Ecotoxicity of engineered nanoparticles to freshwater organisms

A large variety of societal benefits are expected from the development and use of engineered nanoparticles. At present, the majority of ‘nano-products’ put on the market can be classified as consumer products, whereas future applications are expected to have more widespread and societal benefits in areas as diverse as cancer treatment, groundwater remediation and industrial coatings. Nanoparticles are used to give the products new and improved characteristics. Yet exactly these new and nano-specific properties might be a cause of concern in a health and environment context. In order to ensure adequate protection of humans and the environment, a pro-active effort to understand, identify and minimise potential risks is needed at an early stage in the innovation process. However, due to the fundamentally different nature of nanoparticles as discrete entities, compared to ‘conventional’ water-soluble chemicals, many aspects of commonly used test methods for evaluation of potential adverse environmental effects make their applicability to nanoparticles questionable. For this reason the overarching aim of this PhD project has been to acquire information, which can be disseminated and applied in relation to appropriate test methods for identifying potential adverse effects of nanoparticles; this is of great relevance from both a scientific and regulatory point of view. An important aspect of this project was the acquisition of experience in testing nanoparticles in aqueous test systems – both through practical lab-based studies as well as literature studies. The process of testing nanoparticles in aquatic ecotoxicity tests has been as important in the project approach as the test outcomes. Applied test methods have included acute and chronic toxicity tests as well as bioaccumulation studies with freshwater filter feeder Daphnia magna, sediment feeder Lumbriculus variegatus and green alga Pseudokirchneriella subcapitata. The results made it possible to identify major scientific and methodological challenges in the testing of nanoparticles compared to ‘conventional’ chemicals. It has been highlighted that while it is possible to obtain dose-response relationships for nanoparticles, such tests often raise as many questions as they answer. Issues requiring further attention include nonlinear concentration-aggregation relationships, adhesion of nanoparticles to the cell and organism surfaces, problems of relating effects directly to properties of the primary nanoparticles, dynamic two-way interactions between nanoparticles and organisms as well as how to best quantify and qualify exposure in a dynamic system. Particularly for algal growth inhibition tests, methodological issues were raised related to biomass quantification methods. Here, a combination of several techniques is recommended. The combination of visual inspection, cell counting and fluorescence measurement of acetone-extracted pigments was found to give additional insight into the nature of the observed effects. A separate task was to explore the role of nanoparticles in chemical mixtures, elucidated through experimental and conceptual studies. Interaction between nanoparticles and co-contaminants in chemical mixtures may result in changes in the bioavailability and toxicity of the individual compounds. A conceptual model for interaction scenarios between nanoparticles and co-existing environmental pollutants was developed. Experimental results showed that several types of nanoparticles (e.g. TiO2 and C60) have a large adsorption capacity for some heavy metals and organic chemicals. Nonetheless, cadmium adsorbed to TiO2 nanoparticles was found to be bioavailable to D. magna, L. variegatus and P. subcapitata. TiO2 nanoparticles were seen to attach to the surface of P. subcapitata and to mainly be located in the gut of D. magna. In D. magna the presence of TiO2 resulted in increased uptake of cadmium but not in overall increased toxicity. Interaction studies (especially binary multiple-dose studies) may be considered premature at present due to the additionally increased test system complexity. This may hamper the interpretation of test results both compared to interaction studies for conventional water-soluble chemicals and ecotoxicological tests of only nanoparticles. However, interaction studies using single doses of nanoparticles may still provide indications of the potential of nanoparticles to influence mixture toxicity and how this occurs. Also, when fundamental general test procedures for testing of nanoparticles are in place, the role of nanoparticles in chemical mixtures is an important field for future studies. In the light of these findings, it is recommended that research in the field of nanoeccotoxicology is prioritised towards the methodological challenges that have to be overcome in order to obtain meaningful results. Compared to conventional water-soluble chemicals, additional considerations regarding test procedures are needed to gain an insight into the underlying mechanisms. The influence of particle behaviour in the test system on observed effects and actual toxic mechanisms remains to be explored further. As a supplement to traditional endpoints such as mortality, reproduction and bioaccumulation, it is crucial to perform exploratory in-depth investigations of biological processes and analytical methods to ensure the robustness and applicability of test methods and test endpoints. A focus on methodological problems, test system dynamics, ecotoxicokinetics and effects mechanisms should therefore be encouraged.

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