1. Introduction

Manufactured nanomaterials (MNMs) are increasingly used as additives or active components in various applications and commercial products, e.g. textiles, sunscreens, paints, drug delivery systems, cosmetics, medical devices for diagnostic, textiles, etc. Since MNMs exhibit altered physical and chemical properties compared to bulk materials, such as higher reactivity or higher specific surface area, and since they may show enhanced uptake due to their size, potentially hazardous biological outcomes are a concern with obvious relevance for an adequate understanding of the fate and possible adverse effects of MNMs in the environment. The project FENOMENO funded by FP7 ERA-NET (SIINN) is an integrative project aiming at an understanding of the impact of end-of-life manufactured nanomaterials (MNMs) on the environment. Even though MNMs are mostly removed during wastewater treatment [1], the remaining MNM levels in the effluents are significant and the mostly transformed MNMs [2] may show an increased toxicity for aquatic organisms due to their modification during the WWTP process. The biological impact of wastewater-borne MNMs (TiO2 and Ag MNMs) on different trophic levels and their bioaccumulation within a relevant food chain (algae-Daphnia-fish) have been investigated with innovative analytical and experimental approaches.

2. Materials and Methods

Several model WWTPs were conducted with nano-Ag and nano-TiO2 according to OECD Guideline 303A. The collected effluents were used to perform acute and chronic tests with Daphnia magna and Oncorhynchus mykiss according to the OECD guidelines 202, 211, 215 and 305. Animals were exposed to nano-Ag and nano-TiO2 in three different media (Figure 1). Tissue samples of the different test organisms were analyzed for changes in the levels of several biochemical markers [lipid peroxidation; activities of acetylcholinesterase (AChE), lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST)]. Furthermore, uptake and elimination kinetics of the MNMs were investigated by quantitative ICP-MS and ICP-OES analysis.

3. Results and discussion

3.1. Chronic Effects

Pristine nano-Ag and nano-Ag supplemented to WWTP effluents showed a significant effect on the reproduction of D. magna. After 21d exposure to nano-Ag up to a concentration of 64 µg/L, a dose-
dependant decrease of released neonates per adult could be found. For treated effluents absolutely no effect could be shown.

For nano-TiO₂, no effects on the reproduction of *D. magna* could be shown up to a concentration of 28 µg/L regardless of the tested media. Only after chronic exposure to the very high concentrations of 5 mg/L and 10 mg/L significant effects could be shown.

Neither nano-Ag nor nano-TiO₂ showed an effect on the growth of juvenile rainbow trouts after 28 days of exposure.

The analysis of biochemical markers showed that several effects induced by chronic exposure to MNMs were observed. However, no general effect pattern could be identified.

### 3.2. Bioaccumulation

Total Ag and total TiO₂ levels were measured in several tissue samples in *D. magna* and *O. mykiss* following exposure via the water or food. Pristine nanomaterials showed a significantly higher uptake into the test organisms compared to supplemented and treated WWTP effluents.

![Figure 2: Total Ag content in rainbow trout tissue after 28d exposure with nano-Ag. Bars are means of five samples ± SD and those with different letters are statistically different to each other, those with asterisks are statistically different to the control (p ≤0.05) according to Tukey’s test.](image)

### 4. Conclusions

Nano-TiO₂ showed no chronic effects in *D. magna* and *O. mykiss* at environmentally relevant concentrations, independent of the tested media. For nano-Ag, the WWTP process lead to reduced chronic effects and a significantly lower bioavailability of the tested nanomaterials.

### 5. References


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