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Review

Prioritizing research needs for analytical techniques suited for engineered nanomaterials in food

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A B S T R A C T

Background: The ability to detect, characterize, and quantify engineered nanomaterials (ENMs) in foods is needed to not only understand consumer exposures to ENMs but also to comply with recent changes to European food labeling laws relevant for ENMs. Significant challenges exist, however, in terms of relying on currently-available analytical tools and techniques suited for ENMs in food, as most of these are still in development.

Scope and approach: Based on a literature review, this analysis highlights the current state of knowledge in the area of analytical tools relevant for ENMs in food and then proposes strategies to prioritize near-term research and decision support efforts based on selected high-priority ENM-food applications.

Key findings and conclusions: After reviewing available analytical tools and techniques as well as challenges to using these, we identify and select six ENM-food applications as being of high-priority based on their current use as well as their potential to cause adverse health impacts in vulnerable life stages. Based on these findings, we recommend "fast tracking" the development of analytical techniques suited for high priority ENM-foods as one strategy to prioritize near term research efforts. We also recommend implementing screening-level approaches to first detect ENMs in food followed by characterization techniques to provide minimal ENM characterization information, including elemental composition, size, and size distribution. These strategies may help focus research efforts and decision support priorities in the near-term while the full suite of analytical tools are developed specifically for ENMs in food.

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1. Introduction

The ability to detect, characterize, and quantify engineered nanomaterials (ENMs) in foods is needed to not only better understand consumer exposures to ENMs, but also to comply with recent changes to European food labeling laws that now require foods with ENMs to be labeled (i.e., European Commission Regulation No. 1169/2011) (European Parliament and Council, 2011; European Commission, 2014a, 2014b, 2014c). There are, however, significant challenges to using and relying on current analytical techniques, as most tools and methods relevant for ENMs in food are still in development (Aschberger et al., 2014; Contado, 2015; Linsinger et al., 2013). While there are several tools robust enough to detect, characterize, and quantify ENMs in their raw or pristine states, the application of these techniques to ENMs in more complex media such as food are not yet fully developed, especially if they are used to support legally-mandated food labeling requirements. To date, there is no single analytical method suitable for ENMs in all types of food matrices, and therefore a combination of approaches will likely be needed (Aschberger et al., 2014; Singh, Stephan, Westerhoff, Carlander, & Duncan, 2014).

This review highlights the current state of knowledge in the area of analytical tools relevant for ENMs in food and then proposes strategies to prioritize near-term research and decision support efforts based on selected high-priority ENM-food applications. These were defined as ENM-food applications that are most commonly reported in the literature and may potentially impact vulnerable life stages, such as pregnancy, infancy, and early development, given available toxicological data (e.g., Mohammadipour et al., 2014; Rollerova et al., 2015; Snyder et al., 2015; Yamashita, et al., 2011; Yin et al., 2015; Zhang, Gurunathan, & Kim, 2015) as well as information on current use and consumption patterns (Siega-Riz et al., 2010; USDA, 2014). While this analysis focuses on vulnerable life stages, subsequent analyses could extend this methodology to include different population subtypes, e.g., immunocompromised individuals, alternative demographic groups, or even specific types or groups of ENM-food

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applications (see e.g. Cockburn et al., 2012). Given the evolving state of scientific knowledge regarding ENM safety and the need for near-term decision support (Grieger, Baun, & Owen, 2010; Linkov, Anklam, Collier, DiMase, & Renn, 2014), this paper presents one approach to identify potentially high priority ENM-food applications based on available data and suitable analytical techniques that may be used in subsequent safety evaluations. The objectives of this paper are therefore to: i) provide an overview of suitable analytical tools for ENMs in foods, ii) identify a subset of high priority ENM-food applications in terms of their potential to impact more vulnerable life stages, iii) identify analytical tools that may be used for these high priority ENM-food applications, and iv) recommend strategies to prioritize research in the field of analytical techniques relevant for ENM-food safety.

1.1. Methodology

This paper is based primarily on a comprehensive literature review of analytical tools to detect, characterize, and quantify ENMs in food. This involved a literature search of publically-available databases such as Pubmed, JSTOR, Scopus, and Google Scholar for research papers and reports focused on ENMs in food. The following search terms were used in these search engines to identify publically available peer-reviewed scientific articles and reports: analytical tools, characterization, detection, engineered nanomaterials, food, measurement, techniques, and tools. In addition, the authors' knowledge in fields of ENM risk assessment, decision-support, ENM regulation, nanotoxicology, detection, and analytical tools and techniques was also employed in this work.

2. Results

2.1. Overview of current analytical challenges

Analytical methods are needed to determine whether ENMs are present, the physico-chemical properties of the ENMs (e.g. size, size distribution, morphology, composition, aggregation/disaggregation state), and the concentration or quantities present in a given food sample (Linsinger et al., 2013; Szakal et al., 2014). Unfortunately, there are currently a number of challenges related to using analytical methods for ENMs in foods. Some of these include: i) separating and analyzing ENMs in (complex) food matrices, given the interactions between ENMs and a variety of substances in food that can alter ENM physico-chemical parameters (Aschberger et al., 2014; Bouwmeester, Brandhoff, Marvin, Weigel, & Peters, 2014), ii) distinguishing ENMs from natural or unintentionally-formed nanoscale materials (Aschberger et al., 2014), iii) detecting organic ENMs (e.g. ENMs including carbon based, polymers, lipids, proteins, polysaccharides), as most analytical tools are better equipped to analyze inorganic ENMs (Bouwmeester et al., 2014), and iv) developing tools and approaches that can be routinely applied, especially for regulatory purposes (Aschberger et al., 2014), such as reference materials and standardized procedures (EFSA., 2013) (Fig. 1).

To detect and analyze ENMs in foods, it is often necessary to separate or extract the ENM from the food matrix. This may involve processes such as dehydration of the sample for imaging or extraction into solution for liquid-based analytical techniques. Extraction processes present some challenges due to potential alterations of the original state of the ENM in food as well as interactions between the ENMs and other substances in food (Aschberger et al., 2014; Bouwmeester et al., 2014). Due to these interactions between ENMs and other materials in the food matrices, results obtained after analyzing ENMs in food may not necessarily reflect the conditions present in the original food matrix (Bouwmeester et al., 2014). This challenge may be particularly important if information is required regarding ENM-food conditions at the point of consumption. To analyze samples that are most representative of the ENM-food matrix to be consumed, it has therefore been recommended to use minimum sample preparation (Bouwmeester et al., 2014). Discussions have also been raised whether analytical methods should be used during the early processing stages (e.g. ingredients) or during later processing stages of food (e.g. finished product). While it's important to analyze foods at the point of consumption, interactions between ENMs and other materials in foods may create additional difficulties to analyze results based on commonly used detection methods (Graf, Behsnilian, Hetzer, Walz, & Greiner, 2014).

Distinguishing ENMs from other organic materials in the food (e.g., naturally-occurring lipids, proteins, micelles, polysaccharides) is also another obstacle to overcome in the analysis of ENMs (Aschberger et al., 2014; Singh et al., 2014; Stamm, Gibson, & Anklam, 2012). Detecting organic ENMs in food is also challenging, especially compared to detection abilities of inorganic ENMs which are better developed. While a few techniques may be suitable for organic ENMs, such as electron microscopy (EM), imaging techniques for organic ENMs are currently limited due to their low electron density which is similar to the food matrix (Bouwmeester et al., 2014). Separation techniques (e.g. asymmetric field-flow fractionation (FFF)) also have to account for the micelle-like structure of organic ENMs, which should be treated carefully (Bouwmeester et al., 2014). For these reasons, only a few studies have reported efficient methods to extract or separate organic ENMs from foods or to analyze them in their original food matrices (e.g. Singh et al., 2014; Sk, Jaiswal, Paul, Ghosh, & Chattopadhyay, 2012).

Another significant issue is the lack of robust, validated analytical methods or suitable reference standards to detect, identify, and quantify ENMs in foods (Aschberger et al., 2014; Blasco & Picó, 2011; Contado, 2015; EFSA., 2013; Linsinger et al., 2013; Stamm et al., 2012), as well as the lack of comparability across lab analyses (Stamm et al., 2012). Validated, standardized analytical test methods are needed to ensure harmonization and mutual acceptance of data across tests and testing facilities (Bouwmeester et al., 2014), while reference materials are needed to provide a consistent test material in order to make comparisons more direct. While there are a number of methods currently in development, none of these have been documented to be completely fit for purpose or validated according to harmonized and internationally-agreed upon standards (Stamm et al., 2012).

To date, there are only a few readily available or certified reference materials for ENMs in food (Grombe et al., 2014; Roebben et al., 2013), such as silica nanoparticles in tomato soup, silver nanoparticles in chicken meat paste (Grombe et al., 2014), and pure SiO_2 nanoparticles (Stamm et al., 2012). One challenge to developing reference materials is the stability of solutions, as ENMs tend to quickly aggregate in solution, providing barriers to producing high quality ENM solution standards (Gschwind, Aja Montes, & Günther, 2015). As ENM-food combinations may present unique conditions, the development of validated analytical methods and reference materials is hampered by the diversity of ENMs and applications in which they may be used (Heroult, Nischwitz, Bartczak, & Goenaga-Infante, 2014; Szakal et al., 2014). There is also a need to develop proficiency testing programs that can provide accreditation, certification, and verification of the analytical proficiency of laboratories. To date, only one study has been reported to demonstrate the proficiency of laboratories for the analysis of ENMs, which used silica nanoparticle samples in an aqueous solution (Lamberty et al., 2011).

There are also challenges related to properly preparing samples

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