Ecotoxicity of photocatalytically active titanium dioxide nanoparticles: A model study with *Caenorhabditis elegans*.

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Background

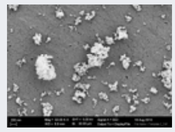
Titanium dioxide nanoparticles (TiO_2 NPs), which are increasingly used in a variety of consumer products, enter the aquatic environment with urban and industrial waste waters. According to modeled data, TiO_2 NPs are expected to accumulate in the sediment phase with a rate of 273 to 1409 µg kg^{-1} y^{-1}[1]. The ecotoxicological relevance of TiO_2, however, is still poorly understood. This ongoing study aims to elucidate the ecotoxicological effects of TiO_2 NPs on the aquatic environment based on lab experiments using *Caenorhabditis elegans* as a model for for the elucidation of specific toxic responses. As many of the commercially used TiO_2 NPs are photocatalytically active, the main focus of this study will be the impact of UV-exposure on the toxicity of the nanomaterial. This poster shows first results on the effects of unirradiated particles towards *C.elegans*.

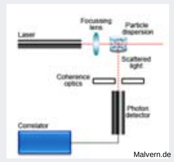
Materials and methods

The standardized bioassay ISO 10872, a chronic test using the nematode *Caenorhabditis elegans* as model organism, is applied for the nanomaterial testing. Ecotoxicological endpoints are reproduction, fertility and growth.

The testmaterial P 25 Aeroxide (Degussa AG) has a primary particle size of 21 nm and a crystal structure of 75% anatase and 25% rutile. It is dispersed in ultrapure water without stabilizers using magnetic stirring and an ultrasonic bath.

The particle size distribution in the TiO_2 dispersions introduced to the test are determined by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM).





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Results and Discussion

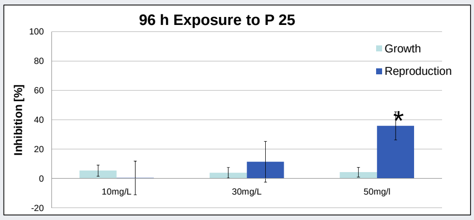

*Fig. 2: Inhibition of growth and reproduction of C.elegans exposed to TiO_2 for 96 h [mean inhibition and CV in %]; * p-Value <0.01 (one way ANOVA)*

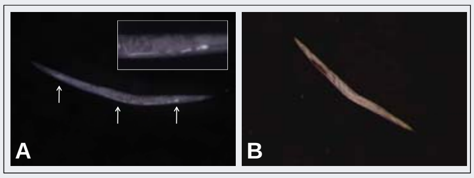

Fig. 1 Stereomicroscope images
A: C.elegans exposed to 20mg/L TiO_2 for 96 h;
B: C.elegans exposed to TiO_2 -free media for 96 h (Control)

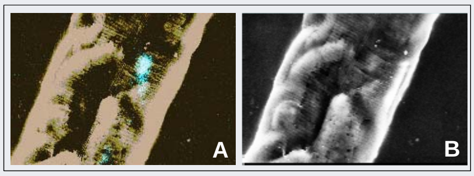

Fig. 2: Critical point dried worm exposed to 20 mg/L TiO_2 for 96 h,
A: EDX mapping of Titanium with 20 keV
B: SEM image, SE detector, 5 keV

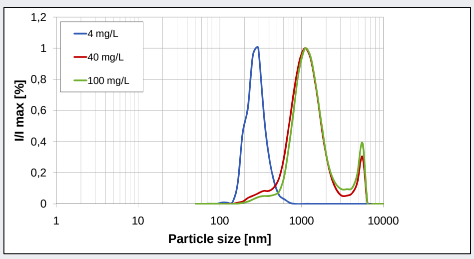

Fig. 3: Particle size distribution at concentrations of 4, 40 and 100 mg/L TiO_2 gained by DLS; shown as standardized "Mean Intensity"-values.

Conc [mg/L]	Media	PDI	Average particle size*[nm]	CV [%]
4	H_2O	0,6071	285	21
40	H_2O	0,4673	1439	19
100	H_2O	0,4564	1637	22
2	H_2O+ M9	0,6322	647	15
20	H_2O + M9	0,4683	1688	29
100	H_2O + M9	0,563	1111	41

*Tab. 1: DLS measures of TiO_2 dispersions in H_2O, pure and introduced to M9 media. * Average Intensity mean values (10 repeated measures); PDI: Poly Dispersity Index.*

Chronic effects

Significant effects on reproduction were observed after 96 h exposure to TiO_2 NPs (35% inhibition of the reproduction rate at 50 mg/L).No significant effects on growth rate were observed up to 50mg/L.

Long-term- effects

No significant effects on reproduction and growth rate were observed up to 50mg/L after 12 days exposure to TiO_2 NPs.

Uptake of TiO_2 particles

Agglomerated particles are visible in the intestinal tract of about 80% of the worms after 96 h exposure to TiO_2 (Fig. 1). Titanium was located subcutaneously with Energy Dispersive X-ray (EDX)-analysis (Fig. 2).

Elimination of TiO_2 particles

Agglomerates are egested completely after 96 h in TiO_2 free media, visible (no figure shown).

Particle size distribution

All dispersions show a wide range of particle sizes, which is also reflected in the relatively high PDI values (tab.1). Particle sizes in the dispersions increase with increasing particle concentration.

Agglomeration behavior

According to the DLS data (table 1), addition of M9 media to TiO_2 dispersions leads to agglomeration and formation of larger particles. Agglomeration and sedimentation of the particles is also visible macroscopically.

Conclusions

Reproduction of *C.elegans* is inhibited significantly by exposure to TiO_2 NPs at concentrations of 50 mg/L.

This study provides strong evidence for ingestion and egestion of agglomerated TiO_2 NPs by *C.elegans*.

The problem of agglomeration effects in this bioassay is currently unresolved.

The effects of unirradiated TiO_2 observed in this study do not indicate an environmental risk regarding the expected environmental concentrations.

Future perspectives

The further studies aim at investigating

- photo induced toxic effects of P 25 as a photo catalytic substance
- the influence of P 25 on toxicity of co-contaminants elucidating the importance of photo-modification and photo-sensitization processes
- the impact of TiO_2 -contamination on sediment toxicity.

The further studies aim at establishing

- Scanning Electron Microscopy to monitor particle size distribution during ecotoxicological testing.

Reference
[1] Gottschalk, F.et al., 2009. Modeled Environmental Concentrations of Engineered Nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for Different Regions. *Environmental Science & Technology*, 43(24), 9216-9222.

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