Summary

In the last two decades, the field of nanotechnology has been rapidly expanding and has been experimented with in various applications, such as consumer products, nanomedicine, as well as engineering and materials. The growing concern of potential health and environmental risks associated with ENPs has triggered various safety regulations around the world. However, the understanding of the toxicity level of these ENPs is still underestimated as the influence of water chemistry such as NOM on the fate and toxicity of ENPs, the quantification of ENPs trophic transfer through food chains and relative affecting factors are still poorly studied. In accordance with the scientific questions, the main conclusions reached in this thesis are summarized below:

1) How does NOM affect the stability and toxicity of **individual ENPs** to aquatic organisms?

The case study of **Chapter 2** determined the impacts of humic substances (HS) as a NOM analog on the aquatic stability and single toxicity of CeO_2NPs to three organisms with different exposure characteristics. With the addition of HS at a concentration ranging from 0.5 to 40 mg C/L, the stability of CeO_2NPs was significantly improved, and the stabilization depended on the concentration of HS. Toxic effects of CeO_2NPs on algae and on daphnids were reduced by different concentrations of HS, while the toxicity towards zebrafish larvae was enhanced. A concentration-dependent influence of the addition of HS on the toxicity of CeO_2NPs was observed to different organisms. Furthermore, relationships between particle stability with

toxicity and between aquatic species with toxicity were found in the condition of HS. These findings emphasize the important role of NOM in stabilizing the nano-suspensions and the different impact on CeO_2NPs toxicity towards different aquatic organisms.

2) How does NOM affect the fate, accumulation and toxicity of **ENP mixtures**?

In **Chapter 3**, the fate, joint toxicity and accumulation of a mixture of CuNPs and ZnONPs in *Daphnia magna* influenced by the presence of Suwannee River natural organic matter (SR-NOM) were investigated. Different concentrations of SR-NOM have no significant impact on the hydrodynamic diameter and zeta potential of the binary mixtures. The only exception was that the co-agglomeration behavior of ENP mixtures was significantly alleviated after adding 20 mg/L SR-NOM. The addition of SR-NOM didn’t affect significantly the apparent joint toxicity of CuNPs + ZnONPs. Whereas, SR-NOM changed the contribution to total toxicity and enhanced metal bioaccumulation of ENP mixtures. The presence of SR-NOM increased the relative contribution of dissolved metal-ions released from metal-based ENPs to the joint toxicity. Particularly, the released Zn-ions dominated the toxicity of the binary ENP mixtures with the co-existence of SR-NPM. This is due to the agglomeration and sedimentation of CuNPs and the complexation of the released Cu-ions with SR-NOM. Moreover, the accumulation of Cu and Zn in the mixtures of CuNPs and ZnONPs in daphnia was both remarkably increased by the addition of SR-NOM.

3) To what extent do ENPs **transfer** in particulate, and ionic forms and how does the particle size and number change in different organisms?

The trophic transfer of CuNPs through an aquatic food chain consisting of algae, daphnia, and mysid was quantified in **Chapter 4**. The number-based concentration and size of Cu particles in different tropic level species were quantify by single particle inductively coupled plasma mass spectrometry (sp-ICP-MS). A limited extent of trophic transfer of total CuNPs or Cu ions was observed from algae to the mysid. Meanwhile, the particulate Cu biomagnified from the algae to the mysids as a result of the trophic transfer factor value higher than 1. Additionally, the number concentrations of Cu particles in different trophic levels were in the order of 10^{13} particles/kg wet weight. The size of the particulate Cu was determined from 22 to 40 nm throughout the food chain without significant changes. These results exhibited that tracing the particulate fraction of ENPs is as important as tracing the ionic fraction along the trophic transfer.

4) How do **particle sizes** and **food chain types** affect the trophic transfer of ENPs and their subsequently biodistribution and toxicity to the predators?

In order to compare the influence of food chain types on the trophic transfer extent, the length and food source were considered in **Chapter 4 and 5**. The transfer of CuNPs through three simulated freshwater food chains were constructed including from algae to daphnia to mysids, from algae to mysids, and from daphnids to mysids (**Chapter 4**). Trophic transfer was only found in the 2-level food chain from algae to mysids, rather than the other two types food chain. This provided evidence that both the position in the length of the chain as well as the food source have impact on the trophic transfer potential of CuNPs. Moreover, the significant effects on the feeding rates of predator mysids by the dietary CuNPs was found in the transfer processes from daphnia to mysid and from algae through daphnia to mysid.

The trophic transfer of polystyrene particles (PSPs) from daphnia to mysids as a function of particle size (26, 500 and 4800 nm) was investigated (**Chapter 5**). Only a small fraction ranged from 1 to 5% of all sized PSPs ingested by daphnia was transferred to mysids. The extent of trophic transfer is size-dependent decreased in the order of 4800 nm > 500 nm > 26 nm PSPs. No tropic transfer was observed in the predator for all sized PSP treatments. Furthermore, all PSPs were mainly accumulated in the intestinal tract (stomach and intestine) of mysids. Consequently, our findings emphasized that different sized PSPs can transfer along the daphnia-mysids food chain, and the impact of particle size on the potential of trophic transfer shouldn't be neglected.

In conclusion, the findings in this thesis improve the understanding of 1) the relationship between exposure characteristics and toxicity of ENPs, 2) the joint toxic action of ENP mixtures and the comparison to metal salt mixtures, 3) how NOM affects the individual and joint toxicity of ENPs, 4) the extent of trophic transfer of ENPs along aquatic food chains, 5) the influence factors on trophic transfer, and 6) bioaccumulation, distribution and toxic effect on predators. This knowledge would provide a basis for data on individual and joint toxicity, bioaccumulation, and trophic transfer of ENPs for more realistic environmental risk assessment.