Framework to evaluate exposure relevance and data needs for risk assessment of nanomaterials using *in vitro* testing strategies

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Abstract

The Society for Risk Analysis Emerging Nanoscale Materials Specialty Group convened a workshop in September 2014 to examine the use of alternatives to animal testing (or Alternative Testing Strategies, ATS) for nanomaterial (NM) risk assessment. The goal of this workshop was to explore ways to increase confidence in the use of ATS, including *in vitro* methods, for testing NMs and how to incorporate ATS into the risk assessment process in a weight-of-evidence approach. As a part of the workshop proceedings, a multi-stage framework was proposed to optimize the utility of such *in vitro* testing strategies for human health risk assessment of NMs with each stage of analysis considering context-specific information relevant to realistic human exposure situations. The initial stage frames the exposure considerations and scenarios of interest in advance of testing to link aspects such as release points, route of exposure, biological and environmental transformations, dose metrics, and biological targets in subsequent stages. The second and third stages consider relevant characterization and test conditions. The final stage involves evaluating the strength of evidence obtained in the previous stages. This framework is intended to aid risk assessors in evaluating the relevance of data from *in vitro* tests and to optimize the development of new *in vitro* testing strategies based on specific exposure scenarios.

Stage 1: Exposure assessment

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Stage 2: Context-specific NM characterization

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Stage 3: Tailor *in vitro* testing strategy to exposure conditions

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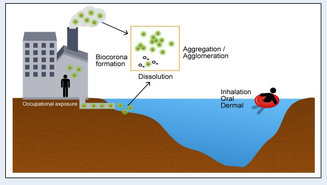
Stage 4: Evaluate strength of evidence for exposure

Proposed framework for assessment of NM risks.
This step-wise paradigm is relevant for risk assessors designing or evaluating *in vitro* toxicity assessments of NMs. The proposed approach provides flexibility to generate information at each stage exclusive of the information from the other stages. However, the data from each stage is important to fine-tune the testing strategies.

Stage 1: Exposure assessment

➢**Determine likely route of exposure and exposure scenarios**

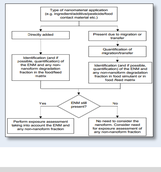


Risk assessment requires evaluation of context-specific NM transformations in a particular exposure scenario. The NM form in one exposure pathway (e.g., inhalation of pristine particles in an occupational setting) may be toxicologically different than the same NM exposed through another pathway (e.g., drinking water while swimming in a lake receiving the same dust)

➢**Exposure monitoring**

A number of techniques are used to monitor NM exposure, and these are mostly developed for an occupational setting (e.g., cascade impactors and particle counters).[1,2]

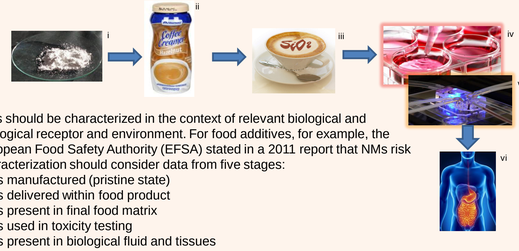
➢**Development of exposure scenarios**



"Prior to commencing the detailed risk assessment of the nanomaterial, anticipated exposure scenarios from the proposed uses should be outlined. These exposure scenarios will contribute to decisions on the extent of the hazard characterization and will provide parameters for the exposure assessment required for the risk assessment."[3]

Stage 2: Context-specific NM characterization

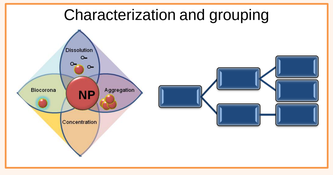
➢**Evaluation of context-specific physical-chemical properties**



NMs should be characterized in the context of relevant biological and ecological receptor and environment. For food additives, for example, the European Food Safety Authority (EFSA) stated in a 2011 report that NMs risk characterization should consider data from five stages:

1. as manufactured (pristine state)
2. as delivered within food product
3. as present in final food matrix
4. as used in toxicity testing
5. as present in biological fluid and tissues

➢**Grouping and read-across**

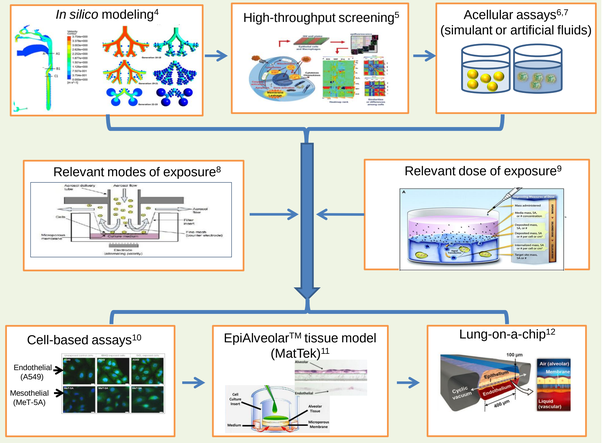


Grouping of NMs can increase the strength of evidence of exposure translation, clarify data needs, reduce the need for testing, and optimize the utility of *in vitro* test results.
Grouping can be based on:

1. p-chem properties such as size, shape, number of walls, and surface chemistry
2. mechanism of action
3. state (bound in matrix, dispersed in liquid, or aerosolized)

Stage 3: Tailor *in vitro* testing strategy to exposure conditions

In silico → HTS → Acellular → Cell-based → 3D tissue/organ systems



Stage 4: Evaluate strength of evidence for exposure

➢**The data generated in stages 1 to 3 of this framework should be used to evaluate the strength of evidence obtained from testing and to determine whether any additional testing is necessary. It can also be used to identify limitations of existing tools and thereby aid in the design of new methods.**

Matrix linking the proposed approach to considerations for assessing NM exposure and risk associated with each stage.

Test	Exposure Source	Dose	Transformations	Biological Response
NM Characterization	How well has the NM or nanoform been characterized in source matrix?	Is the NM introduced to the system in a form representative of the human situation?	What is the significance of possible bio-corona formation, dissolution, and aggregation /agglomeration? How might such transformations affect NM measurements?	How do the identified NM features relate to biological responses of interest? How is response being related to NM dose measurements?
	How is variability in NMs in source matrix evaluated and reported?	How completely are NMs characterized with biologically relevant criteria? What, if any, impacts result from required sample prep processes?	Are indirect indicators, radiolabeling ,or fluorescent tags needed to track NMs? Do they interfere with NM biological activity, detection, or characterization? Can administered NM be effectively differentiated from transformation by-products, conventional scale substances and other NMs endogenous to the biological system?	What parameters of the biological system most influence NM activity and how are they being monitored? What is the significance of possible bio-corona formation, dissolution, and aggregation/agglomeration? How might such transformations affect NM toxicity?
NM Detection	Is analytical equipment compatible with NMs and source matrices of interest?		Is analytical equipment compatible with NMs and biological matrices of interest?	How will NM measurements be correlated with dose-response measures?
		Can NM be detected in key biological matrices?		
		Are method detection limits in range of lowest anticipated dose and biologically important particle sizes and size distributions?		
Dose-Response Correlates	How can dose-response data be linked back to exposure source characterization and vice versa?	What, if any, impacts result from the required sample preparation processes?		How well are findings transferable to other cell types?
	What, if any, assumptions are inherent in the exposure and dose-response assessment?	How well does administered dose compare to delivered dose? How well does the delivered dose represent the realistic exposure concentrations?	How well does delivered dose relate to cellular dose?	Are cells used in the test system the most vulnerable to or representative of NM exposure and its toxic effects? Does the test system simulate mechanical stresses important for physiological response? Does the test system contain microbial and other biological substances that are important mediators of NM toxicity? Does the test system simulate physiology in both diseased and healthy states? What is unique about NM behavior in these states?

Recommendations

➢Determine the exposure potential and identify relevant scenarios, medium, and pathways, if not exposure levels before testing

➢Optimize analytical tools to characterize NMs and assess phys-chem properties throughout the NM lifecycle

➢Correlate phys-chem properties with biological response to identify patterns that will aid in development of read-across strategies that can be used for predicting effects of other NMs that share similar properties

➢Use the proposed tiered framework to develop *in vitro* testing strategies that are driven by anticipated exposure scenarios and appropriate dose metrics

➢Evaluate the strength of available evidence to identify the gaps that exist in the current strategies and also to evaluate the adequacy and quality of collected data for risk assessment

➢Refine the assessment and strength of evidence protocols from binary (yes/no) to scaled as NM detection, characterization, and dose-response correlation methods are improved

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