Advice note 5 – Given the right resources we have the methodology to find the NPs inside the tissues. Not all of the particles that we have found are the original particles.

Advice notes to answer the big five questions

NanoFATE has identified five "Big Questions" important to our understanding of the ecotoxicology of engineered nanoparticles and will help provide key information required to assess the risk that these materials may pose to the environment.

This advice note is in response to the question:
What is a workable way of proving / analysing if nanoparticles are present in tissues or media (re. EU Definition)?

Our approach and findings

NanoFATE partners from Cardiff University carried out detailed studies in zinc oxide and Ag exposed earthworms. We have used a tiered approach to the tracking of ENPs in biological tissues (Fig. 1) applying a set of imaging techniques spanning from CARS to SEM/X-ray and X-ray micro-focus.

Earthworms (*Lumbricus rubellus*) were exposed under standard laboratory conditions to Cobalt-doped ZnO- (1000 or 4000 mg/kg 'substrate' dry weight nominal concentrations) or Zn+Co ions-spiked soils or food. In a second experiment, worms were exposed to food contaminated with Ag ENPs. Control worms maintained in non-spiked soils were also processed.

Fresh tissue samples taken from the mid-intestinal region were processed in a number of ways for micro-focus analysis on the I18 beamline at the DIAMOND Light Source synchrotron (Harwell, UK). High resolution *in situ* micro-focus XAS on sectioned earthworm tissues from the Zn ENPs experiment (Figs 2) yielded a number of significant biological/environmental and technical observations.

No evidence was obtained that earthworms exposed via skin only or via skin and gut accumulated intact or aggregated ZnO ENPs in any cellular or tissue compartment. However, exposure to these ENPs did result in focal increases in the Zn concentration within the body wall musculature (Fig. 2 C) and in the excretory (nephridial) tubules (Fig 2 B).

We were unable to find any mineralized deposits on I18 akin to those observed and analysed on the surface of an earthworm sample by analytical scanning electron microscopy and CARS (data not shown).

We are confident that we were able to detect (using the very unfavourable L-edge signal) Ag in certain key tissues of an earthworm exposed to Ag ENPs but not in an earthworm exposed to Ag ions (Fig.3).

More information

For further information about the work discussed here please contact the Francesco Dondero (fdondero@unipmn.it), leader of the ENP toxicokinetics and toxicodynamics work package.

This project is supported by the European Commission under the Seventh Framework Programme. Theme 4: NMP – Nanosciences, Nanotechnologies, Material and new Production Technologies and Theme 6 – Environment (including Climate Change). Gant Agreement no. CP-FP 247739 NanoFATE

Supporting Figures

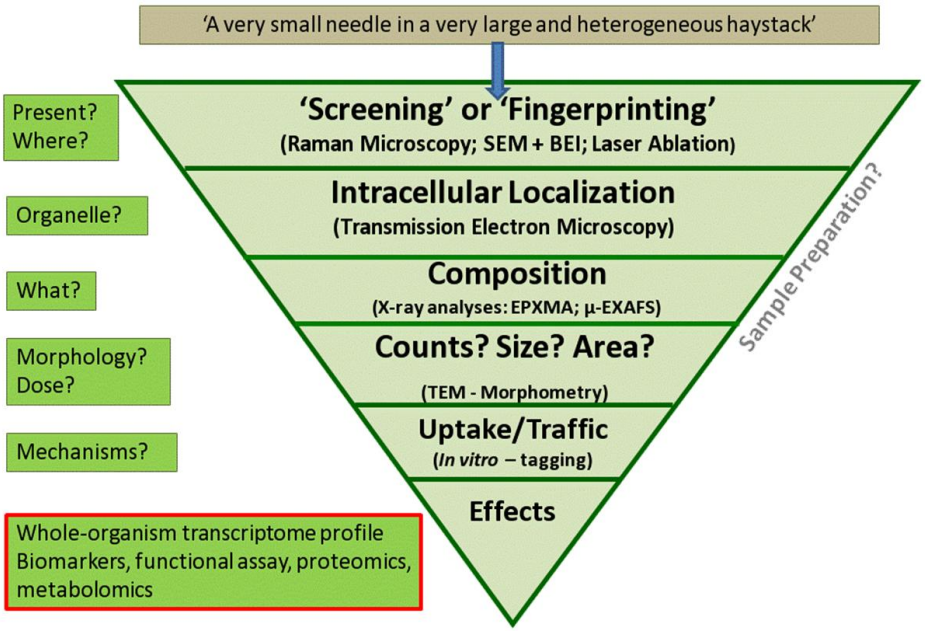

Figure 1: The tiered approach to the tracking of ENPs in biological tissues.

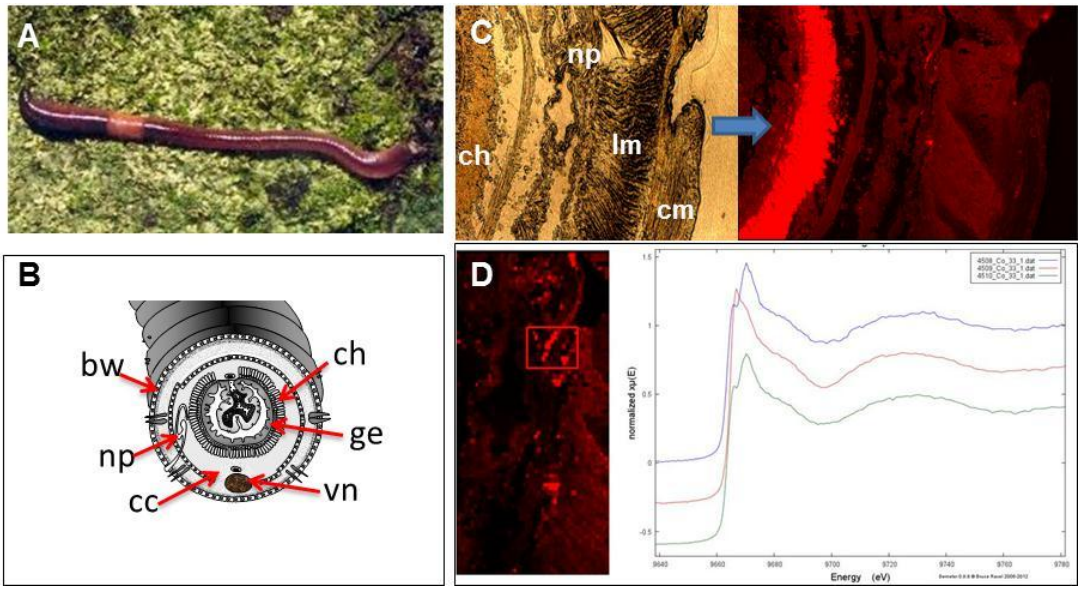

Figure 2: A photograph of a mature 'red worm', Lumbricus rubellus. B: Schematic diagram of the main anatomical features in a transverse, mid-intestinal, region of a lumbricid earthworm. C: Light micrograph, comprised of a region of body wall and adjacent gut wall, from an alcohol-fixed methacrylate section of an mouth-sealed earthworm exposed to 4000 mg/kg ZnO ENPs (left panel), accompanied by a low-resolution low resolution Zn fluorescence distribution map of the same tissue area (right panel). D: High resolution Zn map of the nephridial region depicted in 1C – note the relatively high focal concentrations of Zn in this urine-forming excretory organ; Zn (K-edge) EXAFS spectra derived from selected high intensity pixels in the map. bw = body wall, cc = coelomic cavity, ch = chlorogogenous tissue, cm = circular muscle, ge = gut (intestinal) epithelium, lm = longitudinal muscle, np = nephridium, vn = ventral nerve.

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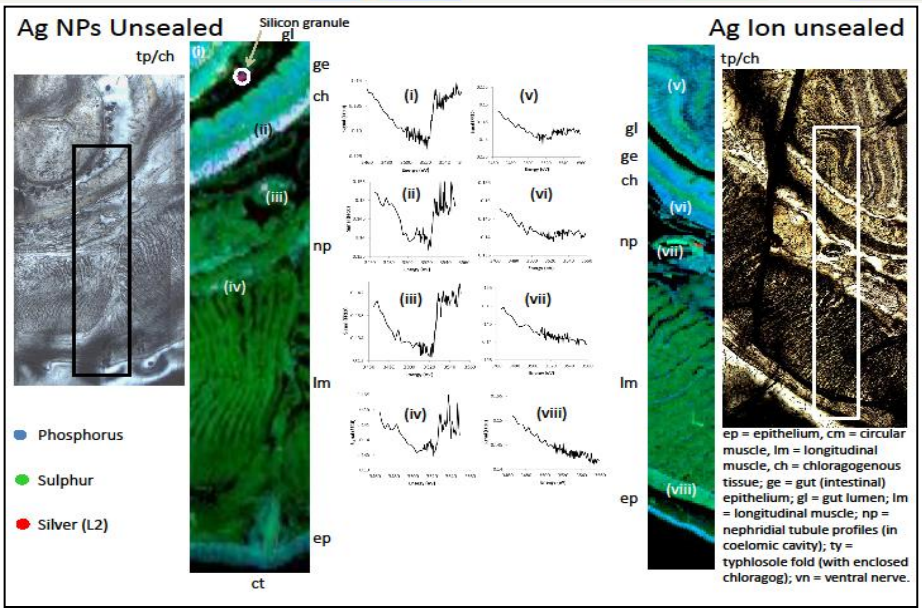

Figure 3: A compendium of images, Ag fluorescence maps, and Ag (L-edge) XANES spectra derived from alcohol-fixed methacrylate-embedded sections of mouth-unsealed earthworms exposed either to Ag ENPs (left panels) or Ag ions (right panels). Note that a discernible, albeit very noisy, Ag L-edge was recorded in the typhlosole chloragocytes (tp), chloragogenous tissue (ch), nephridium (np), and longitudinal muscle (lm) of the worm exposed to Ag ENPs but not in the worm exposed to Ag ions.

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