Engineered Nanoparticle (Eco)Toxicity: Towards Standardized Procedures for Hazard Identification

In the past decade, the use of nanotechnology has led to a large variety of products in the market, and is projected to markedly increase in value in the years to come. The use of manufactured nanomaterials comprises various technological and economic benefits due to their novel physico-chemical characteristics. It is these unique physico-chemical properties that have raised concerns during the last decade regarding the potential risk nanomaterials pose towards human health and the environment. Similar to other chemicals, nanomaterials have to be tested and ranked in order to obtain information on hazard identification, which is an integrated part of risk assessment. The complex nature and behavior of nanomaterials in the different environmental compartments and test systems has made it difficult for the scientific community to conduct robust and reproducible tests, and consequently, for regulatory bodies to take action.

Standard test guidelines developed for conventional soluble chemicals, have been used to test nanomaterials. Concerns have been raised whether these test systems are adequate for addressing particle properties under different testing conditions and assessing toxicological outcomes. In fact, various international organizations (e.g. Organisation for Economic Cooperation and Development and International Organization for Standardization) have recognized the need to amend and refine the current standard tests in relation to nanomaterials. Methodological considerations to standard testing for the purpose of testing engineered nanoparticles (ENPs) in aquatic systems are a central theme in this thesis. The research presented herein has included acute tests with freshwater cladoceran Daphnia magna, genotoxicity tests with bacteria Salmonella typhimurium, as well as acellular and in vitro assays.

An understanding of different physico-chemical properties and specific characteristics of various nanoparticles employed in this project has been attained by reviewing the literature in the field. Specific processes such as agglomeration in aquatic suspension, influence of environmental conditions on toxicity testing, dissolution, phototoxicity and inflammation were identified as important parameters and considered for further toxicity testing of Ag, ZnO, TiO2 and CeO2 ENPs; to investigate specific properties and improve test stability and reproducibility.

The issue of agglomeration of ENPs in aqueous suspensions was investigated by attempting to prepare stable stock and test suspensions of various nanoparticles. A step-wise approach was presented to develop tailored dispersion protocols for (eco)toxicological testing of ENPs, based on the identification of critical issues and parameters for stock dispersion protocol development. This may serve as a basis for the development of a harmonized dispersion protocol for ENPs.

Natural organic matter (NOM) and aging of suspensions prior to testing were employed in an attempt to stabilize aqueous suspensions of three different ENPs. While NOM helped in stabilizing ZnO ENPs suspensions, it caused agglomeration in TiO2 ENP stock suspensions and an underestimate of toxicity for Ag ENPs. Likewise, aging only aided in the stability of ZnO ENPs. It was concluded that NOM can mitigate or eliminate toxicity of Ag ENPs and is not recommended for use. The ratio of NOM to ENP proved to be important in stabilizing non-capped ENPs. Another attempt towards stable suspensions involved adjusting different standard testing parameters, such as the pH and ionic strength of the test media. It was found that point of zero charge measurements should be conducted prior to ecotoxicological studies. Testing media of very low ionic strength at a pH where the ENPs have the lowest agglomerate size should be employed. This will control agglomeration and increase the stability and reproducibility of the test results. For ENPs such as TiO2, toxicity of smaller agglomerates was significantly higher than larger agglomerates, highlighting the importance of size distribution in relation to toxicity. It was recognized that it is difficult to give general advice that is applicable for testing all nanomaterials, thus, a case-by-case evaluation should be conducted.

Another topic in this thesis was to evaluate the feasibility of current methods to screen and rank toxicity of ENPs in a high-throughput manner. Investigation of TiO2 phototoxicity using the umu assay revealed that UV light caused damage to the bacteria and that high ENP concentrations had a shading effect, which were categorized as confounding factors. Similarly, an attempt to measure inflammation response caused by CeO2 and TiO2 ENPs, revealed that the high surface area of ENPs has a high affinity and binding capacity for protein molecules/assay reagents. These artifacts questioned the feasibility of these assays for testing ENPs. The influence of test parameters and confounding factors/artifacts should be taken into account and investigated prior to undertaking nano-toxicological studies. These results indicate that test guidelines need to be revised and tailored according to ENP properties, as test conditions affect toxicity. The information presented in this thesis may help the scientific community and regulators better understand test design and outcomes of nano-(eco)toxicological studies, which in turn may lead to a stronger scientific basis for regulation of nanomaterials.

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In this thesis, the feasibility of these assays for testing ENPs was addressed. The influence of test parameters and confounding factors/artifacts should be taken into account and investigated prior to undertaking nano-toxicological studies. These results indicate that test guidelines need to be revised and tailored according to ENP properties, as test conditions affect toxicity. The information presented in this thesis may help the scientific community and regulators better understand test design and outcomes of nano-(eco)toxicological studies, which in turn may lead to a stronger scientific basis for regulation of nanomaterials.