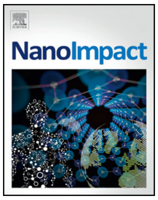
NanoImpact 1 (2016) 9–12

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.journals.elsevier.com/nanoimpact



Full length paper

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

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 15 December 2015
Accepted 15 December 2015
Available online 2 January 2016

Keywords:
Engineered nanoparticles (ENPs)
Agriculture
Nanotoxicity
Phytotoxicity

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 to 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.

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 (ENP) (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 (C_{60}), 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 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

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http://dx.doi.org/10.1016/j.impact.2015.12.002
2452-0748/

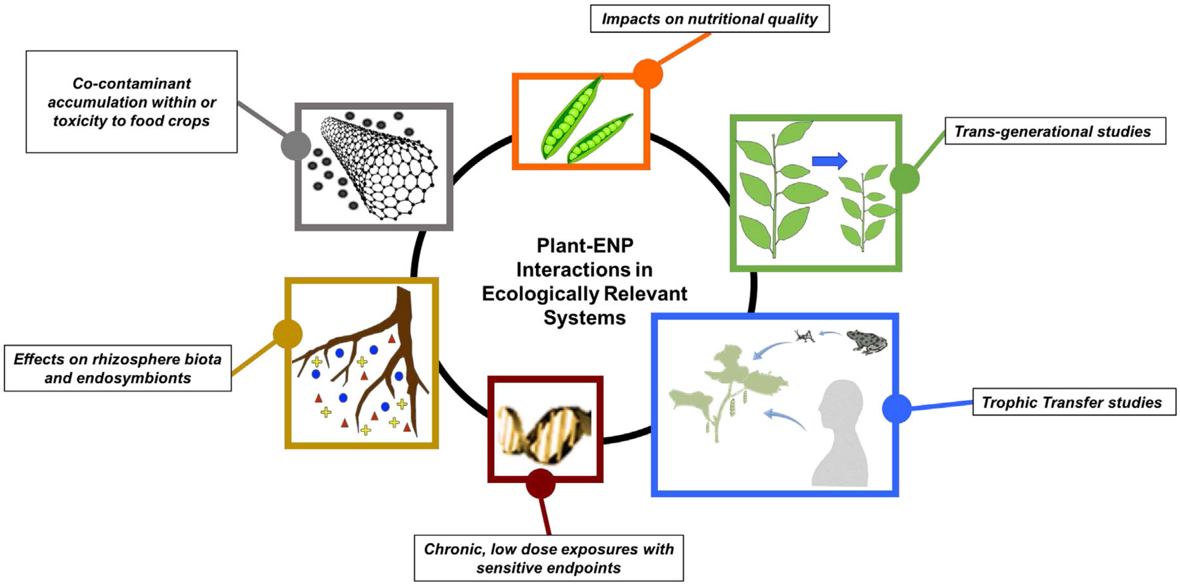

Fig. 1. Key knowledge gaps and recommended research areas that need to be addressed to fully characterize the risks and benefits of engineered nanomaterial use in agricultural systems.

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 CeO_2 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 CeO_2 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 La_2O_3 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