Session: The environment as a reactor determining fate and toxicity of nanomaterials (II)
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The role of invertebrates in a tiered bioaccumulation testing strategy for engineered nanomaterials

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1. Introduction

Due to the increasing usage of manufactured nanomaterials (MNMs) there is an mandatory need for testing the bioaccumulation potential of this materials within the regulatory frame of REACH. The bioaccumulation potential is usually expressed as bioconcentration factor (BCF) determined in flow-through studies with fish according to OECD 305 [1]. The increasing number of different MNMs would lead to an unrealistic workload for testings. Handy et al. 2018 [2] proposed a tiered aproach including stategies for the reduction of animals for in vivo testing.

Four tiers are mentioned, starting with the use of environmental chemistry trigger values and the use of existing chemistry data sets (Tier 1). MNM-specific in silico models and validated screening tools are considered as part of Tier 2. Existing bioaccumulation data sets are reviewed including data from microbes and invertrebrates. Only if concerns appear in Tier 2, an in vivo dietary bioaccumulation test according to OECD 305 is considered (Tier 4), provided that concerns are confirmed by chemico digestibility assays as part of Tier 3. To obtain data from invertebrate tests that are suitable for the bioaccumulation assessment of MNMs as part of Tier 2, appropriate testing procedures are required. Several test methods are available for ecotox studies using different aquatic and terrestrial invertebrate organisms. However, there is a lack of information regarding the suitability of the test systems for MNM testing.

As part of this study, bioaccumulation tests were performed with a range of invertebrate organisms to elucidate the bioaccumulation potential of different MNMs. Based on the results obtained, bioaccumulation endpoints such as bioaccumulation factors (BAF) or biomagnification factors (BMF) are calculated and compared. The pros and cons of the different test systems are discussed.

2. Materials and methods

Bioaccumulation studies were carried out with silver MNMs (NM300K) and titanium dioxide MNMs (NM105). The test media containing the particles were applied in studies on the water flea *Daphnia magna*, the freshwater amphipod *Hyalella azteca* and the freshwater bivalve *Corbicula fluminea*. Alternatively, test diets containing the MNMs were applied. Results were compared with data from studies on terrestrial oligochaetes and isopods. Test systems applied in this study are presented in Figure 1 and were carried out under flow-through or semi-static conditions. Animals were sampled through-out the uptake and elimination periods for the analysis of total metal content by ICP-MS or ICP-OES. In addition to that, tissue samples were further analysed for accumulation of single particles by sp-ICP-MS. Histological investigations were carried out to confirm the results obtained. Endpoints for bioaccumulation assessment were calculated and the trajectory of the tissue concentrations were compared. Differences between the test species with respect to uptake, bioavailability and bioaccumulation of the tested MNMs were identified. The suitability of the different test systems to be used as screening tools as part of a tiered bioaccumulation testing strategy as proposed by Handy et al. 2018 [2] are discussed.

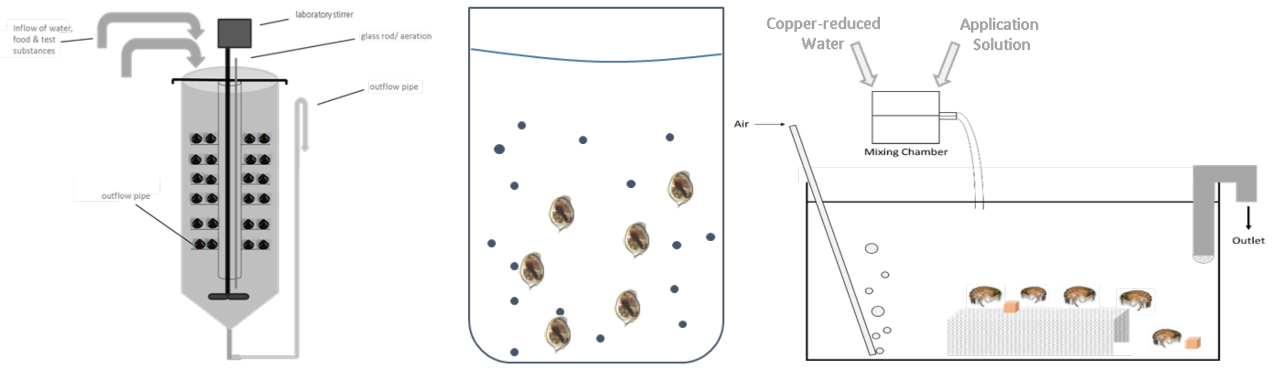

Figure 1: Exposure test systems for bivalves (left) , water fleas (middle) and amphipods (right)

3. Results and discussion

Results of bioaccumulation studies with NM 300K and NM 105, as obtained in flow-through tests with the freshwater bivalve *Corbicula fluminea,* show that constant exposure conditions can be reached. Tissue analysis gave insight into the uptake and elimination of silver and titanium dioxide (Figure 2). Both compounds showed different elimination behaviour which provide clear indications regarding the potential bioavailability of the test compounds. Bioaccumulation endpoints could be calculated, however, the presence of MNMs remaining in the gastrointestinal tract my confound the total burden measurement. In contrast to fish, the exclusion of gut tissue from the body burden determination is difficult or even impossible. The bioaccumulation factors obtained under different exposure concentrations are compared providing clear indications for concentration related effects.

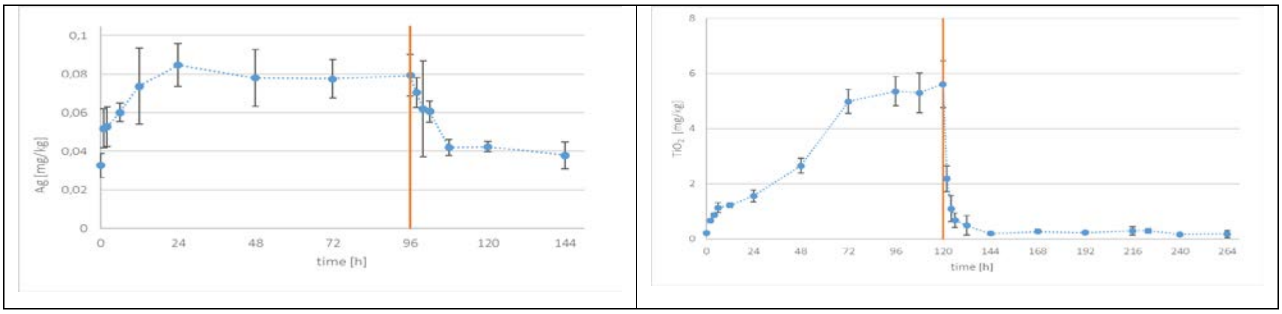

Figure 2. Bioaccumulation study with silver MNMs (NM 300K; left) and titanium dioxide MNMs (NM 105, right). Tissue concentrations of *C. fluminea*.

4. Conclusions

Bioaccumulation studies with invertebrate species can be carried out as part of a tiered bioaccumulation testing strategy. However, test systems have differing suitability for testing MNMs which may require specific adjustments of the test systems or the use of additional analytical approaches to guarantee a sufficient data quality for regulatory assessment.

5. References

[1] OECD (2012) ‘Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure’. OECD Publishing.

[2] Handy, R., Ahtiainen, J., Navas, J.M., Goss, G., Bleeker, E.A.J., von der Kammer, F. 2018. Proposal for a tiered dietary bioaccumulation testing strategy for engineered nanomaterials using fish. Environ. Sci.: Nano. DOI: 10.1039/c7en01139c

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