Electronic Supplementary Material (ESI) for Environmental Science: Nano.


**Supporting information**

# Phenotypic and transcriptional responses associated with multi-generation exposure of *Folsomia candida* to engineered nanomaterials

Jeroen W. Noordhoek*, Fabrizia Pipicelli, Ida Barone, Oscar Franken, Kora Montagne-Wajer, Janine Mariën, Rudo A. Verweij, Cornelis A.M. van Gestel, Nico M. van Straalen, Dick Roelofs

Department of Ecological Science, Faculty of Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

**Corresponding Author**

*Corresponding author, Current address: Jeroen W. Noordhoek: +31 570-697054, e-mail: jeroen.noordhoek@witteveenbos.com, address: Department of Ecology, Witteveen+Bos, P. O. Box 233, 7400 AE Deventer, The Netherlands.

Number of pages: 3
Number of figures: 3

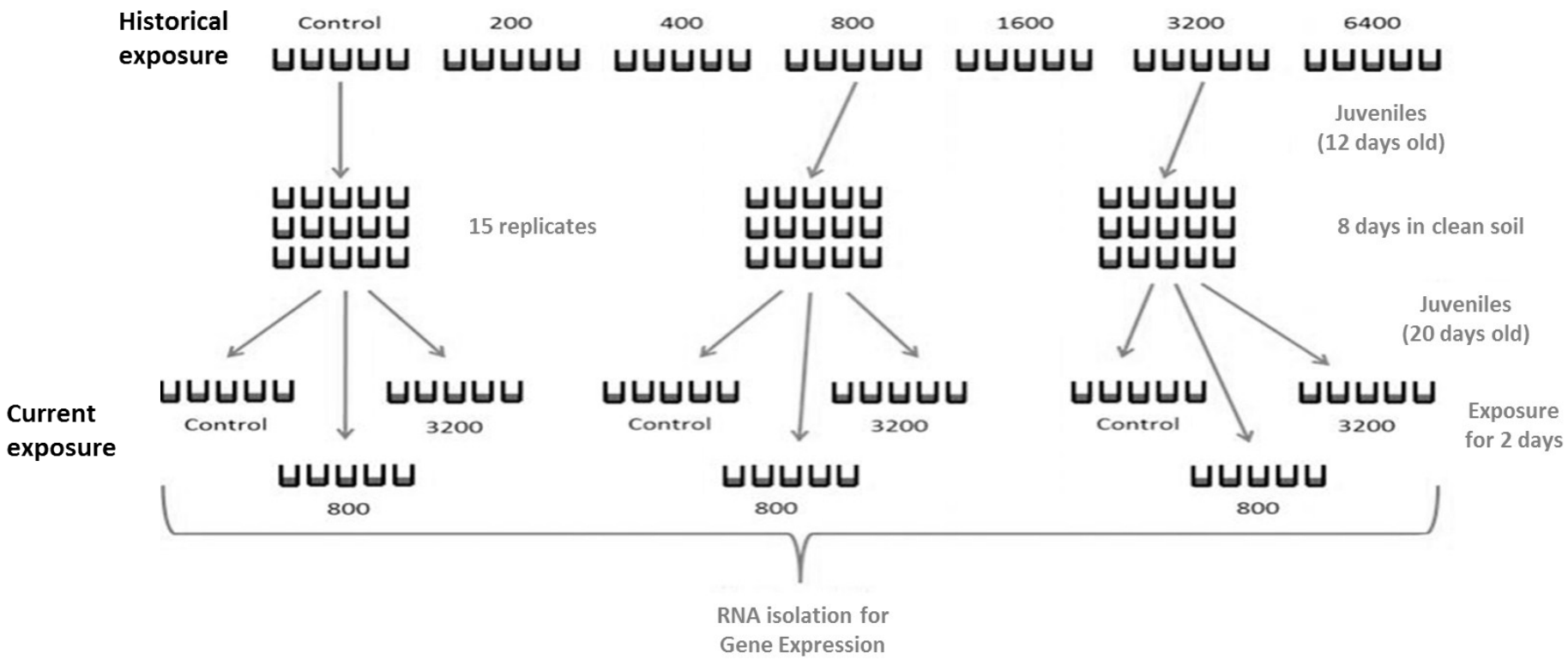


**Figure S1.** Gene expression test set-up. The multi-generation ecotoxicity test with test concentrations ranging from 200-6400 mg/kg, is shown in the top row. From this setup, animals for the gene expression test were collected from three treatments (control, 800 and 3200 mg/kg dry soil) when they were approximately 12 days old. Subsequently, all animals were placed on clean soil for eight days allowing them to become approximately 20 days old, which is the standardized age of *F. candida* in gene expression analyses. Pools of 30-50 animals were then exposed for two days to each of the nanomaterials in jars containing 30 grams of either clean or polluted LUFA 2.2 soil, using five replicates per treatment. Animals were then extracted and deposited in microcentrifuge tubes to be snap frozen with liquid nitrogen. Samples were kept at -80°C prior to RNA isolation. The same setup was used for both CuO-NM and WCCo-NM.

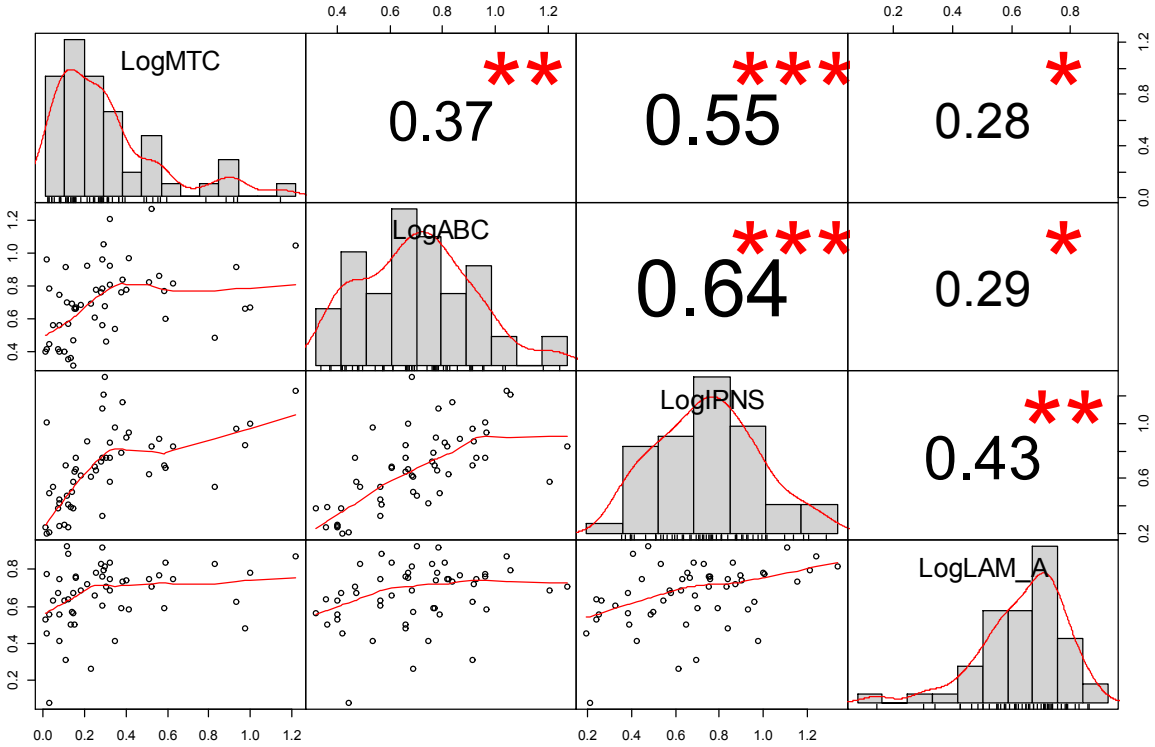


**Figure S2.** Pearson's correlation coefficient (*r*) to assess the relationship between the expression of stress-response genes (MTC, ABC transporter, IPNS, Laminin A) in CuO nanomaterial exposed *Folsomia candida*.

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

*** Correlation is significant at the 0.001 level.

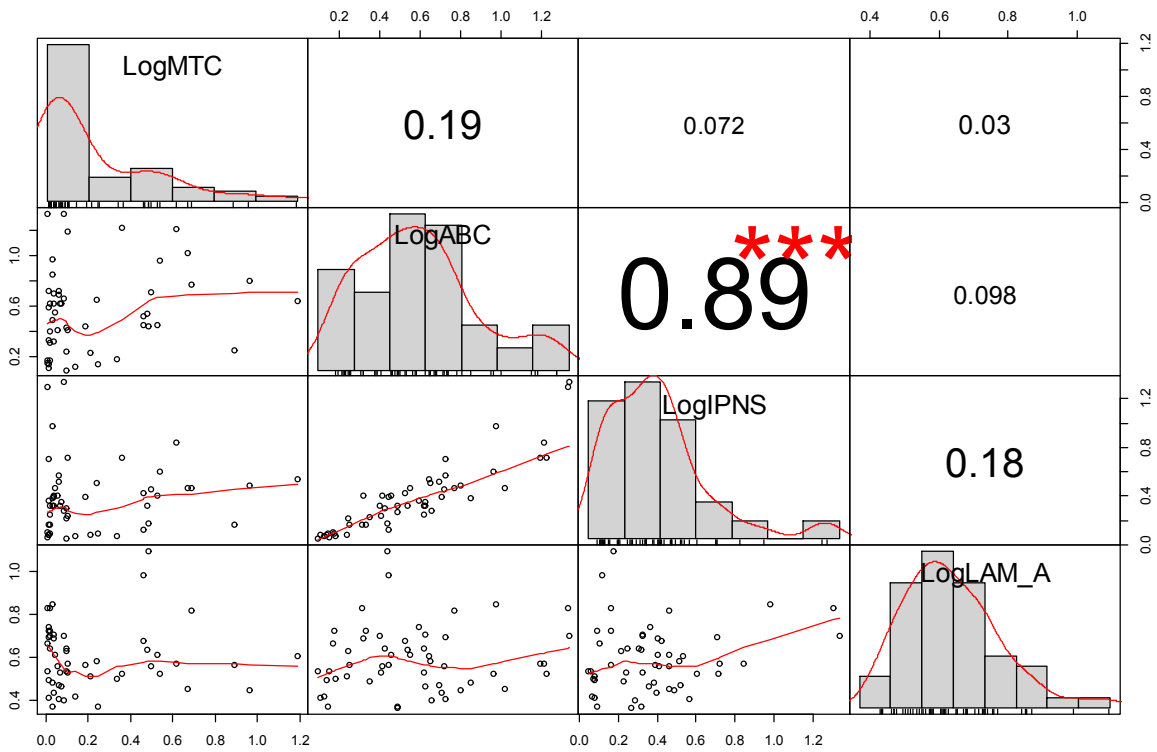


**Figure S3.** Pearson's correlation coefficient (*r*) to assess the relationship between the expression of stress-response genes (MTC, ABC transporter, IPNS, Laminin A) in WCCo nanomaterial exposed *Folsomia candida*. The only significant correlation is between ABC transporter and IPNS (*r* = 0.89).

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

*** Correlation is significant at the 0.001 level