Investigations on the uptake of nanostructural polystyrene in the fresh water mussel *Corbicula fluminea*

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**Introduction**

Polystyrene is one of the mostly used type of plastic and could enter the environment by direct release from industrial products during their usage as well as by their disposal. In the environment, those macro- and microplastics could undergo weathering through biological degradation, mechanical erosion and UV radiation and could thus be transformed into nanoplastic particles. Most nanoparticles (NPs) tend to sediment in water and are supposed to be taken up by benthic species in aquatic ecosystems. Different studies have shown that mussels are able to ingest and to incorporate NPs suspended in water. By this the NPs may bioaccumulate in these organisms and thereby enter the food chain at a very low trophic level. The filter feeding freshwater bivalve *Corbicula fluminea* was used to investigate the uptake of nanostructural polystyrene (nPS) from the water phase. By the use of fluorescent nPS and fluorescence microscopic analysis we were able to investigate the uptake, localisation and elimination of the nPS in the soft body of *C. fluminea* after different exposure and depuration times.

**Methods**

The mussels were exposed to 5 mg nPS L⁻¹ under semi static conditions for 24 hours with a full water exchange after 12 hours. For the following depuration phase lasting 24 hours the mussels shells were brushed and rinsed before transferred into a new, clean water tank. The depuration water was exchanged after 12 hours. Animal and feces were collected each after 12 and 24 hours of exposure and depuration phase.

The soft body was dissected to gain the adductor muscles, the foot, the mantle and the viscera (including the gills and the digestive tract) as isolated compartments. Those compartments, as well as squash preparations were examined using a fluorescence microscope.

**Results**

- **Fig. 1:** *Corbicula fluminea* sampled from the river Niers
- **Fig. 2:** *Corbicula fluminea* exposed to nPS under semistatic conditions
- **Fig. 3:** Compartments of the soft body and squash preparation for the further examination
- **Fig. 4:** Fluorescence hot spots in the gills (A) and in the gut (B) after 24 h of exposure
- **Fig. 5:** Feces collected after 12 h (A) and 24 h (B-D) of exposure and after 12 h of depuration (C & D); no fluorescence was visible after 24 h of deparuration
- **Fig. 6:** Mantle tissue: A (control), B after 24 h of exposure, C after 24 h of depuration. Viscera tissue: D (control), E after 24 h of exposure, F after 24 h of depuration

- **A**
  - Tissues related to the food uptake and digestion showed the strongest fluorescence
  - Nearly no fluorescence visible in the muscle tissue

- **B**
  - nPS exposure induced high filtration activity and excretion of feces
  - Feces showed very strong fluorescence after 12 and 24 hours of exposure, but no fluorescence after 24 hours of depuration

- **C**
  - Tissues related to the food uptake showed decreased fluorescence after depuration

**Conclusions**

The results indicate that the particles are ingested and temporarily adsorbed to the tissue surfaces, but finally pass the digestive system without being incorporated into the organisms tissues and are obviously not bioavailable for mussels. A rapid and effective elimination of the nPS was observed after 24 hours of depuration in the tissues related to the uptake and transport of food. The ingested nPS seems to be concentrated in the feces and eliminated from the soft tissue by the release of the feces. Furthermore, two scenarios of secondary poisoning are likely: i) the uptake of highly contaminated feces or pseudo feces by benthic invertebrates that feed on fecal matter and ii) predators feeding on the NP loaded mussels tissue.

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Most nanoparticles (NPs) tend to sediment in water and are supposed to be primarily taken up by benthic species in aquatic ecosystems. Different studies have shown that mussels are able to ingest and to incorporate NPs suspended in water. By this the NPs may bioaccumulate in this organisms and thereby enter the food chain at a very low trophic level leading to potential further biomagnification.

The filter feeding freshwater bivalve *Corbicula fluminea* was used to investigate the uptake of nanostructural polystyrene (nPS) from the water. By the use of fluorescent nPS and fluorescence microscopic analysis we were able to investigate the uptake, localisation and elimination of the nPS in the soft body of *C. fluminea* after different exposure and depuration times.

During the exposure period the nPS induced a high filtration activity resulting in a strong production of (pseudo-)feaces, whereby the feaces showed a strong fluorescence intensity. Also the soft tissue of the mussel showed a high strength of fluorescence after 24 hours of exposition with different intensities for the different compartments like the mantle, the foot, adductor muscles and the viscer (including the gills and the digestive system). The results indicate that the particles are ingested, are temporarily adsorped to the tissue surfaces, finally pass the digestive system without being incorporated into the organisms tissues and are obviously not bioavailable for mussels. A rapid and effective elimination of the nPS was observed after 24 hours of depuration.