Bioavailability and Bioaccumulation of Silver Nanoparticles in the Rainbow Trout *Oncorhynchus mykiss*

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Introduction

Silver nanoparticles (AgNPs) are present in a wide field of applications and consumer products, covering various industries like agriculture, medical engineering and clothing. Due to their extensive use, AgNPs are likely to be released into the environment, mainly via urban and industrial sewage. Even though AgNPs are mostly retained within the sludge of wastewater treatment plants (WWTPs), a small amount of mainly sulfidized particles still enters the aquatic environment, where they can be taken up by various aquatic organisms and transferred along the food chain.
In this study, uptake and bioavailability of AgNPs via aqueous and dietary exposure were investigated in the rainbow trout *Oncorhynchus mykiss*.

Materials & Methods

Several model WWTPs were conducted according to OECD Guideline 303A. The effluents containing AgNPs (present or supplemented manually) were compared to pristine AgNPs and used to perform a bioconcentration study with *O. mykiss* according to OECD Guideline 305. Fresh zooplankton was collected in a fishpond in southern Germany and enriched with pristine AgNPs. The loaded plankton was filtered, frozen, freeze-dried and processed into agar pellets, which can be eaten by fish. The agar pellets were fed to juvenile *O. mykiss* in a biomagnification study according to OECD Guideline 305 (Figure 1).

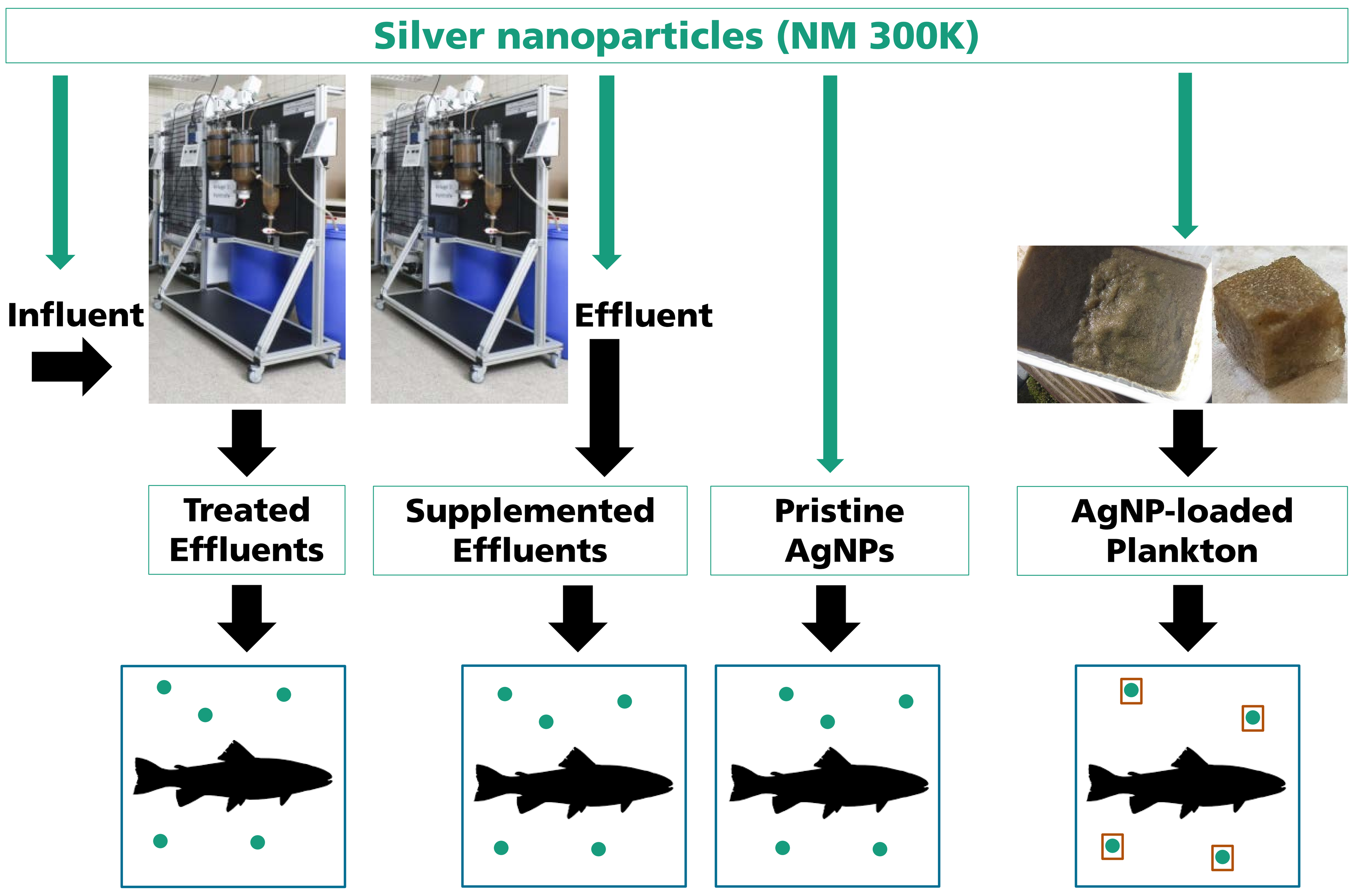

Figure 1: Experimental scenarios for the assessment of the bioaccumulation of AgNPs. Test animals were exposed to (i) WWTP effluent from AgNP-treated plants, (ii) uncontaminated effluent, manually supplemented with AgNPs, (iii) pristine AgNPs and (iv) AgNP-loaded plankton-agar-cubes.

Results

- No significant Ag uptake into the gills and the carcass of the analyzed fish could be found when AgNPs were present in or supplemented to WWTP effluent (Figure 2)
- Contrarily, when supplemented to tap water at a concentration of 12.4 µg Ag /L, AgNPs presumably adsorbed to the gill lamellae, leading to maximum total Ag tissue concentrations of ca. 100 µg/kg and 50 µg/kg in gills and carcass, respectively (Figure 2)
- For the dietary exposure studies, the prepared agar food pellets were measured to show a total Ag concentration of 121.5 µg/kg
- During examination of freeze-dried plankton using energy dispersive X-ray (EDX) detected AgNPs seemed to be sulfidized to a large content, due to the large sulfur signal intensity (Figure 4D)
- After ingestion of the prepared food, pronounced Ag concentrations could be found in the digestive tract up to 34.3 µg/kg (Figure 3)
- No increase of the total Ag concentration was detected in the carcass (Figure 3)

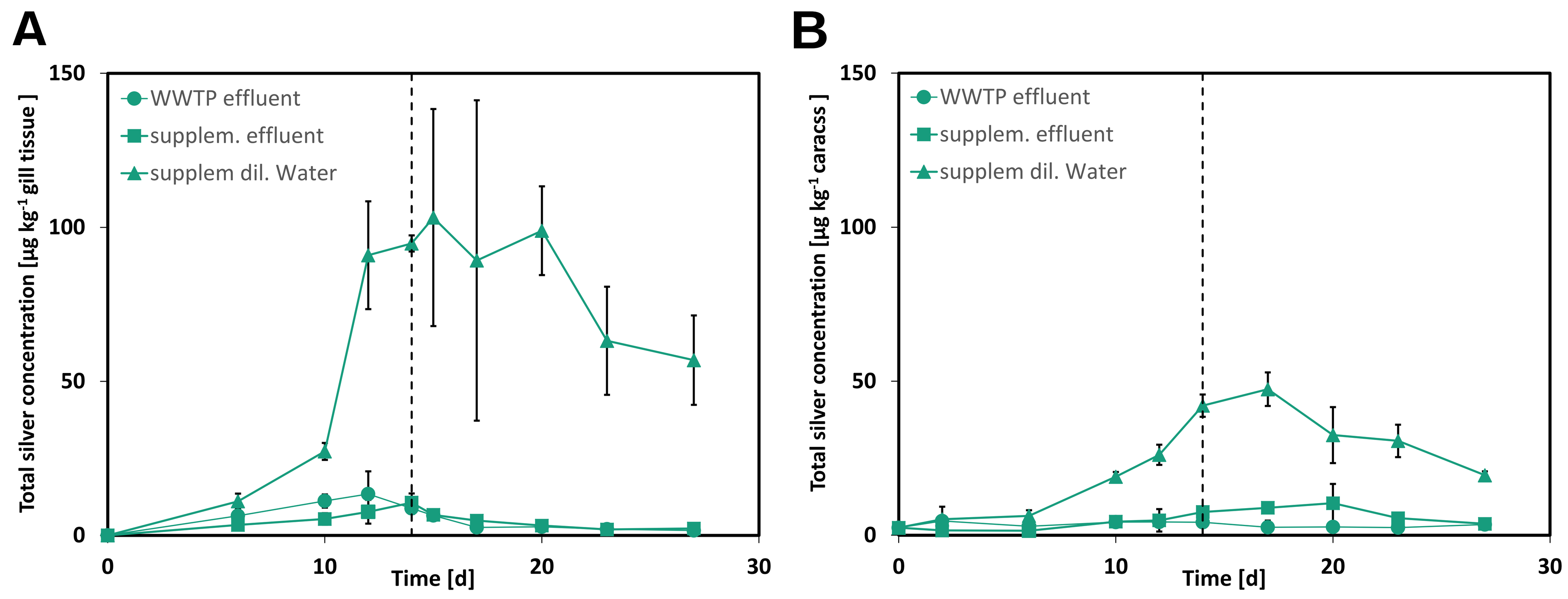

Figure 2: Total Ag concentration in gill tissue (**A**) and carcass (**B**) of *O. mykiss* during 14d exposure with waterborne AgNPs (NM 300K) and 14d of depuration.

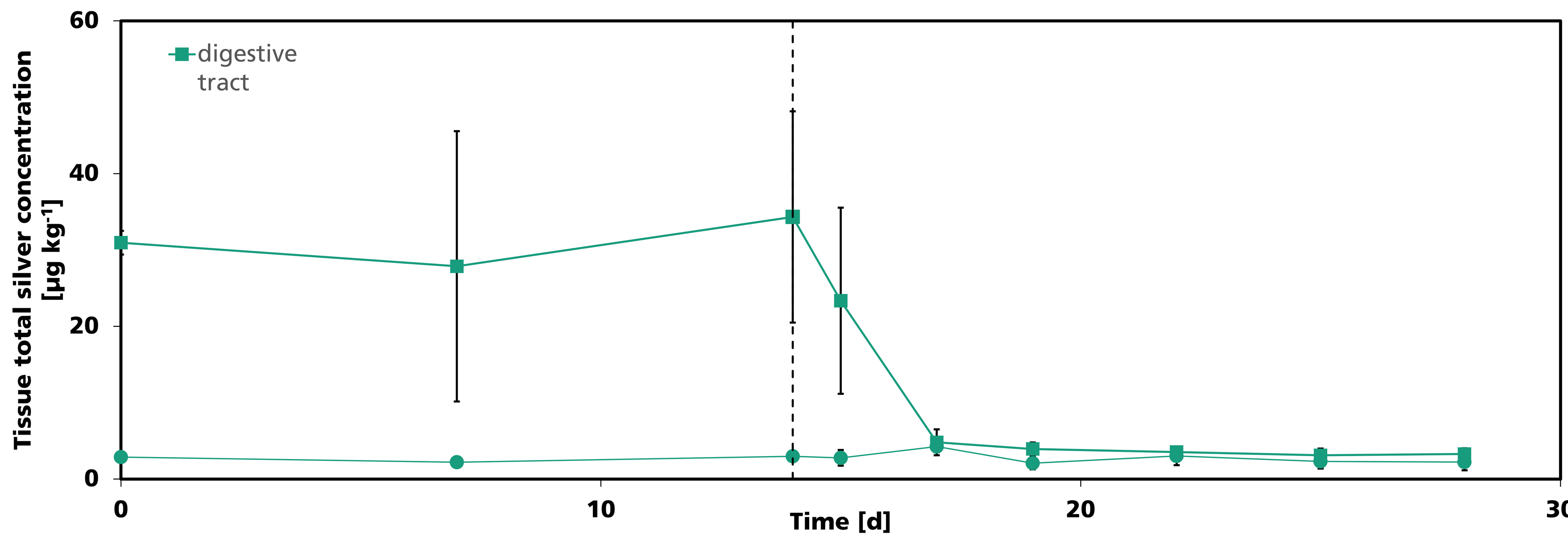

Figure 3: Total Ag concentration in digestive tract and carcass of *O. mykiss* during 14d exposure with dietary AgNPs (NM 300K) in plankton food and during 14d of depuration.

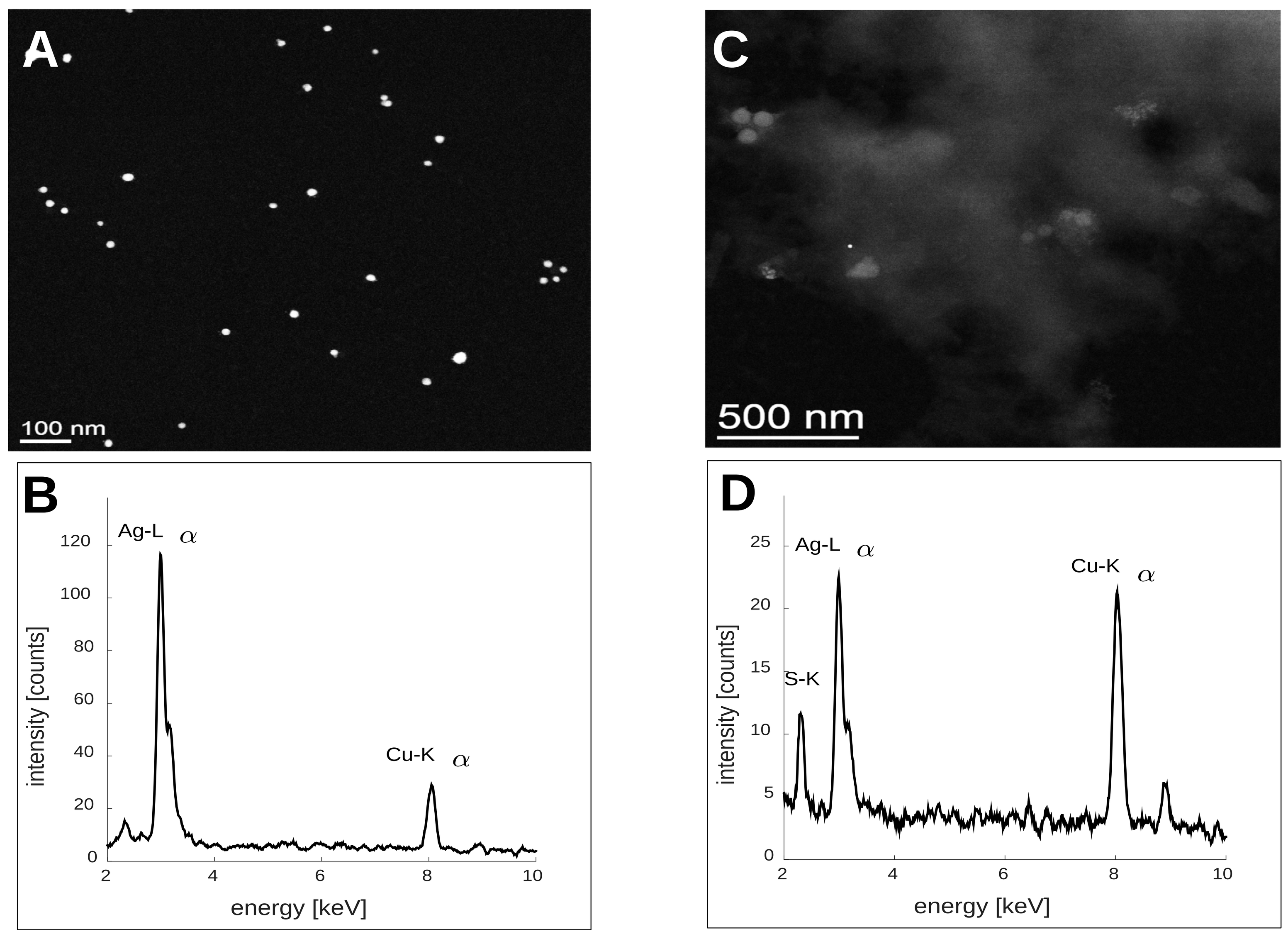

Figure 4: Transmission electron microscopy (TEM) with energy dispersive X-ray (EDX) of pristine reference material (**A & B**) and freeze-dried plankton containing silver nanoparticles (**C & D**)

Conclusions

- The presence of sulfides in WWTP effluents seems to decrease the bioavailability of Ag from waterborne AgNPs
- No or only negligible transfer of Ag through the intestinal walls could be detected after the ingestion of AgNPs in agar food pellets
- The adsorption of AgNPs to the gills of rainbow trouts seems to be the major entry route of Ag species into various tissues of the fish
- Therefore the release of Ag^+ ions is essential, which is dramatically lower, when AgNPs are sulfidized in the effluent matrix of a WWTP

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Due to the increasing use of silver nanoparticles (AgNPs) in the production of various products, the risk of AgNPs being released into the environment rises. Even though AgNPs are mostly retained within wastewater treatment plants (WWTP), a small fraction of predominantly transformed nanoparticles enters the aquatic environment. Besides the numerous studies on ecotoxicological effects, information on the transfer, bioavailability and bioaccumulation of AgNPs in the aquatic food chain is essential for risk assessment. The aim of this study was to investigate bioavailability and bioaccumulation of waterborne and dietary AgNPs in the rainbow trout *Oncorhynchus mykiss*.

Several model WWTPs were conducted according to OECD Guideline 303A. The effluents containing AgNPs (present or supplemented manually) were compared to pristine AgNPs and used to perform a bioconcentration study with O. mykiss according to OECD Guideline 305. Fresh zooplankton was collected in a fishpond in southern Germany and enriched with pristine AgNP. The loaded plankton was filtered, frozen, freeze-dried and processed into agar pellets, which can be eaten by fish. The resulting fish food was stable and displayed a homogenous AgNP distribution. The agar pellets were fed to juvenile *O. mykiss* in a biomagnification study according to OECD Guideline 305. Tissue concentrations during uptake and elimination in *O. mykiss* exposed to AgNPs via water or food were investigated by quantitative inductively coupled plasma mass spectrometry (ICP-MS). Enriched plankton samples were analysed by high-resolution transmission electron microscopy (TEM) with energy dispersive X-ray (EDX).

For waterborne AgNPs present in or supplemented to WWTP effluents no significant uptake into the carcass of the test organisms could be detected. However, exposure to pristine AgNPs was leading to the accumulation of silver in fish tissues presumably driven by the uptake of silver ions released by the AgNPs.

Biomagnification studies showed that pronounced silver concentrations could be found in the digestive tract. However, only a limited biomagnification of silver could be detected.