Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information: Harmonizing Across Environmental Nanomaterial Testing Media for Increased Comparability of Nanomaterial Datasets

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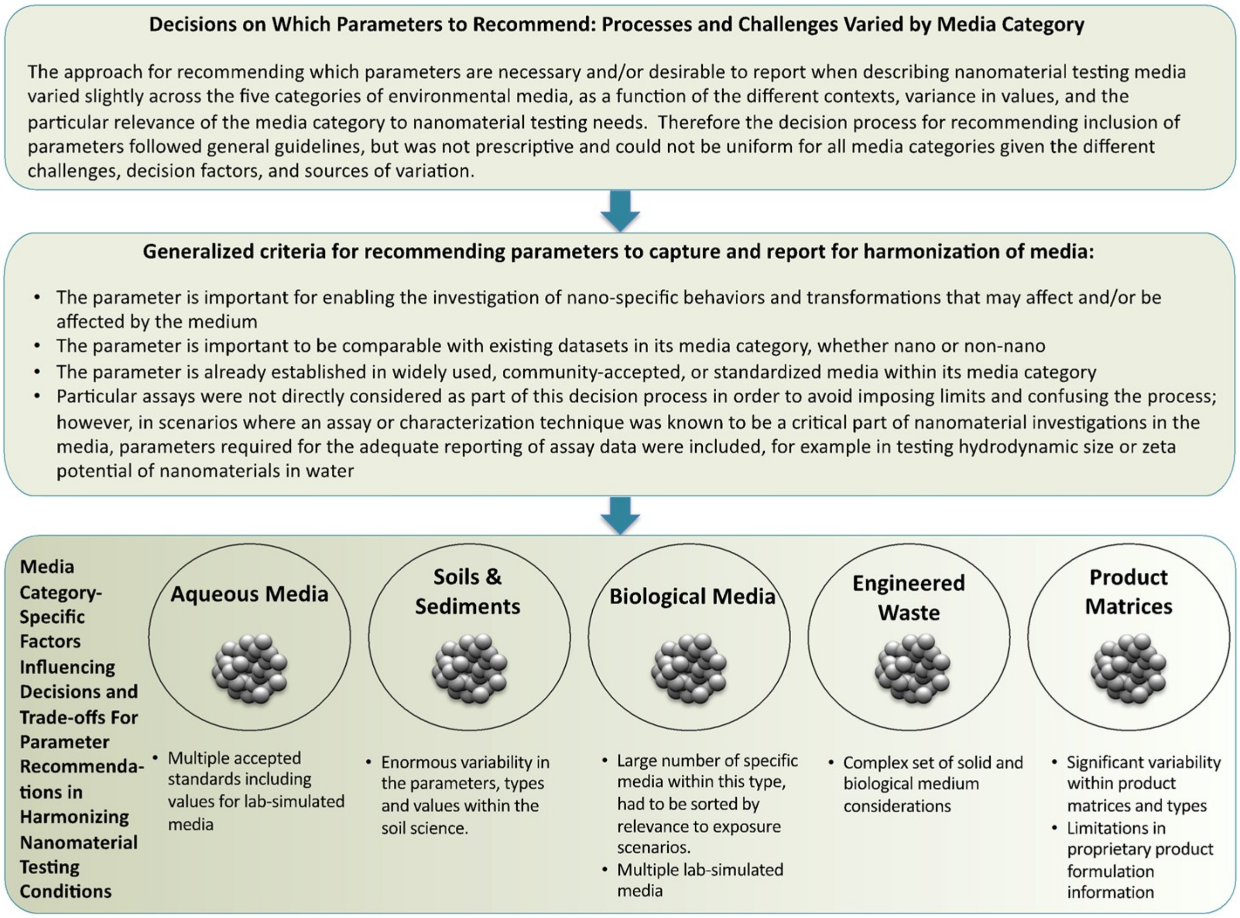

Figure S1: Decision-making process for parameter and medium recommendations

Rationale for not recommending other available standard bioassay test waters.

Some key limitations of the OECD and ISO standard waters limit their broad applicability as a recommended standard freshwater for functional assays of NMs. For the OECD test waters, they are typically specified for individual test organisms to maintain the health of that test organism. It is unclear if the water recommended for algae and *Daphnia magna*, for example, would also be applicable for fish studies. In addition, the common test waters specified in OECD test methods such as M4 and M7 testing contain EDTA and a diverse array of trace elements and vitamins, which could dramatically impact the results for some NMs which may dissolve to varying degrees in the test waters. These media have also rarely been used for environmental fate testing of NMs, making connections between fate processes and toxicity difficult. Many of these limitations also apply to the ISO test media, such as its limited use in studies on the environmental fate of NMs and that it was developed for toxicity testing. Most importantly, this water has a very high calcium concentration (2 mmol/L), so the use of this water is less reflective of the conditions typically found in freshwaters compared to other water media.

Experiment-specific Medium Amendments

Amendments to reference media are proposed for simulation of dissolved organic matter and naturally occuring particles. Naturally occuring organic matter and particles are important components of aquatic ecosystems with, for example, DOC playing an important role in modifying NM surface chemistry, complexing dissolving metals and mediating photocatalytic reactions.(13) Each of the recommended aquatic media described above may be amended with DOC and/or organic particles depending on experimental requirements, although it is critical to carefully describe the DOC composition and concentration.

NOM Amendments

NOM is a ubiquitous and important component of aquatic environments that plays critical roles in the colloidal stability, aggregation, transport, and bioavailability of NMs, as well as NM–contaminant interactions. Thus, NOM is a key component to be included in test systems for aquatic environments. In general, surface waters vary widely in NOM concentrations, ranging from 0.1 to 20 mg L^{-1}.(51, 52) The US EPA moderately hard water matrix that we are recommending for aquatic test systems contains only salts of major cations and anions, and no organic matter or suspended particulates. Therefore, NOM may be added to the test medium to better simulate surface water, marine and estuarine ecosystems in which NOM concentrations are elevated.

However, the types, composition and concentrations of NOM differ substantially among aquatic systems. Furthermore, NOM from different sources may vary significantly in physicochemical characteristics, such as molecular weight, relative contents of aliphatic versus aromatic carbon, the contribution of carboxylate and phenolic functionalities, and the ionization thresholds (i.e.

log K_1). After weighing the advantages and disadvantages, we recommend the addition of "Suwannee River Aquatic NOM" for freshwater and estuarian test systems. This product is concentrated from surface water using reverse osmosis technologies, according to the supplier, the International Humic Substances Society. We further recommend additions that yield a final DOC concentration of 5 mg-C L^{-1}; representing an intermediate concentration within the range of DOC typically found in surface freshwater and estuarine ecosystems.(53) Seawater DOC concentrations should be much lower, at a recommended concentration of 0.05 mg/L. We recommend that NOM not be added to test systems that simulate groundwater and rain water. However, this highly processed product may not be representative of NOM in all natural environments; especially those in which biological productivity is high and contributes to elevated concentrations of complex biological molecules (e.g., polysaccharides, glycoproteins) in solution. We recommend using alginate as an additive for test systems in which the addition of NOM is meant to simulate a highly productive biological system. Alginate is a highly water soluble mixture that is prepared as a sodium salt of alginic acid, which is an anionic polysaccharide isolated from brown algae. It is widely available in large quantities from many commercial suppliers. Because of the potential for variations between suppliers and from batch to batch, we recommend that the alginate be purchased in bulk and characterized fully before being used in aquatic test systems, with complete reporting of the characterisation alongside the experimental test outcomes.

Colloidal Ammendments. The colloids naturally present in aquatic systems consist of an inorganic fraction in addition to the previously discussed NOM.(54) It has also been shown that these background particles can significantly impact the fate of NMs in the aquatic environment, such as by increasing sedimentation.(55) For this reason, it is important to consider this component ubiquitously present in many aquatic systems.

The inorganic colloids naturally present in oxic fresh water with the highest mass abundance are clays, silica and iron oxyhydroxide particles.(54) Although there are many different clay types such as laponite, kaolin and montmorillonite, it is recommended to use a well characterized synthetic clay. This is due to these being (i) widely available (ii) relatively constant quality and (iii) already well characterized. There are no clear standards available for inorganic background particulates. However, Laponite is the recommended clay as it fits the above-mentioned criteria. Extensive descriptions of the characterization of Laponite may be found elsewhere.(56) Silica particles have been used for several applications(8, 57, 58) and many are available for purchase, either synthetic or derived from a natural silica source. It is recommended to use particles smaller than 5 µm and to use solid particulates in order to exclude the extra surface area and reactivity provided by porous particulates.

Background particulates specifically play a role in river and estuarine waters. As clays are the background particulates most often transported to the estuarine systems, the addition of quartz (silica) can be neglected for this system.(54) For river systems both clay and silica particulates are thought to be representative of riverine background particulates. Iron oxyhydroxide particulates were not considered, although they have been shown to have high surface areas in some systems when nano-scale particles are present.(59, 60)

These artificial inorganic background particulates in any one of the aquatic media are not fully representative of a real natural system. A natural system would also include particulate matter related to algae and microorganisms; these are found to be of such relative complexity that suggesting specific measurement requirements would not be helpful to enhancing comparability when they would be added to one of the recommended aquatic media. However, the recommended media and background particulates could be taken as a starting point in studies aimed at investigating interactions of NMs with algae or microorganisms.

Key challenges and trade-offs guiding parameter and benchmark suggestions

Soils, Sediments, and Sludge: The greatest challenges in selecting a reference system for porous media such as soils and subaquatic sediment is the complex and heterogeneous nature of those solids, and their spatial and temporal variability. Finding a reference material that captures the complexity of these systems, but can be standardized, is homogenous, and can be prepared at a reasonable cost for a sufficiently large mass is a great challenge. Clearly, there is no single soil or sediment representative for a broad set of terrestrial systems. Any reference system has to be fit for purpose. For instance, soil toxicity testing requires that the reference soil be within the ecological boundaries required by the test organisms, e.g. in terms of organic carbon content. Similarly a clay-rich soil would not be suitable for transport evaluation in column tests, because particle transport will be virtually unobservable in such soils.(61) However, encompassing a variety of natural systems may increase accuracy of NM assessments, but would induce high costs in regulatory, research and industry communities.

Properly characterized reference systems as described in Table 4 are lacking for most solids that could be considered as a reference media. It is unclear how well the proposed mixture of sand, clay and peat of the commonly selected synthetic OECD reference soil(80) mimics the complex interactions that affect NM fate in natural soils. Organic matter is highly important in the control of NM behavior in soils. The OECD soil uses peat that sorbs NMs strongly compared to other organic material(97) that a reduction of peat quantity has been advised for OECD testing of NMs.(98) Moreover, saturated column experiments cannot be done in the OECD soil because of the high clay content (20 %)(64, 99) and using this reference soil has been shown to lead to variability between toxicity assays.(100)

Activated sludge: Sewage sludge is a biologically active medium and can therefore not be stored over extended periods of time. However, minor differences in activated sludge properties are not expected to have dramatic effects of the behavior of NMs in the activated sludge. Therefore, rather than defining a reference sewage sludge, we recommend that activated sewage sludge from pilot or full-scale treatment plants should be used for experiments. Reporting key parameters of the sludge (Table 8) will then allow comparing the different sludge qualities used in experimental studies.

Treated wastewater: This medium can be influenced by the geological setting. In a setting typically dominated by sedimentary rocks, Ca^{2+} concentrations between 50 and 60 mgL^{-1} are reported in surface waters in Switzerland as well as in wastewater effluents. Surface waters (and treated wastewaters) originating from geological settings dominated by plutonic or metamorphic rocks (e.g. granites and gneisses), show considerably lower Ca^{2+} concentrations on the order of 20 mgL^{-1}. Thus, elevated concentrations of Ca^{2+} caused by the (geological) background may have a more pronounced (and site specific) impact on NM behavior than the increased concentrations of Na^+ and Cl^-, related to the wastewater.

Landfill leachate: Landfill leachates are very variable depending on the type of waste that is deposited. In addition, there is also a temporal component resulting from the degradation of the organic matrix in the waste material, which changes for example pH and dissolved inorganic components. Furthermore, DOC changes its composition and decreases in quantity by more than an order of magnitude over time. The suggested medium compositions therefore only represent one kind of landfill at a certain time of landfill evolution.