Accumulation of Engineered Nanoparticles in Belowground Vegetables: Nutritional Bioaccessibility and Dietary Exposure Risks

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PIs: **Stephen D. Ebbs[1]**, **Xingmao Ma[2]**
[1]Department of Plant Biology, Southern Illinois University; [2]Department of Civil and Environmental Engineering, Southern Illinois University

Project overview and objectives:

The release of engineered nanoparticles (ENPs) into the environment has raised concerns about their potential risks to food safety and human health. There is a particular need to determine the extent of ENP uptake into plant foods. Belowground vegetables that grow in direct contact with the soil are the foods which will likely accumulate the highest concentration of ENPs and present the most significant risk to human health. However, no such information is currently available in literature. This four year project would conduct a systematic evaluation of the accumulation of six different ENPs into the tissues of ten common belowground vegetables, including carrot, parsnip, turnip, beet, radish, and sweet potato, to identify the plant foods from those examined with the greatest potential for accumulation of individual ENPs. Project efforts are also examining the penetration of the ENPs into the edible plant tissues and how basic food preparation steps may remove the tissues with the greatest density of ENPs and thereby reduce dietary exposure. The cornerstone of the project is the inclusion of a physiologically-based extraction test to provide data on nutritional bioaccessibility of the ENPs from those tissues in the gastrointestinal tract. Data from these extraction tests will be used to conduct dietary exposure modeling to estimate dietary risks of ENP exposure. The nutritional bioaccessibility of the ENP will be demonstrated and models projecting age- and gender-specific dietary exposures will be produced to provide a comprehensive picture of the food safety risk posed by these ENPs. Results from this work will allow growers, extension agents, and USDA to make sound decisions on choice of crops for particular ENP-impacted soils. The specific accumulation and dietary exposure scenarios associated with particular ENP and plant combinations would allow for recommendations and/or restrictions concerning which ENP-containing products can be safely applied to human food crops. The research project has six specific aims:

1. Assess the accumulation of ENPs in sand-grown belowground vegetables
2. Assess the accumulation of ENPs in soil-grown belowground vegetables
3. Determine the spatial distribution of accumulated ENPs in belowground vegetables
4. Assay for the nutritional bioaccessibility of ENPs from belowground vegetables
5. Assay for the nutritional bioaccessibility from leafy vegetables, fruits & cereals acquired from other researchers working on ENP accumulation in plant foods
6. Develop models to estimate the dietary exposure to ENPs from the tested plant foods

Progress:

This project is in the early part of its second year. Efforts to date have concentrated on Aim #1 and ENP accumulation experiments have been conducted with four ENPs (ZnO, CuO, Ag, and CeO_2) and five plant species (carrot, parsnip, beet, radish, turnip). These studies have all included plants treated with the ionic counterpart of the metal associated with the ENP (e.g., ZnO or Zn^{2+}) to provide a context for evaluating the uptake and accumulation of the ENP and the subsequent dietary exposure. Toxicological and regulatory limits are typically based on the free or ionic metal. The toxicity of ENPs is currently assumed to be comparable to that of the metal constituent, not the ENP itself. However there is not a conclusive body of data on which to base that assumption. The efforts here seek to establish the distinction between the ENP and the metal ion to help derive relevant dietary exposure endpoints.

Most of the work has used carrot as this species can be grown quickly and effectively on a large scale. Use of this species has also allowed for some initial data to be collected relevant to Aim #3. Since carrots are typically peeled prior to consumption, there have been opportunities to investigate the penetration of the ENPs through the carrot epidermis into the edible flesh. This also concomitantly provide information on how a simply kitchen preparation technique like peeling may influence dietary exposure to ENPs. In comparing the uptake of CuO, ZnO, or CeO_2 NPs by carrots to the corresponding ions, the accumulation of the ionic for has been consistently and dramatically higher than the accumulation of the ENP form. For example, in an experiment where plants were exposed to either ZnO or Zn^{2+} at concentrations ranging over two orders of magnitude, the concentration Zn in the peels was 5-10 higher for Zn^{2+} treated plants as compared to ZnO treated plants. Additionally, the Zn^{2+} penetrated readily into the edible flesh while the Zn from ZnO was restricted entirely to the peel. The results for Ce^{2+} and CeO_2 were the same in that the ionic form accumulated more readily and the CeO_2 was generally restricted to the peel. In a separate hydroponic experiment with carrot, the plants were exposed simultaneously to Cu^{2+} and CuO. The concentration of each used in this experiment were based on a dissolution study conducted with CuO in hydroponic nutrient solution. For this dissolution study, CuO was added and after a period of equilibration, the respective ionic and NP concentrations were determined and used in the same proportion in the hydroponic experiment. Separate treatments applied the ionic concentration alone. Through this approach, information was sought to distinguish the amount of Cu accumulation from the ionic form as compared to the ENP form. The distribution of Cu between peels and flesh was similar to that for the ZnO and CeO_2 experiments above, except that evidence was obtained that implies that the Cu in the edible carrot tissues was both ionic and CuO.

As experiments like those above proceed, fresh plant tissue is being stored for use in the physiologically-based extraction test. While that inventory is still being built up, it has been possible to use the available tissue concentration data from the ZnO experiment to do some preliminary dietary modeling. Models have been generated based for consumption of carrots (peeled or unpeeled) treated with Zn^{2+} or ZnO by an adult male and a young child. The models provided an estimate of dietary exposure (in mg Zn kg body mass^{-1} d^{-1}) and those exposures were compared to the lowest observable adverse effect level (LOAEL) and recommended daily intake (RDI) for Zn. When compared to the LOAEL value (a toxicological endpoint), consumption by either the adult or the child of any carrots treated with ionic Zn^{2+} would results in a dietary intake the exceeded the LOAEL. However for the ZnO, that value was exceeded only for children eating the unpeeled carrots or the peeled carrots treated with the highest concentration of ZnO. In contrast, for most scenarios the presence of the ZnO in peeled carrots increased the nutritional quality of the carrot in terms of the RDI for Zn. These models are preliminary but suggest that dietary exposures to the ENPs tested would be considerably lower than what would occur from the consumption of vegetables exposed to the free metal ion.

In parallel with the studies associated with the six project aims, concurrent efforts are examining the phytotoxicity of the ENPs to the plant species being studied. In addition to effects on growth, development, and yield, efforts are also assessing the influence of ENPs on oxidative stress in these plants and the spatial distribution using confocal microscopy.

Summary of notable results:

- Peels of carrot appear to effectively screen metals from ENPs such as ZnO and CeO_2, reducing the accumulation in the edible tissues. Peeling carrots can remove a large fraction of the metals, potentially reducing the dietary exposure to that element.
- Preliminary modeling efforts suggest that metal accumulation from ENPs like ZnO is considerably lower than from Zn^{2+} and that the potential dietary exposure to adults from Zn originating from ZnO likely to be significant only at high exposure concentrations. However, the lower biomass of children would translate into higher dietary exposures, particularly for unpeeled carrots. However, peeled carrots exposed to either Zn^{2+} or ZnO may have greater nutritional quality for Zn.
- Penetration of ENPs into the edible tissues of belowground vegetables varies by chemical form of the ENP, size of the ENP, and the external concentration applied.

Publications

The research to date has provided sufficient data to initiate the preparation of at least four manuscripts. Two of the manuscripts would report the results from the ZnO and CeO_2 experiment described above with carrot while the second would provide the results of our initial dietary exposure modeling. A third manuscript would be developed from the CuO study above. In terms of the phytotoxicity studies, a fourth manuscript is in preparation that describes the effect of CeO_2 and other chemical forms of cerium on the growth of radish, including data on the spatial distribution of this element in the edible bulb as a function of the form applied.

Collaborations

The project proposal to USDA included a letter of collaboration from Dr. Jason White (Connecticut Agricultural Experimental Station) to link his current USDA-funded work on ENP accumulation in crops with the nutritional bioaccessibility testing and dietary exposure modeling included within this project (Aim #5). Since the launch of this project in 2012, there has been an open line of communication between the two PIs to establish this collaborative link to expand the scope and impact of each funded project. The PI of this project has also been in communication with other PIs working on similar projects to extend this network even further. The goal is to establish the PI and his lab as a focal point for nutritional analysis and dietary exposure modeling across multiple projects. To extend efforts on Aim #3, the PI has been in contact with Dr. Ryan Tappero (Brookhaven National Laboratory) to develop a collaboration that would allow for the application of X-ray absorption spectroscopy to aid in determining the spatial distribution and speciation of the metal in the plant tissues. Finally, the PI has been in contact with Dr. Raymond Glahn (USDA-ARS), a nutritional physiologist. As work progresses, collaboration with Dr. Glahn would provide an animal model to examine ENP absorption into the blood stream and additional parameters to increase the robustness of the dietary exposure models.

References

[1] For further information about this project contact Stephen Ebbs, sebbs@plant.siu.edu