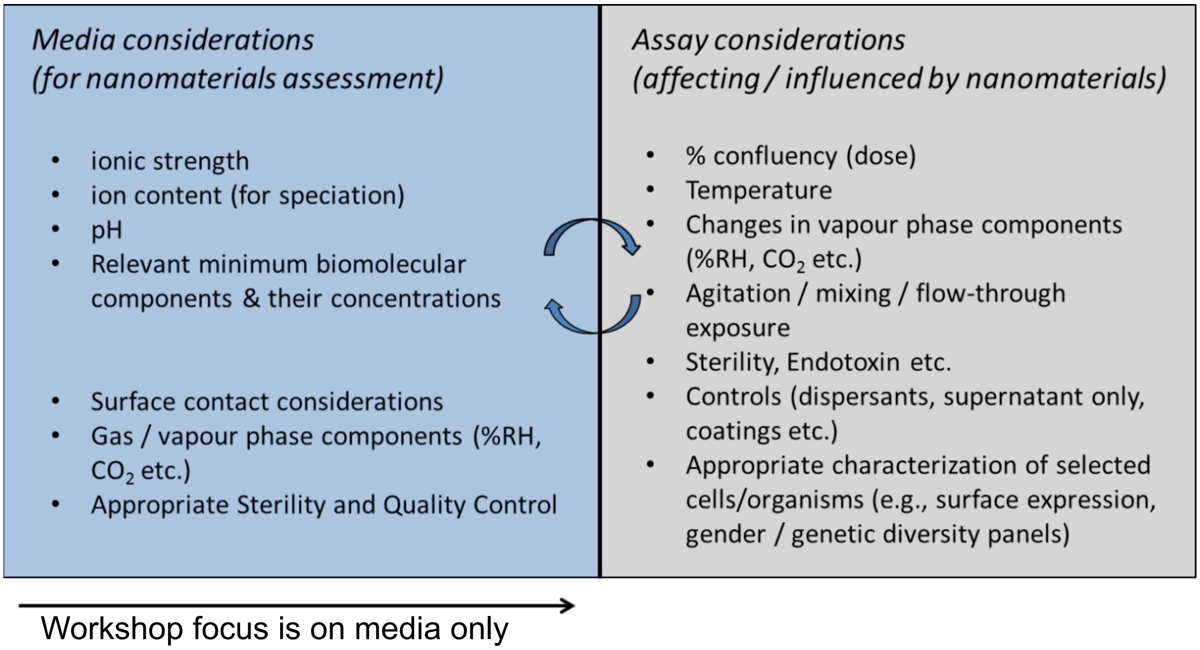

Figure 2: Distinction between media considerations, which are the focus of the current paper, and assay conditions, which while important and not the focus of the current paper. Thus, the key parameters discussed in the previous sections focused on the medium conditions only.

We know the media surrounding NMs are key determinants of the transformations those NMs will undergo, and of the ultimate characteristics of the resulting materials that will be moving through environments and taken up into biota. Because the characteristics and effects of the material are actually a function of the combined system of the material and the media in which it is tested, data characterizing both the material and the media must be reported together to facilitate meaningful analysis. We also know that integrating and comparing multiple datasets is necessary to make progress on understanding behavior and effects of NMs, given the infinite variety of materials and media and the limits of any one individual project. To enable this, we must harmonize data reporting not only on NM characteristics but also of media; and to start toward harmonization, we must select some sample media which are expected to be of particular relevance to guidance and decision making on the part of risk assessors, regulators, and manufacturers. Prior calls have been made for consolidating testing efforts around key functional assays that deliver empirical measurements of how nanomaterials behave in particular systems (e.g. attachment efficiency, dissolution). If such tests are carried out in consistent reference media, the resulting datasets will be comparable, and can propel the nanoEHS community toward both directional guidance for risk purposes, as well as provide a growing mass of meta-data to back out the mechanistic interactions between particle and medium property that governed the FA result.[5]

The hope is that the proposals in this Perspective of primary and secondary parameters to be consistently reported for several key classes of media will be adopted by the broad array of communities engaged in NM testing. In studies where a standard or synthesized medium is relevant, the suggested standardized media should be used wherever possible. The resulting potential for comparison of datasets will be particularly fruitful when data are then entered, as is increasingly the goal, into shared databases (e.g. NanoInformatics Knowledge Commons, eNanoMapper, the developing NanoCommons and EUON).

For several of the more complex media categories here, there are important next steps to be realized in analysis and detection (e.g. characterization of NMs in situ within product matrices), and in agreement of most relevant systems (e.g. insights from life cycle analysis to align product matrices with environmental compartments of their likely release). As these insights emerge, continued harmonization of environmental media for NM testing will be improved. Work in the US-EU nanoEHS Communities of Research to coordinate and harmonize efforts in multiple projects and regions may serve as a platform for continued development and discussion.

SUPPORTING INFORMATION

Supporting information includes additional rationale for recommendations made in this Perspective, additional recommendations for amendments to media, and noted challenges and tradeoffs for guiding benchmark suggestions.

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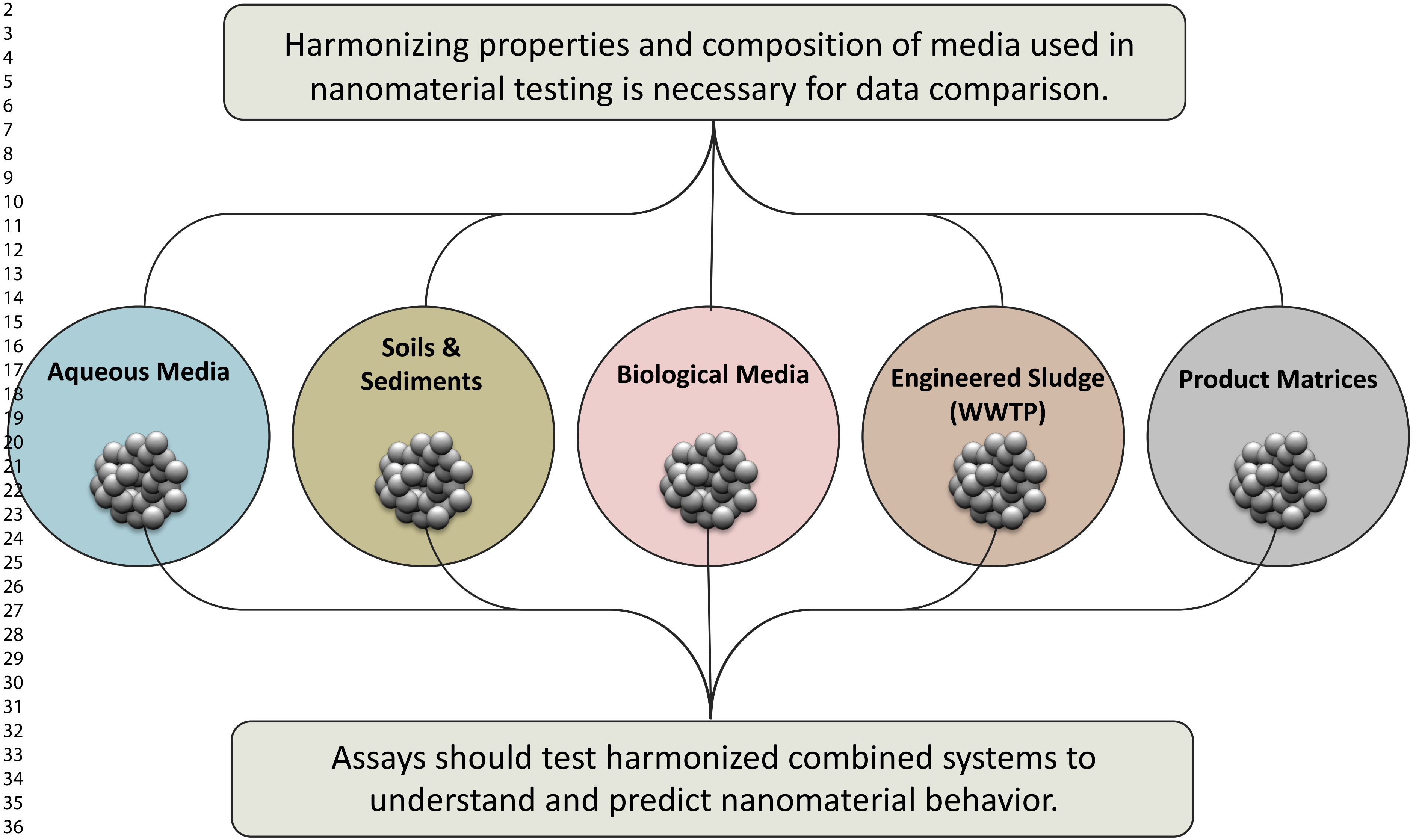

Harmonizing properties and composition of media used in nanomaterial testing is necessary for data comparison.

Assays should test harmonized combined systems to understand and predict nanomaterial behavior.