Toxicity, bioaccumulation and trophic transfer of engineered nanoparticles in the aquatic environment

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Chapter 6

General Discussion

Nanotechnology is considered part of a new renaissance in science, as it has been identified as a key enabling technology that brings prosperity and innovation within a wide range of commercial and industrial applications (European Commission, 2012; Song et al., 2017). However, it is crucial to unravel the fate and effects of ENPs in aqueous media in a world where nanotechnology is accelerating and hence unintended ENPs will be emitted into surface waters. Evaluating nanosafety, making use of standardized first tier screening assays, has shown not to be not sufficient accurate. ENPs will undergo physicochemical processes in freshwater media, which can influence the bio-uptake and -accumulation of ENPs and modify their toxic effects.

In addition, within natural settings it is not unlikely that mixtures of nanoparticle suspensions can be found. Moreover, the presence of natural organic material (NOM) is known to impact the fate and subsequent processes of ENPs up to the potential of trophic transfer through food chains. To add experimental data as well as new knowledge to address the inevitable questions and challenges for the environmental risk assessment of ENPs, we systematically investigated 1) the impact of NOM on the fate, bioaccumulation and single/joint toxic actions of ENPs, 2) the transfer of ENPs from lower trophic levels to higher trophic levels, and the subsequently occurring effect on predators.

6.1. Findings in this thesis

We investigated the individual toxicity of CeO_2NPs in three organisms. A relationship between exposure characteristics with the toxicity of

CeO_2NPs was found. The joint toxic action of CuNPs + ZnONPs was additive or more-than-additive for *D. magna*. A similar pattern was found in the toxicity of the mixtures of Cu- and Zn-salts.

The individual and joint toxicity were affected in the presence of NOM. Different concentrations of humic substances (HS) alleviated CeO_2NPs toxicity to *R. subcapitata* and to *C. sphaericus*. The joint effects of HS and CeO_2NPs were additive and synergistic to *D. rerio*. Additionally, NOM increased the relative contribution of dissolved metal-ions to the joint toxicity. NOM enhanced metal bioaccumulation in the mixtures of CuNPs and ZnONPs.

Trophic transfer was observed for PSPs from algae to mysids, and for CuNPs from daphnids to mysids. A limited extent (TTF < 1) of trophic transfer of total CuNPs or Cu ions was observed from algae to the mysid. Biomagnification of particulate Cu occurred from algae to mysids. The extent of trophic transfer was found to be affected by particle size and the type of food chain. Particle size of CuNPs determined by sp-ICP-MS ranged from 22 to 40 nm in species. No significant changes in the particle size of CuNPs were measured during uptake.

6.2. Implications and future perspectives

Assessing the environmental risk of ENPs is the key to nanosafety and green design (Savolainen et al., 2013). The European Commission emphasized that understanding the effects and concentrations of ENPs in complex and realistic situations is required to better and more effectively assess the risks of manufactured nanomaterials (Science for Environment Policy, 2017). As described above, investigations were performed in this thesis to help unravel the toxicity of ENPs exposed in single and mixture settings, explicitly considering the impact of NOM on the accumulation and toxicity of ENPs and their potential to transfer through food chains. These findings are crucial when developing predictive models for ENPs based on the processes that jointly determine the fate of ENPs and their relationship to uptake and trophic transfer potential. Understanding of the exposure and hazard risk of ENPs can ultimately provide basic data for safe design and process-based environmental risk assessment (Figure 6.1).

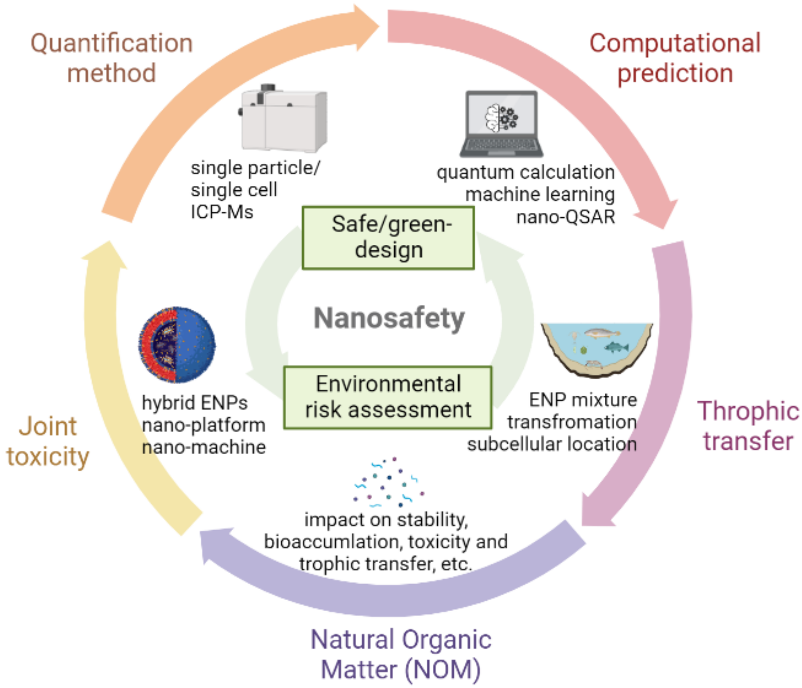

Figure 6.1 Schematic illustration of the implications and future perspectives of ENPs on safe/green-design and environmental risk assessment in this thesis.

6.2.1 Joint toxicity of ENPs

The data obtained on the joint impacts of two ENPs in a mixture could be the stepping stone for prospective new innovations. Nowadays, metal alloys and hybrid nanoparticles are already synthesized, going a step beyond the 1^{st} generation of single element nanomaterials. Consequently, the future co-release of multiple ENPs will inevitably result in mixtures of ENPs in the environment. As found in our case study (**Chapter 2**), the joint toxicity of CuNPs + ZnONPs was greater than the single toxicity of CuNPs or ZnONPs toward water fleas. The mode of action of toxicity induced by CuNPs + ZnONPs was additive or more-than-additive. Synergistic and antagonistic actions were found in other researches, as listed in Table 1.1 (Chapter 1). Hence, the toxic potential of ENP mixtures could be distinct from the summed toxicity of individual ENPs. The risk assessment and risk management should include the joint toxicity of ENP mixtures in complex conditions or under environmentally realistic conditions.

In addition, we found that the toxic mode of action of CuNPs + ZnONPs was similar to the toxic mode of action of Cu^{2+} + Zn^{2+}. This finding indicates that the joint toxic mode of metal salts could be the reference for the evaluation of metal-based ENPs of similar chemical composition. However, the contribution of particles should not be neglected as well. Furthermore, the presence of CuNPs will enhance the bioaccumulation of ZnONPs as found in **Chapter 2**. Previous studies proved that the bioaccumulation of Cu ions was promoted with the addition of Zn ions (Komjarova and Blust, 2008). This suggests that the hazard of Zn-based ENPs will increase in the presence of Cu-based ENPs. For the purpose of safe/green design, Cu and Zn should not be included into the same nano-product.

6.2.2 Trophic transfer

Within our study results we have found that trophic transfer occurred only under specific conditions. The exposure pathway of ENPs to organisms was an important descriptor in this respect (**Chapters 4 and 5**) The confirmed biomagnification of particulate Cu further highlights the potential hazard to human beings. Specifically, the extent of transfer depended on the type of food chains and particle size. CuNPs were found to transfer from algae to mysids, rather than from daphnia to mysids or from algae to daphnia to mysids (**Chapter 4**). Also the larger the particle size of PSPs, the higher the extent of transfer in the food chain, which is counterintuitive and currently lots of researchers are investigating this issue (**Chapter 5**). The study provides evidence that the role of particle size should not be neglected in regulating the bioaccumulation and trophic transfer of ENPs in the aquatic environment. We echo Tangaa et al. (2016) in this respect and plea for a larger scientific understanding on the trophic transfer of ENPs and the affecting factors such as food chain types and particle size. To reveal the availability of ENPs for transfer in a more realistic and complex system, understanding how ENPs transfer through the food web or micro-ecosystem is meaningful and challenging. Furthermore, investigations on the transfer of ENP mixtures along food chains or the food web are warranted in future study, are still a research area.

6.2.3 An inevitable factor —— NOM

Natural organic matter (NOM) is ubiquitous in field setting. Soils, sediments, air and water are carrying large amounts of NOM, whereas organisms for instance shed off skin, mucus and other body fluids. This provides NOM as well death biota (plants, microbes and biota) that are later turned into NOM. It turns out that when translating simplified screening laboratory tests towards realistic settings this parameter is an inevitable and key parameter to consider. Additionally, NOM is also crucial for the fate of ENPs because NOM stabilizes nano-suspensions (**Chapter 2)** and thus have a huge impact on CeO_2NPs toxicity, as it differs across different aquatic organisms. Our research emphasizes how the stabilization of particles with NOM or any other type of NOM affects the toxicity of ENPs. A key element of our findings is that the interactions between NOM and ENPs or other water chemistry factors affecting particle stability can be utilized to reduce the toxicity of ENPs. In addition, we highlighted that NOM enhances the toxicity to fish larvae but inhibits the toxicity induced by CeO_2NPs to algae and water fleas. Further investigations of the role of NOM on the fate of ENPs is thus an essential part of the exposure characterization of specific organisms in the aquatic environment. The same is true for considering multiple ENPs in an exposure media (**Chapter 3**).

6.2.4 Other implications and research area

One main limitation of risk assessment frameworks for ENPs is the lack of a quantitative uncertainty assessment to improve transparency

(Jahnel, 2015). To determine ENPs in aquatic columns and biota, constructing an accurate and suitable quantification method combined with an extraction procedure is crucial. In **Chapter 4**, we used sp-ICP-MS quantified the size and number concentration of CuNPs in the biota using tissue extraction with TMAH. Particle number and mass were found to be equally suited to express bioaccumulation and to trace the ENPs in food chains. Moreover, a newly developed mode of the time-resolved inductively coupled plasma mass spectrometry (ICP-MS) technique, described as single-cell (sc)-ICP-MS, was recently introduced to measure the concentration of elements in cells (Monikh et al., 2019, 2021). However, there is still a lack of a recognized and systematic quantitative analysis method for environmental risk assessment and management. It is a critical challenge to develop more reliable and reproducible measurement techniques and standards for small size and particulate materials (Shatkin, 2020).

Tools for predictive risk assessment and risk management including databases and ontologies were described as one of the research needs and priorities of ENPs for the coming 10 years in the "Nanosafety in Europe 2015–2025" report (Savolainen et al., 2013). Alternative methods of toxicity assessment were proposed by the US National Research Council in "Toxicity Testing in the 21st Century: A Vision and a Strategy" (National Research Council, 2007). There are currently many researchers focusing on the development of these predictive models; what we learned from the thesis results is that also emphasis should be put on the joint toxicity of ENPs in suspension. There is thus an urgent need to create curated and publicly accessible data sets for ENP mixtures (Shatkin, 2020).

To conclude, the research results described in this thesis are stepping stones towards improving the understanding of the processes that determine the actual exposure of a suite of aquatic organisms to exposure media of different compositions, mimicking to an increasing extent natural aquatic systems. Our findings provide a basis, i.e. joint toxicity, trophic transfer potential and effect of NOM, for environmental risk assessment and management in a more realistic and natural aquatic environment. From the evaluation endpoint, feedback and reference were generated for predictive risk assessment as well as building blocks for a green/safe-design of ENPs. Based on current studies, research priorities include further investigations on the toxicity of ENP mixtures, trophic transfer of ENP mixtures, the influence of NOM on trophic transfer of multiple ENPs, development of quantitative analysis methods and standards, and prediction of nanotoxicity.

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