Characterisation of exposure and effects of silver nanoparticles to the earthworm Lumbricus rubellus

N. Van den Brink*, Alterra / Dept. of Ecology & Environment; M. van der Ploeg, Alterra, Wageningen UR; H. van den Berg, Wageningen University / Division of Toxicology; R. Handy, University of Plymouth / School of Biomedical and Biological Sciences; R. Peters, RIKILT, Wageningen University / Residues & Contaminants, RIKILT / Contaminants; P. Waalewijn-Kool, VU University / Department of Ecological Science; G. Koopmans, B. Molleman, Wageningen University / Sub-department of Soil Quality; I. Rietjens, Wageningen University / Division of Toxicology

*Presenting and corresponding author (<u>nico.vandenbrink@wur.nl</u>)

Risks of engineered nanoparticles are under debate, and further scientific underpinning of the assessment of these risks is still needed. Silver nanoparticles (Ag-NPs) are used widely, and relatively many studies have investigated hazards that these particles may pose to organisms. However, up till now relatively little attention has been paid to soil organisms. Literature shows that effects of Ag-NPs on earthworms depend on, among others, soil type, particle size and the surface coating of the NPs (Shoults-Wilson et al 2011a,b). In order to elucidate the hazards of noncoated Ag-NPs to earthworms, we exposed earthworms (Lumricus rubellus) to non-coated Ag-NPs (NM-300K in Tween20, < 20 nm, Fraunhofer). Adult earthworms were exposed for four weeks, after which growth and reproduction was assessed (cf. OECD 222). Animals were exposed to Ag-NPs at 0, 1.54, 15.4 and 154 mg/kg soil and to AgNO₃ as a ionic control (15.4 mg/kg). F1 animals were further exposed to the same levels to which their parents were exposed and effects on stage dependent growth and mortality were quantified. The Ag-NPs in spiking extract and the exposure during the experiments were characterised by using AF4 in combination with UV and ICP-MS, single-particle ICP-MS, Atomic Absorption Spectrometry (AAS) and SEM-EDX. Extensive exposure characterisation showed the occurrence of primary particles in soil extracts, which were approximately 16 nm in size, but also the presence of larger agglomerates was detected. Furthermore, in contrast to Ag-NPs in the spiking solution, Ag-NPs in soils showed traces of Cl, indicating possible deposition of AgCl on the surface of the Ag-NPs. Ag-NPs in the soil with the highest levels showed release of Aq ions in the soil pore water, even after 6 months of incubation. The lower Ag-NP and AgNO₃ treatments of the soil showed only low ionic concentrations in the pore water after 6 months. Effects of Ag-NP treatment were evident at different levels of biological integration. Juvenile mortality was 100% at the highest Ag-NP dose, adult growth and cocoon production were also affected at this concentration. Effects of AgNO₃ were visible at 15.4 mg/kg. In the presentation, more details will be given on the effects of Ag-NPs on histological endpoints and on the population growth rate. These effects will be discussed in relation to the exposure characterisation to elucidate whether Ag-NP toxicity may just be caused by ionic exposure or also through NP-properties.