Effects of ZnO nanoparticles on Algae, Daphnia and Zebrafish larvae.

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Background:

- Incorporation of engineered nanoparticles (NPs) into consumer products is increasing rapidly and contamination of the environment with potential exposure of organisms has become a realistic concern.

- The aquatic toxicity of metal oxide nanoparticles is related to NP physicochemistry and the characteristics of the exposure medium.

- Toxicity of metal NPs has frequently been attributed to dissolution of metal ions; however, the contribution of particles to toxicity (or "nano-specific" effects) has not been resolved for most NPs.

- Zinc oxide, ZnO, NPs are widely used in commercial products because of their chemical stability, strong adsorption and antibacterial property. They are one of the main components in sunscreens due to their effectiveness at shielding UVA.

Our objectives:

- To assess the toxicity of ZnO NPs (coated (nominal size: 152 nm) and uncoated (nominal size: 70-90 nm)), in parallel with the ionic $ZnSO_4$, in three different aquatic organisms: an algae (*Raphidocelis subcapitata*), a crustacean (*Daphnia magna*), and a vertebrate (Zebrafish, *Danio rerio*).
- To evaluate the effects of exposure media on the toxicity of the NPs in Zebrafish larvae.

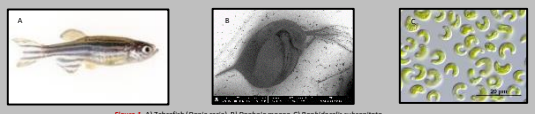

Figure 1. A) Zebrafish (*Danio rerio*), B) *Daphnia magna*, C) *Raphidocelis subcapitata*

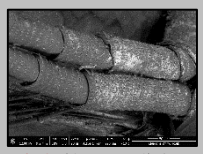

Figure 2. Daphnia magna antennae with ZnO NPs.

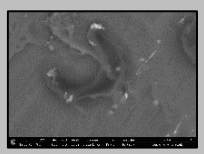

Figure 3. R. subcapitata with NPs clusters.

Method:

- **Zebrafish**: The toxicity of the triethyoxycaprylsilane coated ZnO NPs (NM-111) and uncoated ZnO NPs (NM-110) (1-100 mg/L), and $ZnSO_4$ (1-500 mg/L), to Zebrafish (*D. rerio*) larvae (72-96 hpf) was assessed following 24 h exposure in 3 different media: Sea salt (Egg water) medium (60 mg/L Instant Ocean), OECD medium ($CaCl_2$ 0.294 g/L, $MgSO_4$ 0.1232 g/L, $NaHCO_3$ 0.0647 g/L, KCl 0.0057 g/L) or E3 medium (NaCl 0.290 g/L, KCl 0.013 g /L, $CaCl_2*2H_2O$ 0.048 g/L, $MgCl_2*6H_2O$ 0.082 g/L, 1 % Methylene blue).

- **Daphnia**: Acute immobilization test (48 h, OECD test guideline 202) (at concentrations 0.16-10 mg/L) and chronic reproduction test (21 days, OECD test guideline 211) (at concentrations 180-1400 µg/L) were performed to determine the toxicity of ZnO NPs (NM-110 and NM-111) compared to $ZnSO_4$.

- **Algae**: The toxicity of ZnO NPs (NM-110 and NM-111) in *R. subcapitata* was tested following OECD algal growth inhibition assay (guideline 201). Algal cells were exposed 96 h to ZnO NPs (10-76 µg/L), and samples were extracted for chlorophyll-a and measured as a surrogate measure for algae population growth.

Results:

- In **Zebrafish** larvae, ZnO NPs caused mortality in Sea salt (coated ZnO NPs: LC50 = 13.9 mg ZnO/L (11.1 mg Zn/L), uncoated ZnO NPs: LC50 = 15.0 mg ZnO/L (12.0 mg Zn/L)) and E3 medium (uncoated ZnO NPs: LC50 = 15.3 mg ZnO/L (12.3 mg Zn/L)), but no mortality was observed by either ZnO NPs in OECD medium (*Figure 4*).

- There was no difference in mortality in larvae exposed to either coated or uncoated ZnO NPs (i.e. similar LC50 values) (*Figure 4*).

- $ZnSO_4$ caused mortality in both Sea salt and OECD media, with similar LC50 values (Sea salt LC50: 126.4 mg $ZnSO_4$/L (28.7 mg Zn/L), OECD LC50: 88.5 mg $ZnSO_4$/L (20.1 mg Zn/L)) (*Figure 4*).

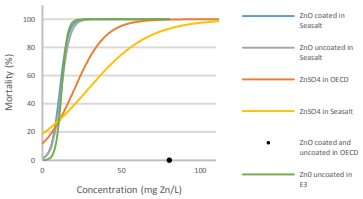

Figure 4. Mortality (%) of Zebrafish larvae (72-96 hpf) after exposure to coated and uncoated ZnO NPs and $ZnSO_4$ in different exposure media for 24 h.

EC50	ZnO NPs uncoated	ZnO NPs coated	$ZnSO_4$
24 h	5.18 mg ZnO/L (4.16 mg Zn/L)	7.72 mg ZnO/L (6.20 mg Zn/L)	33.74 mg $ZnSO_4$/L (7.66 mg Zn/L)
48 h	2.43 mg ZnO/L (1.95 mg Zn/L)	2.41 mg ZnO/L (1.94 mg Zn/L)	9.98 mg ZnO/L (2.27 mg Zn/L)

Table 1. EC50 values from *D. magna* acute immobility test (OECD 202)

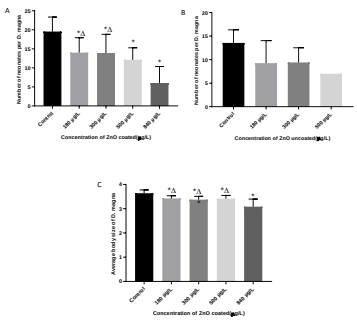

Figure 5: Chronic (21 days) exposure of *D. magna* to ZnO NPs. * indicate statistical significant difference from control, Δ indicate significant difference from the 840 µg ZnO/L group, P-value < 0.05. A) Number of neonates produced per adult when exposed to coated ZnO NPs. B) Number of neonates produced per adult when exposed to uncoated ZnO NPs. C) Average body size after exposure to coated ZnO NPs.

- Exposure of ***R. subcapitata*** to ZnO NPs caused decreased growth (measured as chlorophyll-a concentration) with increasing NP concentrations (*Figure 6*).

- Growth inhibition EC50 values after 24 h, 48 h, 72 h and 96 h after coated and uncoated ZnO NPs exposure is shown in *Table 2*.

EC50	ZnO NPs Uncoated	ZnO NPs Coated
0-24 h	40.71 µg ZnO/L	29.06 µg ZnO/L
0-48 h	28.32 µg ZnO/L	25.29 µg ZnO/L
0-72 h	30.56 µg ZnO/L	17.26 µg ZnO/L
0-96 h	29.59 µg ZnO/L	20.68 µg ZnO/L

Table 2. Growth inhibition EC50 values from *R. Subcapitata* ZnO NPs exposure for 24-96 hours.

- In **Daphnia** acute toxicity tests, the concentration estimated to cause 50 % immobility (EC50) from exposure to coated and uncoated ZnO NPs and $ZnSO_4$ is shown in *Table 1*.

- Results from the chronic exposure showed that coated ZnO NPs, decreased reproduction in *D. magna* significantly in a concentration dependent manner (*Figure 5A*).
- At concentration 840 µg ZnO/L the number of neonates per adult had decreased to 6 compared to 20 neonates per adult in the control group.
- At the highest concentration (1400 µg ZnO/L) all animals died before the end of the experiment.

- For the uncoated ZnO NPs, all organisms died before end of exposure at concentrations 840 and 1400 µg ZnO/L during chronic exposure.
- There was a concentration-dependent but non-significant trend of decreasing reproduction (7 neonates/adult at 500 µg ZnO/L compared to 14 neonates/adult in control) (*Figure 5B*).

- Exposure to coated ZnO NPs also caused a significant reduction in body size for all concentrations 180-840 µg ZnO/L (*Figure 5C*).
- The average body size for the control animals was 3.64 mm whereas the average sizes of organisms exposed to concentrations 180 µg ZnO/L was 3.42 mm, 300 µg ZnO/L was 3.38 mm, 500 µg ZnO/L was 3.42 mm, and 3.09 mm for animals exposed to 840 µg ZnO/L.

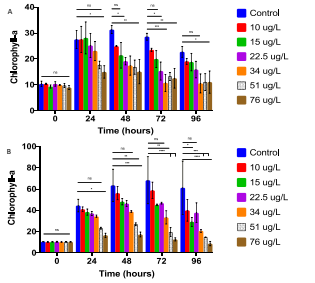

Figure 6: Chlorophyll-a content in samples of *R. subcapitata*, treated with different concentrations of coated ZnO NPs (A) and uncoated ZnO NPs (B), throughout a 96 h period. Samples were harvested at 24 h intervals, starting at 0 h. Data shown as mean ± SEM. Significant differences ($p<0.05$) compared to the control at each point of time and their levels of significance are marked by *.

In conclusion:

Zebrafish:

- **ZnO NPs caused mortality in Zebrafish larvae exposed in Sea salt and E3 media, but not in OECD media.**
- **There was no difference in mortality upon exposure to coated and uncoated ZnO NPs.**
- **$ZnSO_4$ caused mortality in both Sea salt and OECD media, with similar LC50 values.**
- **The toxicity of the NPs was greater than the Zn ion ($ZnSO_4$ was less toxic than ZnO NPs); this being consistent with results from the literature.**

Daphnia:

- **In *D. magna* acute immobility test, $ZnSO_4$ was less toxic than ZnO NPs (when EC50 concentrations are calculated as mg Zn/L). There was no difference in immobilization upon exposure to coated and uncoated ZnO NPs at 48 h.**
- **Exposure to coated ZnO NPs, reduced reproduction significantly in a concentration dependent manner. There was a similar but non-significant trend of reduction in reproduction for Daphnia exposed to uncoated ZnO NPs.**
- **Exposure to coated ZnO NPs caused a significant reduction in body size.**

Algae:

- **Exposure of *R. subcapitata* to ZnO NPs caused decreased growth (measured as chlorophyll-a concentration).**
- **The results suggest that coated ZnO NPs was more toxic to the algal test species than uncoated ZnO NPs.**

This work is part of the project **P**hysiologically **A**nchored **T**ools for **R**ealistic nan**O**materiaL a**S**sessment (760813)
Project website: www.patrols-h2020.eu

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