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ABSTRACT BOOK

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The applied methods indicated that soil microbes from polluted O horizons react differently to additional disturbance than those from unpolluted ones. In the AE horizon the effect of pollution is less evident.

NM01 - Effects and risks of nanomaterials

MO 336

Uptake of carbon nanotubes by the green alga Desmodesmus subspicatus

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Due to their exceptional thermal and electrical conductivity, low density, and extraordinary strength, carbon nanotubes (CNT) are promising for application in different industrial fields. Therefore, potential risks to the environment should be assessed.
This study deals with the bioavailability of CNT for a primary producer, the green alga Desmodesmus subspicatus. Radiolabelled multiwalled CNT (14C-MWCNT) were synthesised by means of chemical vapour deposition of 14C-benzene. A 14C-CNT/L dispersion was prepared in the medium recommended in OECD guideline 201. After subjection to ultrasonication with a microtip, a dispersion containing both crushed agglomerates and single nanotubes was obtained, as was shown by means of transmission electron microscopy (TEM). Algae cells were added and exposed to 1 mg 14C-CNT/L. for 24, 48 and 72h (4 replicates per sampling point). At sampling time, algae and CNT material were successfully separated by means of density gradient centrifugation in 2 steps. After separation, the density of the algae sample was determined by fluorescence measurements before they were gathered on a filter prior to liquid scintillation counting (LSC).
14C-CNT were taken up by the algae over time. Bioavailability of CNT for the algae was confirmed by TEM, as small CNT agglomerates were observed inside the cells. Further experiments, feeding 14C-CNT exposed algae to Daphnia magna, are planned to investigate the biomagnification potential. The interaction of CNT and algae cells will be studied by means of two photon excitation microscopy (TPEM). Furthermore, elimination experiments, in which 14C-CNT exposed algae are transferred to clear water, are performed.

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Evaluation of chronic effects of nanodiamond on Daphnia magna

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Nanomaterials have significant technological advantages but research studies point out to potential ecotoxicological risks, probably due to their new physico-chemical characteristics. Carbon-based nanoparticles and particularly nanodiamond have great interest in industrial and medical applications. The aim of the present study was to overview chronic effects of nanodiamond on reproduction and gut tissue morphology of the freshwater crustacean *Daphnia magna*. Aqueous suspensions of nanodiamond from NanoCarbon Research Institute (Japan), with an average particle size of 20 nm, were prepared and characterized. Results of 21 days toxicity tests showed significant differences in survival and reproduction between exposed and control *Daphnia*, 100% mortality occurred for concentrations higher than 12.5 mg/l and reproduction inhibition was observed for concentrations higher than 1.3 mg/l. Histological observation of exposed females showed that *D. magna* was able to ingest particles/aggregates of nanodiamond from aqueous suspensions, which accumulate within the gastrointestinal tract mixed with food. This accumulation in the gut lumen seems to cause significant changes in gut apical cell surface and epithelial cell degeneration, suggesting that food absorption by intestinal cells can be inhibited. Results indicate that there may be long term inhibitory effects of nanodiamond on food absorption and reproduction of *Daphnia magna*, an ecological significant organism with an important role in regulatory testing of chemicals, waters and wastes. Chronic assessment, including histological observation to follow the tract of ingested nanoparticles, proved to have an added value in environmental protection as part of an integrated environmental strategy.

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Distribution of multiwalled 14C-carbon nanotubes to the zebrafish Danio rerio and visualisation of non-labelled nanotubes in different organs and tissues

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Carbon nanotubes (CNT) are considered one of the most promising materials in nanotechnology. We investigated the bioavailability of radiolabelled multiwalled CNT (^{14}C-CNT) to the zebrafish *Danio rerio* by quantifying uptake over time after both water and dietary exposure.
^{14}C-CNT were synthesized by means of chemical vapour deposition of ^{14}C-benzene in a small-scale batch reactor. Zebrafish were exposed separately via water (1 mg ^{14}C-CNT/L & 8 mg DOC/L) and via a diet of 72h preliminary exposed blackworms (*Lumbriculus variegatus*). The exposure time was set to 48 hours with measurements after 3, 24 and 48 hours. At sampling time, the fish were anaesthetised, killed and dissected into gut, gills, brain and leftover fish. The internal radioactivity of the tissue was measured via solubilising and Liquid Scintillation Counting (LSC).
After exposure via the water in presence of DOC, ^{14}C-CNT were found in both the gut and the gills of the fish. It was observed that fish activity resulted in quicker ^{14}C-CNT agglomeration in the test medium than in controls without a fish. After dietary exposure, ^{14}C-CNT were only detected in the gut. The ^{14}C-CNT were eliminated to the water phase over the course of the experiment. There was no distribution to the brain or other tissues after both water and dietary exposure.
These results show that dispersed ^{14}C-CNT are taken up by zebrafish via both water and dietary exposure. Interaction of CNT with the cells of the fish´s gut and gills will be investigated. Therefore, imaging with transmission electron microscopy (TEM) and two-photon excitation microscopy (TPEM) is under progress.

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Sublethal effects of multi-walled carbon nanotubes on Daphnia

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Relatively little is known about the potential impacts of engineered nanoparticles on aquatic biota. Particularly relevant to aquatic ecosystems are those particles which display increased solubility either through specialized coatings or through an ability to interact with water column constituents such as natural organic matter (NOM). Previous research has indicated that grazing zooplankton (Daphnia magna) are able to ingest lipid-coated single-walled carbon nanotubes (SWNTs) from the water column during their normal feeding behavior. Acute mortality was observed only at high concentrations (>5mg/L). In this research NOM was used in place of a surfactant to stabilize suspensions. Water chemistry (ionic strength, hardness, pH) has been shown to alter the behavior of NOM in natural systems. We hypothesized that these same variables may also affect the toxicity of multi-walled carbon nanotubes (MWNT) stabilized in NOM. The purpose of this research was to examine the potential for sublethal effects to occur following exposure to multi-walled carbon nanotubes suspended in NOM and to determine whether those effects vary with pH alterations.

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Uptake and effects of manufactured nanoparticles on rainbow trout (Oncorhynchus mykiss) gill cells

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The use of manufactured nanomaterials is increasing rapidly and so is the probability that they end up in the environment what rises concerns about eventual toxic effects. Silver is one of the most frequently used nanomaterials in consumer products and is known to be toxic to aquatic organisms in its ionic form. In this study the uptake and the effects of two types of silver nanoparticles (citrate coated, approx. 15 nm; PVP coated approx. 7nm) were studied with most tests also including ionic silver applied as $AgNO_3$. Rainbow trout (Oncorhynchus mykiss) gill cell monolayers were used to determine possible toxic effects like a decrease in membrane integrity, as well as to visualise nanoparticle uptake with light microscopy and transmission electron microscopy (TEM). Moreover, gill cells were grown in multilayers on permeable support for studying the transport of silver through epithelial layers. Silver concentrations where measured by inductive coupled mass spectrometry (ICP-MS). Gill cells showed a reduction of membrane integrity of about 20% after a 48 h exposure to manufactured silver nanoparticles as well as ionic silver. Nanoparticles were taken up into monolayered gill cells where they accumulated near the nucleus. TEM studies showed silver nanoparticles in cells were present mostly in multilammelar bodies. Moreover silver was transported through gill cell multilayers after a 48h exposure to 10 mg/L of silver nanoparticles. Transport rates varied between 80 µg/L and 1.3 µg/L and were depending on the trans epithelial resistence (TER) of the epithelial layers. This study shows the uptake of silver