PERSPECTIVE

Measurement of Nanomaterials in Foods: Integrative Consideration of Challenges and Future Prospects

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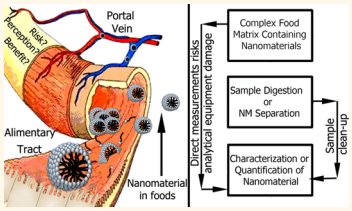
ABSTRACT The risks and benefits of nanomaterials in foods and food contact materials receive conflicting international attention across expert stakeholder groups as well as in news media coverage and published research. Current nanomaterial characterization is complicated by the lack of accepted approaches to measure exposure-relevant occurrences of suspected nanomaterials in food and by broad definitions related to food processing and additive materials. Therefore, to improve understanding of risk and benefit, analytical methods are needed to identify what materials, new or traditional, are "nanorelevant" with respect to biological interaction and/or uptake during alimentary tract transit. Challenges to method development in this arena include heterogeneity in nanomaterial composition and morphology, food matrix complexity, alimentary tract diversity, and analytical method limitations. Clear problem formulation is required to overcome these and other challenges and to improve understanding of biological fate in facilitating the assessment of nanomaterial safety or benefit, including sampling strategies relevant to food production/consumption and alimentary tract transit. In this Perspective, we discuss critical knowledge gaps that must be addressed so that measurement methods can better inform risk management and public policy.



Nanomaterial engineering technologies have the potential to revolutionize industrial food systems, addressing issues related to health and sustainability. Some nanomaterials have unique physicochemical properties that can be exploited for beneficial effects on foods, leading to increased shelf life, enhanced flavor release, and increased absorption of nutrients and other bioactive components. As food products using new nanotechnologies reach commercialization, there is a need to anticipate, to understand, and to manage both potentially positive and negative effects that might result from nanomaterial consumption.[1,2] Along with increasing disagreement about what constitutes true nanomaterial exposure, there is a rising perception that studies supporting risk management have lagged far behind the advancing technology. Indeed, hazard-based studies far outpace exposure studies for nanomaterials.[3] Evidence suggests that this growing knowledge gap is leading to a public perception that there are more risks associated with nanomaterials than benefits.[2,4]

The ability to detect and to measure a given nanomaterial at key time points in the food lifecycle is critical for estimating the nanoscale properties of interest that dictate manufacturing consistency and safety,[5−7] as well as understanding potential beneficial or adverse effects from food intercalation. For food safety, these time points do not simply include when the food is processed or resides on a store or home shelf; rather, these time points also comprise all succeeding scenarios as the food is prepared and transits through the alimentary tract after ingestion. Meeting this basic requirement is extraordinarily challenging

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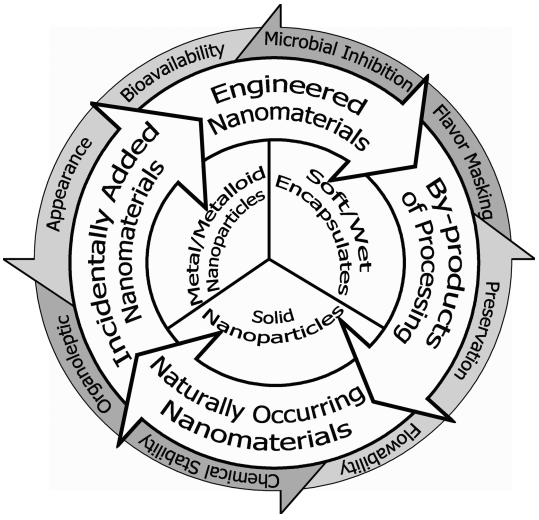

Figure 1. Three concentric movable dials demonstrate the permeations and combinations relating the types of nanomaterials to the end applications. The inner core represents the three main classifications of nanoparticles added to/existing in foods. Regardless of which is the starting point, the next wheel rotates to show that they can all be incidentally added nanomaterials, engineered, and so forth. The outer wheel rotates, showing the final application of the different classes of nanoparticles.

due to the complexity of food matrices, thermodynamic instability of nanoparticles, and diversity of the alimentary tract and is further complicated by the uncertainty of what specifically should be measured to assess potential for *in situ* biological interactions of interest and where.

This Perspective aims to describe the state of the science for nanoscale measurement methods development as applied to foods and the alimentary tract and, more importantly, to identify the critical methods' knowledge gaps that must be addressed to inform appropriate risk management and public policy. This Perspective draws from the combined work of experts participating in the NanoRelease Food Additive (NRFA) project, an international multi-stakeholder effort that aims to address method development needs for nanoscale materials currently used in commerce.[8]

Why Is This Problem So Difficult? There are several factors that complicate the development of methods to detect and to measure nanomaterials in foods and food contact materials. First, whether naturally present or intentionally added to foods, the potential applications and impacts of nanomaterials within these matrices are diverse (Figure 1). The general range of elements used in foods where appropriate nanoscale measurement approaches are needed to clarify debate on nanomaterial safety include silicon, silver, titanium, zinc, calcium, and iron, as well as combinations of elements found in clays and multiple forms of organic carbon, such as lipids, proteins, and polysaccharides. These nanomaterials may be present in different geometries and range from "harder" metals and metal oxides to "softer" liposomes. Solid nanoparticles, for example, can affect appearance, bioavailability, microbial inhibition, flavor masking, preservation, flowability, chemical stability, and organoleptic characteristics in foods, regardless of whether they are of natural or manufactured origin. Simply considering differences in size, origin, composition, chemistry, and uses of these various nanomaterials means that a variety of analytical approaches may need to be considered for different nanomaterials and the necessary decision outcomes.[9] Furthermore, it is extremely important to recognize and to differentiate nanoscale materials that are naturally present in the food supply. For example, in simple dairy systems, a plethora of associated colloids, biopolymeric nanoparticles, and nanoemulsions exist and have existed prior to the advent of industrial processing.[10] Traditional manufacturing processes of grinding and spray-drying can also produce nanoscale variants of natural materials that would otherwise not exist in such a size range. Distinguishing such "legacy" nanoscale materials from newly introduced nanoscale materials and from background levels of nanoscale materials is a challenge to both method development and to problem formulation for risk assessment and risk management.

A further characterization challenge includes the consideration of physicochemical changes as a nanoscale material moves from formulation and preparation through incorporation into foods and finally through consumption and absorption. These complexities that affect the biological interactions of the nanomaterial could include the effective nanomaterial size (including agglomeration and aggregation), solubility/dispersibility, chemical form, chemical reactivity, surface chemistry, shape, and porosity. For example, dissolution, aggregation, or

agglomeration, whether occurring before or after ingestion of a food, may effectively *remove* the nanomaterial by converting it to a conventional particle or solute. By contrast, degradation or disaggregation through digestion in the alimentary tract could increase nanoscale material bioaccessibility relative to that measured prior to ingestion. Thus, it is important to consider changes in nanoscale material properties, to the extent possible, as well as changes to the detection and characterization requirements at time points relevant to understanding nanoscale characteristics during ingestion and alimentary tract transit. Nanoscale materials in relatively pure initial form at the time of manufacture tend to have reproducible characteristics and physicochemical properties, including particle number size distributions, particle shape, and surface properties, that are relatively straightforward to measure. Difficulty in analysis and our relative inability to predict biologically relevant characteristics increases as pristine nanomaterials undergo physical and/or chemical changes during food processing, packaging, aging, and during alimentary tract transit (Figure 2). Incorporation of engineered and naturally occurring nanoparticles into foods can influence not only their agglomeration but also their reactivity in food matrices, thus requiring separation/pretreatment prior to characterization.[11] Numerous preanalysis steps, including degradation of the food matrix and separation of the compounds of interest from background nanomaterials, are required to isolate the nanomaterial for characterization (Figure 3). During these steps, it is critical that sample preparation be performed in ways that allow us to predict "released" nanomaterial characteristics relevant to biological interaction. A demonstration that nanomaterials can be extracted is not necessarily biologically relevant depending upon the extraction conditions. Likewise, the characterization of extracted nanomaterials is not necessarily blind to chemical changes induced by the separation from the food matrix.

The inherent complexity of the mammalian alimentary tract creates additional complications for results-driven nanomaterial characterization. The lumen of the alimentary tract cannot be viewed as a single compartment or step in the lifecycle of a nanomaterial in food because it is dynamic, changing in ways that are potentially important to the nanomaterial properties and driving potential biological interactions of nanomaterials as they pass through complex biological environments (Figure 4). The pH, ionic strength, composition, and absorptive surfaces of the alimentary tract vary considerably, and the composition of the food matrix changes during digestion. In addition, the microflora with which nanoparticles interact may change dramatically in terms of both species and numbers. It has been shown that silver nanoparticles exposed to stomach fluid undergo changes in size, shape, and composition, and the rates of these changes are dependent on particle size.[12] If size, including the influence of agglomeration/aggregation, can change with environmental conditions, then real-time monitoring of engineered nanomaterial (ENM) behavior within biological environments will be difficult. Therefore, the analysis must always be designed to answer a specific question, instead of allowing the analysis parameters to dictate the questions being asked. The best places within the alimentary tract to measure and to characterize nanomaterials in food are likely to be different for different nanomaterials and may

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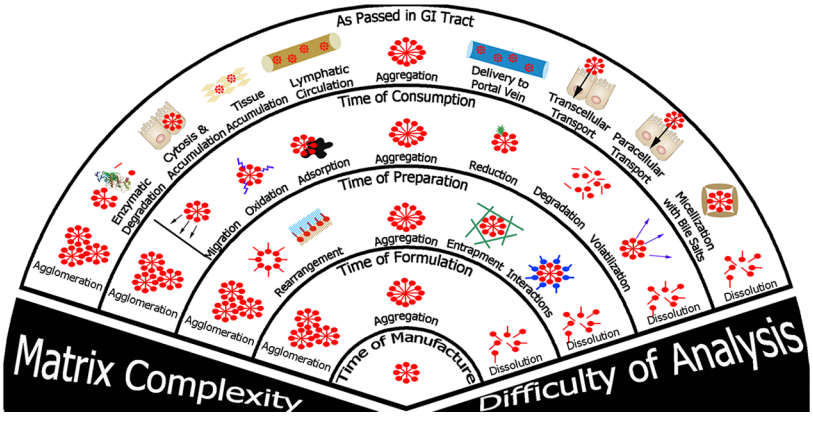

Figure 2. Potential modes of destabilization through the lifecycle of a nanomaterial from the time of manufacture to potential biological interactions in the alimentary tract and the complexity/difficulty of sample quantification and detection. GI, gastrointestinal.

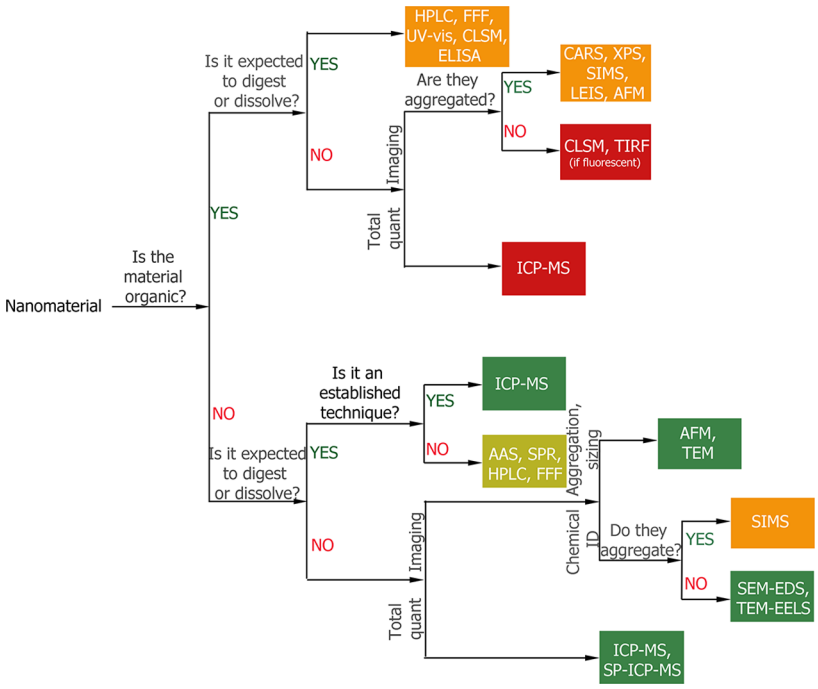

Figure 3. Example decision tree for the selection of analysis methods for nanomaterials in foods depending on the type and state of the material. AAS, atomic absorption spectroscopy; AFM, atomic force microscopy; CARS, coherent anti-Stokes Raman scattering; CLSM, confocal laser scanning microscopy; ELISA, enzyme-linked immunosorbent assay; FFF, field flow fractionation; HPLC, high-performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectrometry; LEIS, low-energy ion scattering; SEM-EDS, scanning electron microscopy with energy-dispersive spectroscopy; SIMS, secondary ion mass spectrometry; SP-ICP-MIS, single-particle inductively coupled plasma mass spectrometry; SPR, surface plasmon resonance; TEM, transmission electron microscopy; TEM-EELS, transmission electron microscopy coupled with electron energy loss spectroscopy; TIRF, total internal reflection fluorescence; UV–vis, ultraviolet–visible; XPS, X-ray photoelectron spectroscopy.

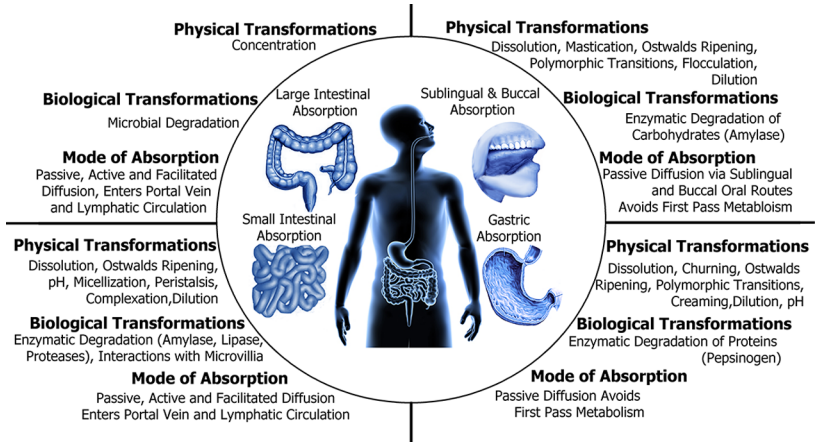

Figure 4. Endogenous modifications to nanomaterials within the alimentary tract during transport from time of consumption to excretion.

depend upon their use, composition, chemistry, behavior (*e.g.*, agglomeration), and the specific risk or benefit question being targeted.

What Approaches Are Currently Available? Due to the complexity and wide chemical and physical disparity of nanomaterials at different points in time from processing to ingestion, it is likely that no single method will suffice to characterize the potential benefits or risks that these materials may present to the consumer. A combination of methods aimed at assessing specific questions will likely be needed to ascertain the best analytical results (Figure 3). Detection methods differ based on the specific questions being addressed, such as the following:[13–16] (1) Are nanoscale materials present, or have they dispersed into their molecular components and therefore ceased to be nanomaterials? (2) Do the nanoscale materials have a consistent size and shape, or have they become chemically altered due to biological interaction such as aggregation/disaggregation? (3) Has there been

chemical transformation of some surface or core component (*i.e.*, oxidation or reduction), or is the concentration of some component changing? (4) Is it necessary to ascertain the *amount* of nanomaterial present and *where exactly* the nanomaterial is located, or is it appropriate to ascertain only if it is present?

Inorganic nanomaterials, such as silver, gold, and silica nanoparticles, have the most established detection techniques. If the primary question is "are *inorganic* nanoparticles merely present or not?", then numerous techniques such as flame atomic absorption spectroscopy,[17] surface plasmon resonance,[18] and inductively coupled plasma technology (ICP) coupled with either mass spectrometry (MS), atomic emission spectroscopy (AES), or optical emission spectroscopy (OES) may be used.[19] Alternatively, if the question of interest is "have these nanomaterials changed shape or do they have modified porosity?", then electron microscopy (EM) is advantageous. Finally, if the question is "does the core material concentration or surface chemistry become modified or changed?", then other more specific techniques are required.[13−16]

Surveys of available measurement methods consistently reveal that there are numerous techniques available to find, to quantify, and to measure the properties of inorganic ENMs. The same cannot be said for organic ENMs composed of polymers, lipids, proteins, and polysaccharides, which are more complicated to analyze than elemental nanomaterials.[13−16] Once nanomaterials are extracted from the food matrix or intestinal cells, some of the aforementioned techniques may be utilized to determine the presence of organic nanomaterials. Rapid screening techniques such as enzyme-linked immunosorbent assay (ELISA) kits for antibody-based detection and flow cytometry may also be useful.[20] Analysis of mixed nanomaterials, such as those with inorganic cores and organic coatings, requires a set of complementary techniques, often including EM combined with sample surface chemistry-based methods such as X-ray photoelectron spectroscopy (XPS), secondary ion mass spectrometry (SIMS), low-energy ion scattering (LEIS), atomic force microscopy (AFM), scanning probe microscopy (SPM), or scanning tunneling microscopy (STM).[13−16,21,22] For many companies, these forms of advanced instrumentation are often too costly and time-consuming compared with other characterization techniques such as high-performance liquid chromatography (HPLC) and dynamic light scattering (DLS).[13−16]

Well-validated imaging methods for characterizing inorganic- or organic-based nanomaterials in foods are not currently widespread, mostly due to the challenges of attaining informative data from complex matrices. For example, both EM and chemical imaging techniques provide successful nanomaterial image data when the samples have large changes in contrast (optical and/or chemical) between the nanomaterial and the surrounding matrix, creating a challenge for locating materials such as carbon nanotubes within carbon-rich cells and tissue. In addition, the labile nature of nanomaterials creates a complication in that sample preparation methods can result in image data that cannot distinguish between concepts such as ENM migration to one location *versus* ENM agglomeration. A potential solution to some of these troubling issues often includes labeling organic ENMs *via* fluorescent tags or radiolabels, but it is unclear whether such modifications to organic nanomaterials will sufficiently change their chemical or physical characteristics to render them poor models of their unlabeled versions. For the foreseeable future, reliable methods for imaging nanomaterials in food matrices and alimentary tract cells/tissue will likely be less developed than those based on presence detection.

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In general, targeted generation of new analytical approaches for organic nanomaterial sampling, detection, and quantification, as well as imaging of both inorganic and organic nanomaterials, are needed to assess the risk of nanomaterials in foods, food contact materials, and the alimentary tract. In addition, methods that depend on tagging organic ENMs will need sufficient investigation to ensure that such procedures do not substantively alter the behavioral characteristics of the nanomaterials within living systems.

WHAT ARE THE PROSPECTS FOR THE FUTURE?

Although certain detection techniques and methods may become standardized to address some nanoscale material exposure decisions, several critical knowledge gaps need to be addressed to ensure that product developers, regulators, and consumers understand *and agree to* their use to facilitate safety evaluation of any particular nanoscale material within our food supply. Ideally, detection and characterization methods for nanomaterials in foods and the alimentary tract would accomplish the following: (1) *directly* observe nanomaterials or confirm their absence in the "as eaten" food or in various compartments of the alimentary tract; (2) distinguish

exogenous nanomaterials of interest from others such as those created endogenously; and (3) not interfere with the ability to comprehend the form of the nanomaterial as experienced biologically (Figure 5). Although many analytical methods exist, no single method is capable of fully characterizing a nanomaterial in every situation; thus, it is necessary to develop sample collection/handling protocols and new methods or combinations of existing analytical methods in order to obtain conclusive results (Figures 3 and 5). This is especially true if characterization of the chemical composition and distinct locations of nanomaterials within the entire food process lifecycle is desired.

Whether the focus is on detecting and characterizing ENMs migrating from food packaging, ENMs intentionally added to foods, or ENMs in various stages of interaction with biology, data are also needed on the *composition*, *form*, and *quantity* of a nanomaterial in its pristine form and within simple matrices before being added to the packaging or food. Efforts focused first on pristine particle analysis will help us determine limits of detection, create standardized measurement protocols and data reporting metrics, and provide a better understanding of how the properties of ENMs change over time in simple media. This is a particularly targeted area for future studies that explore the application of emerging detection methods, and even well-established detection methods, to a variety of ENMs in their pristine states. The lack of these types of data will severely limit the ability to link toxicological, transformational, migrational, and exposure-related studies, as well as critical correlations between the properties of the initial starting material and the ultimate effects of the ENM in the systems being studied. The lack of such correlations between effective dose, transformation, and exposure will severely slow the construction of predictive frameworks for ENM behavior in complex systems.

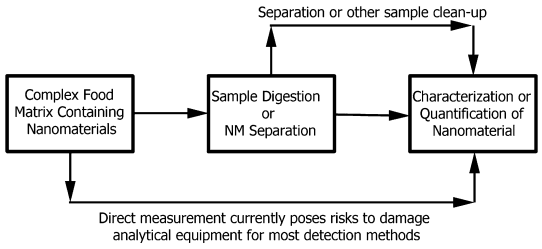

Figure 5. Method development strategy to detect or quantify nanomaterials in complex food and biological matrices. NM, nanomaterial.

Before the properties of a food or tissue sample can be measured, the sample must be prepared in a manner that is compatible with the analytical instrument of choice. Unfortunately, virtually every analytical method currently used for ENM characterization requires that ENMs be extracted from their native environment, or that the environment be digested, destroyed, or critically altered so that the ENM is in a state that can be measured. This introduces two issues that can compromise the value of analytical results. First, although some sample preparation methods, such as alkaline digestion for some nanoscale particles and matrices, are widely thought to be comparable across laboratories, sample preparation methods are generally not standardized, which means that comparing analytical results from one laboratory to another should be done with caution. Second, little is known about how the sample preparation technique affects acquired data on ENM characteristics; therefore, it is difficult to know whether samples that have been prepared in a certain way offer data that are a realistic representation of ENMs in their native environments. This Perspective highlights a need for a better understanding of how sampling affects detection outputs and identifies a need for analytical methods that require less destructive or transformative sampling techniques.

Assuming that the sample preparation is standardized, one fact that seems to be conserved over a majority of the research studies considered by NRFA participants is that ICP-MS in conjunction with transmission electron microscopy (TEM) are often viewed by the scientific community as being the minimum (and often sufficient) tools needed for adequate detection and characterization needs. ICP-MS provides a compositional analysis, and TEM fulfills the need for information on ENM *form* and *location*. Even this combination of powerful techniques, however, results in information gaps. While often employed as the sole detection method, ICP-MS provides no information about ENM form. Like all microscopy techniques, TEM is vulnerable to nonrepresentative sampling and the lack of utility at low analyte concentrations. These observations point toward an urgent need for compositional analysis techniques that can provide information on both ENM presence *and* form, in addition to alleviating some of the complexities and shortcomings of traditional TEM sample preparation approaches. Methods development targeted toward the elucidation of synergisms between existing or emerging detection techniques that minimize sample preparation, keep costs low, and do not require significant expertise will likely help to maximize efficiency and ensure that research studies produce meaningful and comprehensive data. As often exhibited in the face of analytical challenges, expressed need becomes the promise of opportunity. Many detection methods are close to being useful for routine analysis of nanoscale materials in foods[13−16] but need additional development to standardize protocols, develop realistic detection limits, and

establish matrix compatibilities, among other issues. Furthermore, the tremendous variety of forms, analysis modes, and contexts for measurement suggests a continual need to anticipate and produce standard reference materials in different food matrices for both inorganic and organic nanomaterials. It is perhaps too grandiose to expect that in the near future we will be able to detect and to characterize each type of nanoscale material at all time points within a food-based lifecycle. However, if method development continues to be aimed at specific questions regarding "presence", "how much", and "where/when", then sustainable product development and safety decisions for more of those lifecycle points will become populated with well-informed answers.

This Perspective highlights a need for a better understanding of how sampling affects detection outputs and identifies a need for analytical methods that require less destructive or transformative sampling techniques.

Although nothing can truly replace a study conducted in a real organism, a real food, or a real packaging material, real systems such as these feature complexities and uncertainties that can obscure meaningful structure–function relationships. NanoRelease Food Additive project participants identified valuable model systems that can be more conducive to systematic evaluations of how ENM characteristics determine their behavior in more complex environments.[23,24] Such model systems include theoretical or mathematical models of ENM migration into foods from packaging or *in vitro* models that can mimic the conditions of an intestinal organ without the need to handle and sacrifice live animals. Unfortunately, in many of these cases, the model systems require validation in order to confirm that they are good predictive models of true ENM behavior. The continued allocation of resources toward the development and validation of model systems that can predict the transformative behavior of ENMs in complex biological systems can help inform the measurement development of a wide range of analytical methods.

To achieve informed risk management and meaningful public dialogue on ENMs in food, there is a need for widely accepted methods and data to assess their presence in foods, physical fate once ingested, physiological behavior, and uptake into the body. In this respect, the work of NRFA project experts provides an important foundation.[8,9,12–16,23–25] However, information gathered through their efforts must be fitted within a bigger picture analysis of ENM applications across disciplines and stakeholders.[26–32] Joint interdisciplinary analysis must be further incentivized in order to allow active sharing of knowledge among chemists, physicists, toxicologists, food technologists, instrument vendors, and other important stakeholders. This collaboration is essential for ensuring that nanoscale material development and use reflects key societal, industrial, and ecological needs. Moving beyond the isolation of critical information within individual knowledge domains and stakeholder groups will leverage nanotechnology and the nanoscale from an area of perceived uncertainty and debate into a rich field of potential.

Conflict of Interest: The authors declare no competing financial interest.

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