

Acute and Reproductive Toxicity of Nano-sized Metal Oxides (ZnO and TiO₂) to Earthworms (*Eiseniafetida*)

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Supplementary information:

Scanning Electron Microscopy:

Scanning electron microscopy was used to further confirm the results of the particle size analysis (Figure S1). SEM analysis confirmed that ZnO and TiO₂ particles were still in the nano range (100 nm or less) for concentrations of 1 mg/L. Likewise, SEM indicated a qualitative change in particle morphology, confirming that aggregation occurred in 10 mg/L ZnO and TiO₂ solutions with large particle sizes between 200-300 nm. However, from the DLS data analysis, it was observed that ZnO particles are not aggregated until a concentration of 10 mg/L. The differences in observation between the DLS data and SEM observations may be attributed to the problems of sample preparation for SEM. The sample preparation technique for SEM might induce aggregation during drying. Thus DLS is a more accurate technique for the determination of particle size distribution of nanoparticles in dilute solutions. More aggregation was observed in TiO₂ solutions

compared to ZnO solutions (Figures S1b and S1d). The SEM images in Figure S1 are representative of a wide range of sample images.

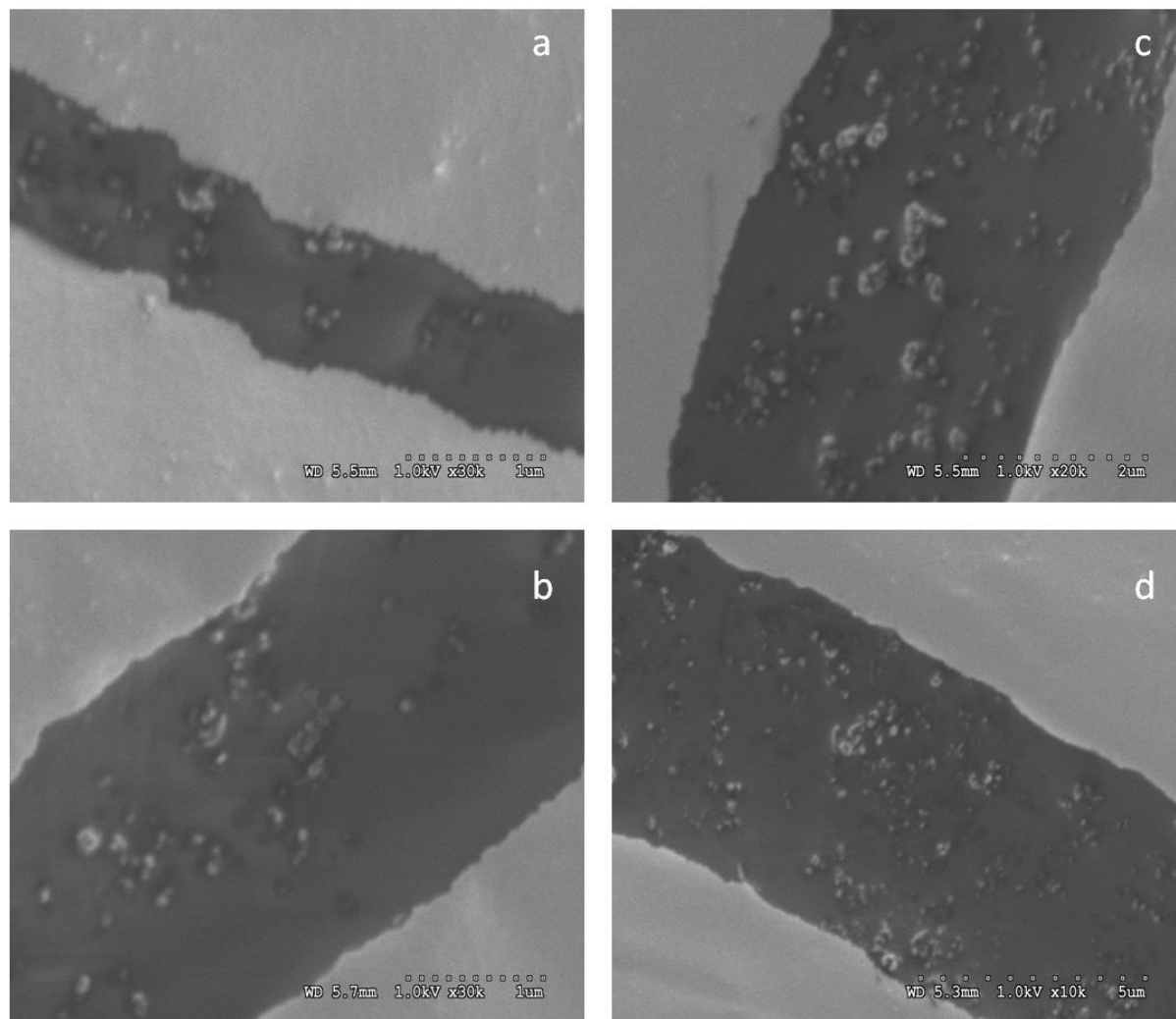


Figure S1. Vacuum filtered samples of ZnO and TiO₂ imaged using scanning electron microscopy: (a) ZnO 1 mg/L, (b) ZnO 10 mg/L, (c) TiO₂ 1 mg/L, (d) TiO₂ 10 mg/L).

Particle sizes of ZnO and TiO₂ solutions with a concentration of 1 mg/L were observed to be in the nano range (≤ 100 nm). However, in the 10 mg/L, aggregation was

observed by the increase in particle size (≥ 100 nm). More aggregation was observed in the TiO_2 solution than in the ZnO solution.

Dynamic Light Scattering:

The size distributions of the oxide nanoparticles were analyzed (Figure S2 & S3), It is observed that the aggregated solutions contain a mixture of both aggregated larger particles ($>100\text{nm}$ in diameter) and non-aggregated smaller particles.

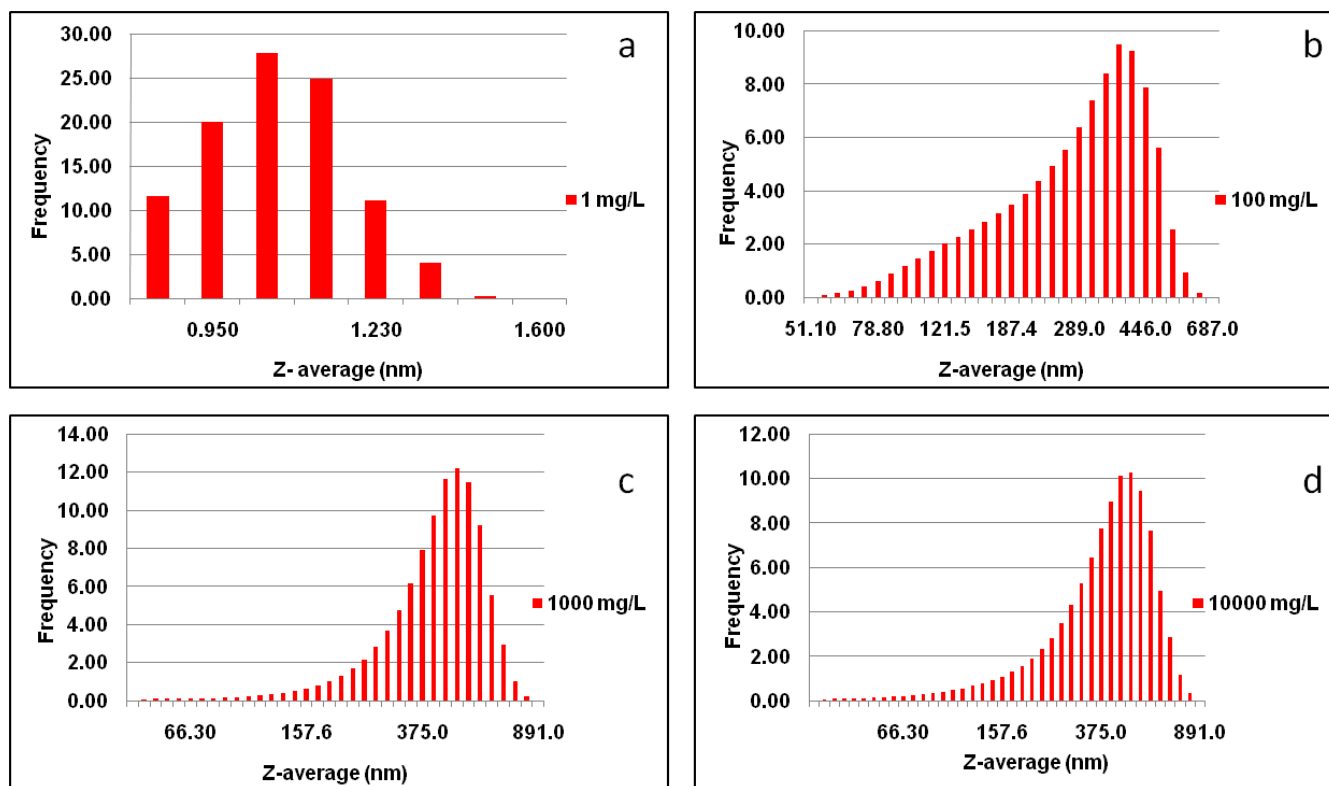


Figure S2: Particle size distribution of ZnO particles at concentrations of (a) 1mg/L, (b) 100 mg/L, (c) 1000 mg/L, and (d) 10000 mg/L.

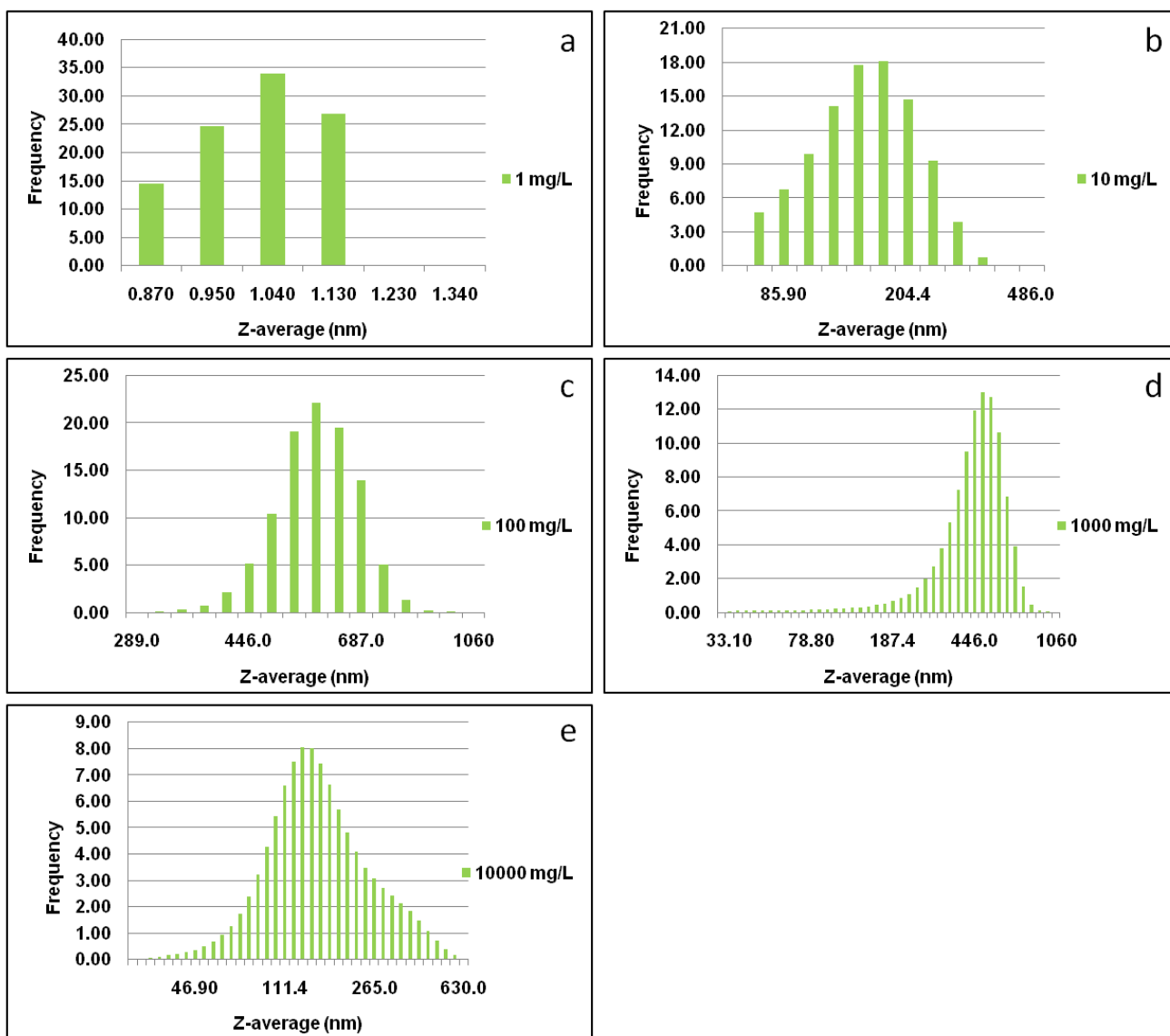


Figure S3: Particle size distribution of TiO_2 particles at concentrations of (a) 1 mg/L, (b) 10 mg/L, (c) 100 mg/L, (d) 1000 mg/L, and (e) 10000 mg/L.