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Considering the widespread applications of nanoparticles (NPs) in consumer and industrial products, concern regarding the toxic effects of NPs to humans and the environment has been sharply growing in recent years. Although the growing concern has led to increasing efforts to assess nanotoxicology, the underlying mechanisms of toxicity of metallic NPs are still largely unclear. To date, it has been widely recognized that the toxicity of NPs is highly dependent on the physicochemical characteristics of the environment in which the NPs are introduced (the exposure medium) and the physicochemical properties of the metallic NPs. To improve our understanding of the toxicity of NPs, research is required that integrates the impact of the physicochemical characteristics of NPs and the impact of differences in water composition with the toxicity profiles of NPs. However, the majority of hazard assessment studies of metallic NPs only determined their toxicity without providing thorough information regarding behavior, fate and accumulation of NPs, let alone that efforts were undertaken to integrate this information with the observed toxicity of NPs. Suspensions of metallic NPs typically are a mixture of particles and ions. Evaluation of the issue whether the main sources of toxicity of NP suspensions are either the NPs or their released ions, is critical for environmental risk assessment and management of released NPs. Nevertheless, most of the studies regarding nanotoxicology have not separated the relative contribution of the particles themselves and their released ions to the observed toxicity. In this thesis, studies integrating fate, accumulation and the related toxicity of CuNPs to *Daphnia magna* across different exposure regimes were systematically conducted. Furthermore, the impact of water chemistry on the relative contribution of particles and their released ions to the observed toxicity of suspensions of CuNPs, and on the particle-specific toxicity of CuNPs was evaluated.

In Chapter 2, the relative contribution of particles and their released ions to the toxicity of suspensions and accumulation of CuNPs in *D. magna* was quantified by using the response addition model. For this study, CuNPs (with a pristine size of 50 nm; spherical shape; advertised surface area, 6-8 m$^2$/g) and ZnONPs (with a pristine size of 43 nm; spherical shape; advertised surface area, 27 m$^2$/g) were used to prepare exposure suspensions. It was found that at the LC50 (lethal concentration at which 50% of the organisms dead) of the CuNP and ZnONP suspensions, the relative contributions of ions released from CuNPs and ZnONPs to the observed toxicity were around 26% and 31%, respectively. This indicates that particles rather than their released ions were the dominant descriptor for the observed toxicity. Taking accumulation as an endpoint, similar patterns of relative contributions of particles and their released ions were determined at exposure concentrations close to the LC50 (approximately 0.1 mg/L and 1 mg/L of CuNPs and ZnONPs, respectively). This implies that there is likely a direct causal relationship between accumulation and toxic effects of NPs. On the other hand, at low exposure concentrations of CuNPs and ZnONPs (around 0.05 and 0.5 mg/L, respectively), the dissolved ions shedding from the NPs were predominantly accumulated by the daphnids. The relative contributions of particles and their released ions to the overall
observed accumulation in organisms are hence dependent on the concentration of NPs to which the organisms are exposed.

In Chapter 3, the toxicity of suspensions of CuNPs (with a pristine size of 50 nm) to *D. magna* upon modification of the exposure conditions was investigated. This was done by aging the suspensions of CuNPs for up to 7 days and by varying the water chemistry parameters including pH and dissolved organic carbon (DOC) concentration. The LC50 value for suspensions of CuNPs to *D. magna* decreased by about 30% after 7 days of aging, compared to the LC50 of freshly prepared suspensions of CuNPs. The LC50 value of suspensions of CuNPs increased by more than 12-fold upon addition of DOC at concentrations ranging from 0 to 10 mg C/L. There was a 3-fold increase in the LC50 value of suspensions of CuNPs upon changing the pH of the exposure medium from 6.5 to 8.5. Furthermore, it was found that within 7 days of aging of the exposure medium (at pH 7.8 and without addition of DOC), the overall observed toxicity could be mostly ascribed to the particles present in suspension (around 70%). However, the presence of DOC at concentrations ranging from 0.5 to 10 mg C/L or the reduction of pH of the exposure medium from 8.5 to 6.5 reduced the relative contribution of particles to the observed toxicity. We thus conclude that the toxicity of CuNP suspensions and the roles of particles and their released ions regarding the observed toxicity are highly dependent on the exposure conditions.

In Chapter 4, the effects of water chemistry parameters (pH, divalent cations and DOC) on the behavior and ultimate fate of CuNPs (with a pristine size of 25 nm; specific surface area, 30-50 m$^2$/g; shape spherical), were systematically investigated. The results demonstrated that divalent cation content was the most influential factor for the aggregation behavior of CuNPs, which explained around 46% of the variations of the aggregation of CuNPs in exposure media across a range of water chemistry. DOC concentration was the next most influential factor, which could explain 23% of the variation of the aggregation of CuNPs. The interaction between divalent cations and DOC also played an important role in influencing the aggregation of CuNPs, accounting for 11% of the change of aggregation of CuNPs. DOC concentration was the predominant factor for the variation in dissolution profiles of CuNPs. The percentage of ions released from CuNPs in the water column was decreased by 3-5-fold by the addition of DOC at concentrations ranging from 5 to 50 mg C/L after 48 h of incubation, compared to the case without addition of DOC. pH in the range from 6 to 9 only accounted for 5% of the variation of dissolution of CuNPs. In addition, the sedimentation profile of CuNPs was mostly influenced by divalent cation content and DOC concentration and the interaction of divalent cations and DOC. These results are helpful for improving our understanding and prediction potential on how and to what extent environmental factors affect the behavior and fate of metallic NPs upon a range of environmental conditions.

In Chapter 5, the impact of water chemistry on the particle-specific toxicity of CuNPs to *D. magna* was determined. CuNPs with a pristine size of 25 nm were exposed to *D. magna* upon modification of the water chemistry parameters (i.e., pH, divalent cations and DOC). It corroborated that the toxicity of the suspensions of CuNPs decreased with increasing pH and with increasing concentrations of divalent cations and DOC. This is the net effect of water chemistry on the toxicity of particles and ions released from CuNPs. Based on the response
addition model, it was found that the toxicity of the particles was in most cases lower than the toxicity of ions released from CuNPs. The particle-specific toxicity of CuNPs decreased with increasing pH and with increasing concentrations of divalent cations and DOC. The variations of the particle-specific toxicity were related to their behavior and fate upon varying exposure conditions. The toxicity of CuNP suspensions was also dependent on the exposure modality: in the presence of DOC at a concentration ranging from 5 to 50 mg C/L, the toxicity of CuNP suspensions to *D. magna* in the dynamic exposure treatment was 25%-40% higher than the toxicity of CuNP suspensions with the same chemical composition under static exposure conditions. The dissolution profiles of CuNPs upon the addition of DOC in the static and dynamic exposure treatments were found to be similar within 48 h of incubation, whereas the extent of aggregation of CuNPs was smaller within the 48 h of incubation in the dynamic treatments than in the static treatments when DOC was added. Hence, the higher toxicity of CuNP suspensions as found in the dynamic exposure treatments, could be attributed to the enhanced stability of CuNPs in the aquatic medium. These results indicate that stabilized particles are more bioavailable to *D. magna* than non-stabilized particles.

To conclude, this thesis provides further insight in understanding the behavior, fate, accumulation and toxicity of CuNPs across different exposure conditions. Furthermore, our study emphasizes that studies integrating the dynamic physico-chemical characteristics of NPs into their toxicity profiles are needed in order to comprehensively interpret the hazards of NPs to the environment. Also, the importance of water chemistry in determining the relative contributions of particles and their released ions to the observed toxicity of NP suspensions and in determining the particle-specific toxicity of NPs, is clearly highlighted.