X-ray based micro- and nanoimaging of nanoparticles in exposed biota

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There are still large uncertainties associated with the potential hazards of engineered nanoparticles (NPs; i.e. <100 nm in size in at least one dimension) released to the environment. The toxic effects of engineered nanomaterials have been related to the ability of NPs to cross biological barriers. This paper discusses the importance of using state-of-the-art analytical techniques in examining biota exposed to three different types of NPs, namely Co, Ce and U. The major challenges in assessing the fate and impact of NPs in complex media (e.g., soils and water) are to identify their transfer across biological membranes and obtain valuable information on the uptake routes, internal distributions as well as the toxicity mechanisms of mixed nanoparticles (NPs). In these studies *Caenorhabditis elegans* (*C. elegans*) nematodes and Atlantic Salmon (*Salmo Salar*) were used as model organisms for exposure studies on, respectively, terrestrial environments and aquatic organisms. Nano-XRF and nano-CT provide a useful tool hereby to study the internal distribution at the submicrometer and nanometer scale.

The studies in this work highlight the use of state-of-the-art sub-micron XRF at the PETRA-III P06 micro-probe and the ESRF ID16A nanoimaging beamlines to investigate the internal distributions of Ce and Co within *C. elegans*. In addition, at the former beamline, more complex organisms were examined, more specifically, the internal distribution of U within exposed Atlantic salmon gills. The main method employed was 2D XRF, which was combined with 2D XRF tomography where possible. Moreover, high resolution lab source X-ray absorption CT (XRadia MicroXCT-400) was employed as an overview tool in order to locate areas of interest. By means of 2D XRF the biodistribution of the selected metals in target organs was visualized. At the P06 endstation the use of the MAIA XRF detector made it possible to obtain such elemental distribution maps with a very short measurement time (<50 ms per pixel). 2D XRF was successfully performed on nematodes in an aqueous agarose solution between Si_3N_4 windows, resembling better natural conditions and ensuring the preservation of the internal structures of the nematode. At the ID16A endstation, with a beamsize of 20 x 37 nm^2 (h x v), elemental distributions, of nematodes exposed to Co, in the nanometer range could easily be obtained. At the same beamline it was possible to perform phase contrast nano-CT to obtain morphological information with a resolution down to 50 nm. Elemental distributions of Co and U, in *C. elegans* and Atlantic salmon respectively, in virtual cross-sections were examined by 2D XRF tomography. In both cases uptake was proven without the need for physically cutting preventing any source of cross-contamination.