Report

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C. Svendsen - Nanoparticle immunotoxicity using an environmental sentinel as a model.
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Summary of main achievements and outcomes; The earthworm *Eisenia veneta* was successfully exposed to various metal based nanoparticles (NP). Exposures were undertaken *in vivo* of individual earthworms in aqueous and soil media, and *in vitro* of extracted immune cells in physiological saline. Exposures were conducted to ZnO nanopowder (Sigma-Aldrich Ltd., Dorset), and three types of TiO_2; namely optisol® (Oxonica, Begbroke Science Park, Oxfordshire), Degussa P25 and T805 (Evonik Degussa, Germany), as per the grant. Additionally we tested CdS particles (donated by Paul Christian at Birmingham via CEFAS Weymouth). Tissue metal analysis is underway.

The main outcome has been that **during both *in vivo* and *in vitro* exposures we have seen little or no immunotoxicity caused by the metal NP even at high doses**. The only impairment of immune activity was observed following exposures to ZnO NP, and the effects were much lower than those caused by matching concentrations of $ZnCl_2$ (based on metal mass). **These results support the growing body of evidence that toxicity of metal NP does not exceed that of their ionic form.** We have met all the objectives set out in the grant, although the RAMAN work needs elaborating.

Exposure validation: The aqueous and physiological saline solutions of the NP used for exposures were made up and stirred prior to use (but not sonicated). This was to obtain the most natural exposure conditions possible. Partical size distributions and aggregation were tested using the NanoSight and EM equipment, under the expert quidance kindly made available by Alison Crossley at Oxford University, Begbroke Science Park. For ZnO NP @ 35mg NP/l aggregates proved to be in the 150 – 300 nm range. For TiO_2, the Degussa P25 NP @ 25 mg NP/l were mainly present as 5-10 particle aggregates with very few small and large (>500 nm) aggregates. Degussa T805 NP @ 17.5 mg NP/l proved very hydrophobic and had few particles <20 nm, some at 100-300nm, but was mostly present as 500nm aggregates. Oxonicas TiO_2 Optisol® @ 7.8mg NP/l had particle sizes of around 10-100nm, but was mainly present as 250-400nm aggregates. The Birmingham CdS NP were too small to be observed on the NanoSight, indicating that individual particles of 5nm do not aggregate to more than 20nm aggregates, and most likely are present as single particles.

ZnO NP vs $ZnCl_2$ *in vivo*: Initial *in vivo* exposures to ZnO NP vs. matched $ZnCl_2$ showed no effect on immune activity of either Zn form up to the top dose of 12.5mg/l. An *in vivo* exposure was then set up in soil using controls and two concentrations (viz. 250 & 750mg Zn/kg soil) of both $ZnCl_2$ and ZnO NP. After 21 days exposure to $ZnCl_2$, somatic growth was reduced at 750mg Zn /kg soil while reproduction declined at both concentrations. Exposure to ZnO NP had no significant effect on growth and only reduced reproduction slightly at 750 mg Zn/kg soil. Immune activity was significantly lowered by both $ZnCl_2$ treatments, while ZnO NP treatments had no detectable effect.

TiO_2 NP *in vivo*: No effects were observed on any parameter after exposures to Degussa P25 NP @ 25 mg NP/l, Degussa T805 NP @ 17.5 mg NP/l, or Oxonicas TiO_2 Optisol® @ 7.8mg NP/l.

ZnO NP and TiO_2 NP *in vitro*: 24 hour exposure of immune cells to TiO_2 NP: P25 @ 12.5mg/l, T805 @ 8.75 mg/l & Optisol @ 3.9mg/l resulted in no effects on immune activity, while exposure to ZnO NP 35 mg/l caused a significant drop in immune active cells from 90% in the control to 55% in the treated cells.

CdS NP results: Comparing *in vivo* exposures to CdS NP and $CdCl_2$ showed that survival was affected by $CdCl_2$ concentrations above 2.5mg Cd/l with 100% mortality occurring at 10mg Cd/l. In the CdS NP treatments however, there was no mortality at 20mg Cd/l. The $CdCl_2$ exposures lead to reduced immune activity at concentrations as low as 3 mg Cd/l, while again there was no effect of 20 mg Cd/l CdS NP. Follow up *in vitro* exposures showed no effect of $CdCl_2$ (0.625-10 mg Cd/l) nor CdS NP (1.25-20mg Cd/l) to immune activity and there was no masking effect on immune cell survival, indicting that only strong cells had survived to be assessed.