Nanotechnology in agriculture: Next steps for understanding engineered nanoparticle exposure and risk

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ABSTRACT

The potential uses and benefits of nanotechnology in agriculture are significant, including producing greater quantities of food with lower cost, energy, and waste. However, many questions regarding the risk of these approaches in food production remain unanswered. A robust literature assessing the toxicity of engineered nanomaterials to terrestrial agricultural plant species has begun to develop. However, much of this literature has focused on short term, high dose exposure scenarios often conducted in model media. Although important for determining inherent nanomaterial hazard, these studies are inadequate for assessing the actual risk posed to agricultural systems, including for sensitive receptors such as humans. Although the existing literature is somewhat contradictory, it is notable that the overall findings seem to suggest low to moderate toxicity to terrestrial plant species. However, what is now needed is a systems-level approach investigating more subtle yet potentially more significant impacts of nanomaterial exposure in agricultural systems, including the use of a range of more sensitive endpoints that can mechanistically characterize toxicity. This article will identify these and other key knowledge gaps and also highlight critical next steps for understanding the balance between nanotechnology applications and implications in agriculture and food production.

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1. Introduction

The use of nanotechnology in agriculture has created a great interest, offering the potential for significantly enhanced agricultural productivity and efficiency with lower cost and less waste (Scott and Chen, 2013; Kah, 2015). Importantly, the emergence of these applications in agriculture and other sectors has also raised safety concerns over environmental and human health; the resulting field of nanotoxicology has developed in an effort to answer critical questions of hazard, exposure and ultimate risk.

Since 2000, over 10,000 articles have been published that investigate the environmental health and safety of engineered nanoparticles (ENPs) (nanoEHS), with more than 50% of those studies occurring in the last three years (Krug, 2014). Early (2006–2010) efforts at the Organization for Economic Cooperation and Development (OECD) focused on a priority list of ENP, which included fullerenes (C60), SWCNTs, MWCNTs, silver, iron, titanium dioxide, aluminum oxide, cerium oxide, zinc oxide, silicon dioxide, dendrimers, nanoclays and gold nanoparticles. The desire was to evaluate the intrinsic characteristics of each material, with OECD testing strategies and evaluation based on “physical–chemical properties, environmental degradation and accumulation, environmental toxicology and mammalian toxicology.” It is worth noting that only a limited number of these studies were focused on terrestrial plant species. For example, of the 10,000 papers published since 2000 on nanoEHS, less than a third addressed plant species. However, more recently a number of reviews on plant-NM interactions have been published (Rico et al., 2011; Miralles et al., 2012; Gardea-Torresdey et al., 2014; Yin et al., 2012; Ma et al., 2015). What is clear is that the majority of plant-ENP investigations have focused on high dose, short exposure scenarios, often have been conducted in simplified or model media. Although these types of investigations are a necessary first step when beginning to evaluate the hazard of a potential class of emerging contaminants, the resulting data set is insufficient for addressing more complex issues of exposure and actual risk.

In reviewing the growing number of studies in this area, it is clear that there are many contradictory findings but notably, the majority of the work suggests low-to-moderate overall phytotoxicity in terrestrial plant species. There are obvious exceptions to this trend but again, many of these findings of negative effects are at high (and likely unrealistic) doses. Also, notably lacking in many of these studies is soil as the exposure media; given what is known about the behavior of other contaminants in complex natural matrices such as soil, one may predict significantly lower toxicity than observed in model media (Schwab et al., 2015). Given this lack of clear overt phytotoxicity, the research community should now refocus efforts on more subtle systems-level processes that can be investigated under conditions of environmental relevance. For example, negative effects on processes such as nutrient...
cycling/acquisition or plant-microbe interactions (nitrogen fixation, mycorrhizal symbioses) may in fact pose greater risk to agroecosystem function and integrity. A semi-comprehensive list of topics and scenarios in need of investigation is below. This should not be interpreted as a list of items to be treated separately but instead as the integrated basis for a systems-level approach to accurately and quantitatively understand ENP fate and effects in agricultural systems (Fig. 1).

2. Low dose exposures with sensitive endpoints

As noted above, much of the existing plant-ENP interactions literature is populated with high dose, short term exposures and relatively insensitive endpoints (germination, biomass, pigment production) that offer little guidance in understanding the mechanisms of action. In a recent review, Holden et al. (2014) presented a comprehensive evaluation of studies reporting environmental hazard in different environmental matrices and compared this to modeled or measured environmental concentrations. Even though there is some overlap between the concentrations used in toxicity studies and those predicted from modeled/measured outcomes, the authors noted that the majority of the studies did not test ENPs across the lowest concentration ranges and studies were routinely exceeding the highest predicted concentration (≤0.001 to 1 ppm for water compartments and ≤0.001 to 1000 ppm for biosolids). For example, from 134 studies evaluated concerning plant nanotoxicity, only one study reported using sub-ppb levels (Holden et al., 2014); most used much higher ENP concentrations. Soil-based studies need to include exposures at relevant environmental concentrations; although these precise levels are not known due to uncertainties associated with modeling environmental ENP concentrations and limited information of the quantity production of ENP, it is clear that exposures in the hundreds to thousands of mg/kg are highly unlikely (except in spill scenarios) and that doses in the 1−100 mg/kg range are much more realistic. These exposures should occur over the full life cycle of the species of interest so that impacts on all stages, including edible tissue/food quality, can be assessed. Last, in addition to traditionally used gross parameters such as growth and yield, regulatory and research efforts would benefit greatly from the inclusion of more sensitive and mechanistic endpoints. For example, “omic” based endpoints (transcriptome, metabolome, proteome) can provide highly detailed and mechanistic information on plant responses to exposure and those molecular level effects can then be correlated to the more standard physiological and biochemical endpoints to provide a more complete understanding of toxicity/effects. However, it is important to mention that if one expands the number of endpoints, the chances of mistakenly observing an effect that does not exist increases, potentially confounding interpretation of results.

3. Trans-generational studies

Although toxicity has not consistently been demonstrated, there has been strong evidence across many studies showing the translocation of ENPs to plant shoots and edible tissues (Rico et al., 2011; Hernandez et al., 2013). This presents a direct and obvious risk to food safety but importantly, studies regarding the influence of ENP-exposure across multiple generations is largely unknown. Wang et al. (Wang et al., 2013) reported inhibited growth and development in second-generation tomato plants whose “parents” were exposed to CeO2 ENPs at low doses (10 mg L−1). The long term impacts on seed integrity and food safety across multiple generations and exposure regimes remains completely unexplored.

4. Trophic transfer studies

Limited information has become available recently concerning the trophic transfer of ENPs within terrestrial food chains (Judy et al., 2011; Unrine et al., 2012; Koo et al., 2015; Hawthorne et al., 2014; De La Torre-Roche et al., 2015). To date, the data have been somewhat contradictory, with select studies suggesting transfer and biomagnification and others not. In our laboratory, the uptake of CeO2 from soil by zucchini and subsequent transfer to crickets and wolf spiders was found to be particle size dependent (ENP greater than bulk). However, no such particle size dependence was observed for bulk and NP La2O3 accumulation and transfer from soil to lettuce, crickets, and mantids (Hawthorne et al., 2014; De La Torre-Roche et al., 2015). Clearly much work remains to be done, with a focus on soil-based long term, low dose studies where receptor response along the food chain is monitored through the use a range of sensitive endpoints.

5. Impacts on nutritional quality

It is known that ENPs interact significantly with both organic and inorganic constituents in soil. It is possible similar element/nutrient specific interactions could impact the availability and accumulation of specific plant macro- and micronutrients, as well as the synthesis and
metabolism of specific biomolecules. For example, Majumdar et al. (Majumdar et al., 2015) conducted a proteomic analysis of kidney bean seeds exposed to CeO₂ ENPs (63–500 mg/kg) in two soil types. The findings demonstrated not only an up-regulation of stress-related proteins but also a down regulation of genes associated with nutrient storage, suggesting significant changes in seed nutritional content and quality upon ENP exposure. Importantly, the plants showed no overt signs of stress or toxicity; the implications of these findings for nanotechnology use in agriculture could be far reaching and clearly much additional work is needed.

6. Co-contaminant effects

To date, a few studies have addressed how co-exposure to ENPs can influence the fate and effects of organic and inorganic co-contaminants. Given the large numbers of additional “analytes” of interest being added to agricultural systems (pesticides, fertilizers), ENP-interactions with these constituents may be significant. For example, carbon nanomaterials are known to associate strongly with hydrophobic organic chemicals. One can envision a range of interactions; carbon nanomaterials could bind persistent pesticides and reduce potential residue levels in the plant/edible tissues, could damage root membranes to facilitate residue uptake, or could bind to intentionally added agrichemicals to reduce pest control efficacy. In a soil-based study from our laboratory, carbon nanotubes consistently decreased the accumulation of weathered chlordane by four crop species in a dose-dependent fashion but the effects with fullerenes were more varied, with the nanomaterial actually increasing pesticide accumulation in some species (De La Torre-Roche et al., 2012). Although the mechanisms of these interactions are not known, the fact that carbon nanomaterial morphology so dramatically impacts pesticide fate is certainly a finding of great interest. Whether these types of interactions occur between other ENP and contaminants (i.e., heavy metals) is a question that remains completely unexplored. Given the implications for food safety, work in this area is strongly recommended.

7. Rhizosphere processes, key symbiotic bacteria and fungi

The rhizosphere or plant root zone is an area of intense microbial and enzymatic activity and many symbioses form that are critical to plant health and crop productivity. Species-specific plant root exudates (organic acids, hormones, secondary metabolites) signal and encourage growth of a specific prokaryotic and eukaryotic microbial community that is important not only to the plant but also to overall ecosystem health. Seemingly subtle changes in the community induced by ENP exposure could have far reaching implications, including alterations on plant productivity. For example, Priester et al. (2012) demonstrated that although NP CeO₂ had modest effects on soybean health, rates of nitrogen fixation were dramatically reduced and levels of nitrogen fixing bacteria within root nodules on the legume were significantly lower. Given the importance of many of these plant-microbe processes as broad ecosystem services (i.e., nitrogen fixation), studies thoroughly characterizing ENP effects on this area of the plant–soil interface are of critical importance.

8. Impacts of exudation and microbial activity on particle fate and dynamics

As just noted, plants secrete many organic compounds through their roots (20% or more of the fixed photosynthetic carbon) including polysaccharides, proteins, enzymes, phyto-hormones, and secondary metabolites that serve as important molecules in the rhizosphere. Given the dynamic nature of ENP dissolution and aggregation, the impact of this highly catalytic and active rhizosphere on particle fate and disposition is likely significant but remains unknown. Root exudates released into the rhizosphere will directly impact physical properties such pH, CEC, and salinity, all of which will then subsequently effect ENP aggregation, dissolution, and oxidation/reduction. Also, the active rhizosphere microbiome will produce proteins and other biomolecules that may affect ENP fate. For example, amino acids such as cysteine present in proteins and natural organic matter have been shown to increase initial ENP aggregation rates but not long-term aggregation size (Maurer-Jones et al., 2013). As the plant grows and transitions from vegetative to reproductive growth, exudation patterns will change, causing a cascade of changes in the rhizosphere that could impact ENP fate. Also, as organic matter accumulates in the root zone, it will alter the stabilization and agglomeration of ENPs in soil; studies have shown that 1–30 mg of carbon/L will significantly decrease ENPs aggregation rates (Majumdar et al., 2015). Understanding the impacts of plant root exudation and rhizosphere microbial activity under soil-based conditions and realistic exposure scenarios is critical for meaningful assessment of ENP fate in the environment.

In addition to the previously mentioned research areas that will significantly advance our current knowledge and understanding of ENP fate in agricultural systems, there needs to be a clear recognition of the importance of robust experimental design, as well as potential artifacts and confounding factors, while performing this work. This includes the use of appropriate analytical methods for ENP characterization and handling as well as potential artifacts and byproducts that could confound data interpretation. Another factor to be considered is the influence of the ENP application technique into soil (e.g. powder, dispersed in solution, spray formulation). Studies have reported stronger effects (inhibition or stimulation) in earthworms, plants, and soil microflora when TiO₂ and Ag ENPs were “wet” spiked to soils in comparison with “dry” amendment (Hund-Rinke et al., 2012). While it is possible that the amendment technique can influence particle fate and effects in soil media, research assessing ENP physicochemical changes during mixing processes and as a result of their heterogeneous distribution in soils is limited. Thus, characterization after addition to the soil matrix is recommended in order to avoid misinterpretations regarding unexpected changes in ENP dissolution and agglomeration that subsequently influence particle availability. Additionally, appropriate storage conditions may be important, including accounting for particle stability, oxidation, transformation, and other physicochemical changes. Wherever possible, orthogonal test methods should be used (Petersen et al., 2014). Inclusion of appropriate controls such as bulk particles and ion treatments is a key part of robust experimental design. Demonstrating that an ENP is phytotoxic and is of little inherent value; what is important is demonstrating whether that toxicity is different in magnitude or mechanism from corresponding bulk or ion exposures. When reporting data, there is need for the inclusion of detailed methodologies specifying test methods, characterization, handling and storage conditions of ENPs; journal-specific Supplemental Information sections are ideal locations for such information (Petersen et al., 2014).

In summary, current plant-ENP studies have answered some of the first important questions about ENP hazard and although the literature is somewhat contradictory due to inherent variations in methodology and experimental design; the majority of studies indicate low-to-modest overall phytotoxicity. However, the current body of nanotoxicological studies present only the first step and are an insufficient basis for an accurate and thorough estimation of ENP risk in the environment. A broader, more ecologically relevant systems approach is needed that includes long-term studies under environmentally realistic scenarios and with sensitive endpoints. Although these efforts will require greater time, energy and expense; the widespread use of nanotechnology in agriculture and other sectors mandates that this work be done.

References


