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

Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms

Nico W. van den Brink[1], Anita Jemec Kokalj[2], Patricia Silva[3], Elma Lahive[4], Karin Norrfors[5], Marta Baccaro[1], Zahra Khodaparast[3], Susana Loureiro[3], Damjana Drobne[2], Geert Cornelis[5], Steve Lofts[4], Richard D. Handy[6], Claus Svendsen[4], Dave Spurgeon[4], Cornelis A.M. van Gestel[7]

1. Department of Toxicology, Wageningen University, Wageningen, The Netherlands

2. Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana Slovenia.

3. Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal.

4. Centre of Ecology and Hydrology (CEH-NERC), Wallingford, UK

5. Department of Soil and Environment, Swedish University of Agricultural Sciences, Uppsala, Sweden

6. Department of Biological and Marine Sciences, Plymouth University, Plymouth, UK

7. Department of Ecological Science, Faculty of Science, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Online information

Modelling uptake including Stored Fraction based on Ribeiro et al 2017

Here we report the data and the modelling results including statistical output discussed in the main manuscript based on the study by Ribeiro et al[1]. All analyses were performed with Genstat, 19th Edition (https://www.vsni.co.uk/software/genstat/).

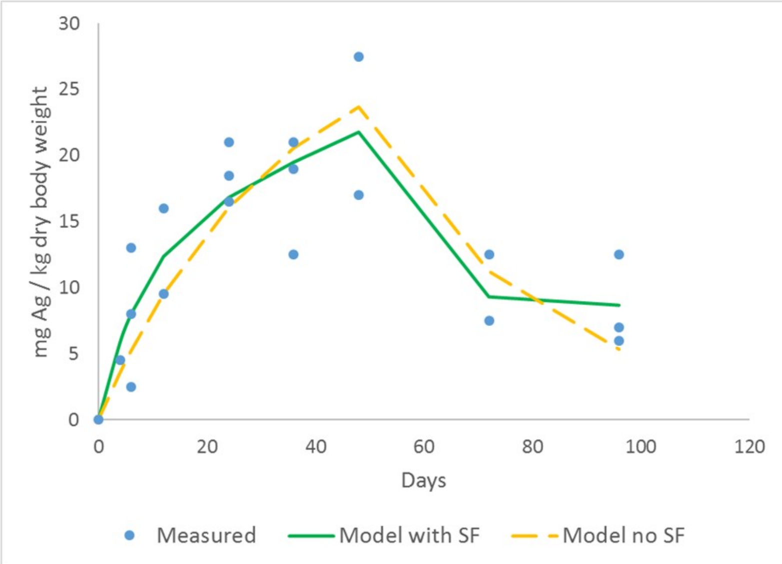

Figure OI1. Measure and modelled concentrations of Ag in Daphnia magna (mg Ag/kg bodyweight (d.w.) based on Ribeiro et al. 2017[1]. Blue dots, measured data, green line: model including SF (equations 3a and 3b), yellow line: model not including SF (equation 1 and 2). Animals were transformed from exposed to clean media at day 48. For experimental details see[1]. For statistical details see below.

Model no SF (equation 1 and 2) using data of[1]

Response variate: Daphnia concentrations

Nonlinear parameters: k_1, k_2

Summary of analysis

Source	d.f.	s.s.	m.s.	variance ratio	F pr.
Regression	2	3771.7	1885.87	97.89	<.001
Residual	18	346.8	19.26		
Total 20	4118.5	205.93			

Percentage variance accounted for 61.2

Estimates of parameters

Parameter	estimate	s.e.
k_1	0.1897	0.0253
k_2	0.03107	0.00654

Model with SF (equation 3a and 3b) using data of[1]

Response variate: Daphnia concentrations

Nonlinear parameters: k_1, k_2, SF

Summary of analysis

Source	d.f.	s.s.	m.s.	variance ratio.	F pr.
Regression	3	3874.4	1291.48	89.96	<.001
Residual	17	244.1	14.36		
Total	20	4118.5	205.93		

Percentage variance accounted for 71.1

Estimates of parameters

Parameter	estimate	s.e.
k_1	0.363	0.121
k_2	0.1240	0.0587
SF	0.0991	0.0327

Case study i: Ag uptake from enchytraeids exposed to Ag_2S NPs with and without correction for mass loss.

Materials and methods

The enchytraeid species *Enchytraeus crypticus* has been cultured at the Vrije Universiteit for several years. The animals were cultured in plastic trays containing a layer of agar prepared with an aqueous extract of standard Lufa 2.2 soil. The cultures were maintained in a climate room at 16 °C in total darkness, and fed twice a week with a mixture of oat meal, dried yeast, yolk powder, and fish oil[2].

Adult age-synchronized adult *Enchytraeus crypticus* were exposed for 14 days to Lufa 2.2 soil (pH_{CaCl2} 5.7, 3.7% organic matter, CEC 8.96 $cmol_c$/kg) spiked with 20 nm Ag_2S NPs at a nominal concentration of 10 mg Ag/kg dry soil. Ten adult enchytraeid worms were placed in glass jars containing 25-30 grams of soil moistened to 50% of its water holding capacity. The jars were covered with perforated aluminium foil and stored in a climate chamber at 20 °C with a 16h/8h light/dark cycle and 75% Relative Humidity. Twice a week, moisture content of the soil was checked by weighing the jars and moisture loss replenished by adding demineralized water if needed. The animals were fed with a few flakes of oatmeal weekly.

At different time intervals, test jars were destructively sampled to collect animals for determining Ag uptake kinetics. After 14 days, the animals in the remaining test jars were transferred to clean soil, and over a period of 14 days sampling took place at different times to assess Ag elimination. At each sampling time, three replicate test jars were sampled, and the enchytraeids collected by hand sorting. To void their guts, the collected animals were incubated for 24 hours in a nutrient solution according to ISO[3], composed of 294 mg/L $CaCl_2.2H_2O$, 123.3 mg/L $MgSO_4.7H_2O$, 5.8 mg/L KCl, and 64.8 mg/L $NaHCO_3$.

Soil was analysed for total silver content after digestion using the "bomb-method". For that purpose, 130 mg of dry soil was placed into a Teflon destruction bomb and 2 ml of a 1:4 mixture of concentrated HNO_3:HCl was added. The bombs were incubated for 7 hours at 140 ^oC. After cooling, the bombs were opened and 8 ml of demi-water was added. The samples were measured using a flame Atomic Absorption Spectrometer (AAS; Perkin Elmer AAnalyst 100). Certified reference material (ISE sample 989 of River Clay from Wageningen, The Netherlands) with a known concentration of 2.8 mg Ag/kg was used in order to ensure the accuracy of the Ag analysis. The measured Ag concentration in the reference material was 104% (±0.9, n=4) of the certified value.

For the Ag analysis, the animals were individually frozen, freeze dried and digested in a 7:1 mixture of HNO_3 (65%; Baker Ultrex II Ultra Pure) and $HClO_4$ (70%; Baker Ultrex Ultra Pure). After evaporation of the acid, the dry residue was taken up in a small volume of 0.1 M HNO_3. Analysis for Ag was done by graphite furnace Atomic Absorption Spectrometer (PinAAcle 900T AAS). Detection limit was 0.005 µg Ag/L.

Case study ii: modelling approaches with different PBPK model definitions

In this section the statistical outputs from the different scenarios discussed in the main manuscript are presented. All analyses were performed with Genstat, 19th Edition (https://www.vsni.co.uk/software/genstat/).

Scenario: Predict

Nonlinear regression analysis

- Response variate: Modelled worm concentrations with k_1 and k_2
- Nonlinear parameters: k_1, k_2

Summary of analysis

Source	d.f.	s.s.	m.s.	variance ratio	F pr.
Regression	2	1217.2	608.593	129.05	<.001
Residual	30	141.5	4.716		
Total	32	1358.7	42.458		

Percentage variance accounted for 41.7

Estimates of parameters

Parameter	estimate	s.e.
k_1	0.06277	0.00774
k_2	0.04094	0.00741

Scenario: Predict separate k1

Linear regression analysis

- Response variate: Measured worm concentrations
- Fitted terms: Modelled worm concentrations with separate k_1 for soil and NM

Summary of analysis

Source	d.f.	s.s.	m.s.	variance ratio	F pr.
Regression	1	1212.7	1212.7	257.5	<.001
Residual	31	146	4.709		
Total	32	1358.7	42.46		

Percentage variance accounted for 41.8

Estimates of parameters

Parameter	estimate	s.e.
Slope	1.28	0.08

Scenario: Predict separate k1, based on pore water

Linear regression analysis

- Response variate: Measured worm concentrations
- Fitted terms: Modelled worm concentrations with separate k_1 for pore water and NM

Summary of analysis

Source	d.f.	s.s.	m.s.	variance ratio	F pr.
Regression	1	1212.5	1212.5	257.23	<.001
Residual	31	146	4.714		
Total	32	1358.7	42.46		

Percentage variance accounted for 41.7

Estimates of parameters

Parameter	estimate	s.e.
Slope	1.02	0.06

Scenario: Predict separate k2

Nonlinear regression analysis

- Response variate: worm concentration
- Nonlinear parameters: k_1, $k_2$fast, $k_2$slow

Summary of analysis

Source	d.f.	s.s.	m.s.	variance ratio	F pr.
Regression	3	1241.8	413.937	102.73	<.001
Residual	29	116.9	4.029		
Total	32	1358.7	42.458		

Percentage variance accounted for 50.2

Estimates of parameters

Parameter	estimate	s.e.
k_1	0.0852	0.0143
$k_2$fast	0.0545	0.0282
$k_2$slow	0.0183	0.0132

Case study iii: Modelling uptake of Ag by earthworms, including dissolution of Ag-NMs and adsorption/desorption to the soil

The dissolution rate constant k_{diss} of Ag_2S was obtained from batch dissolution data of Levard et al.[4] by assuming first order dissolution kinetics:

$$\frac{d[Ag^+]}{dt} = k_{diss}[Ag^+]$$ which solves to $$\left(1 - ln\left(\frac{[Ag^+]}{[Ag^+]_0}\right)\right) =- k_{diss}t$$

The latter equation allows obtaining k_{diss} as the slope of the linear part of the dissolution curve as shown in Figure OI1. The dissolution data for Ag_2S NMs having a molar Ag:S ratio 0.019 was taken from Levard et al.[4], because this ratio was closest to the NMs used in the case study as explained in the main manuscript.

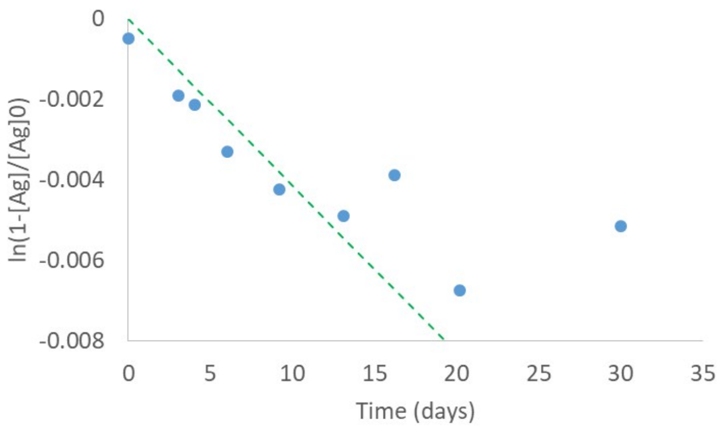

Figure OI2. Fitting of the kinetic dissolution model (dashed straight line) to experimental dissolution data from Levard et al.[4] for Ag_2S NM with Ag/S ratio of 0.0192 (dots).

Similarly, adsorption rate constants were obtained from Zhan et al. 2013 [5], who reported desorption rate kinetic data for Ag^+ from three different soils. One of these soils (Oliver soils) was deemed most similar to the soil used in the studies by Baccaro et al.[6], in terms of chemical properties (See table OI1). While it is obvious these soils differ substantially still, other soils had either a too high pH or a too high clay content, which would induce different interaction mechanisms of Ag^+ with these other soils. The Olivier soil was therefore seen as a compromise.

The adsorption kinetics of Zhan et al.[5]

$$\frac{d[Ag^+]}{dt} =- k_{ads}[Ag^+]$$ which solves to $$ln\left(\frac{[Ag^+]}{[Ag^+]_0}\right) =- k_{ads}t$$ and allows obtaining k_{ads} as the slope of the linear part of $\ln([Ag]/[Ag]_0)$ as a function of t (Figure OI3). This provided the value k_{ads}= 0.0288. Using the Freundlich constant, the desorption rate constant was subsequently calculated (See main manuscript).

Table OI1. Basic properties of the Olivier soil [5].

Property	Olivier soil	Baccaro et al.[6]
pH	5.80	5.2
TOC (%)	0.38	5.4
CaCO3 (%)	< LOD	0.2 %
CEC (cmol kg^{-1})	8.6	22.94
Sand (%)	5	81.7

Silt (%)	89	11.7
Clay (%)	6	6.6

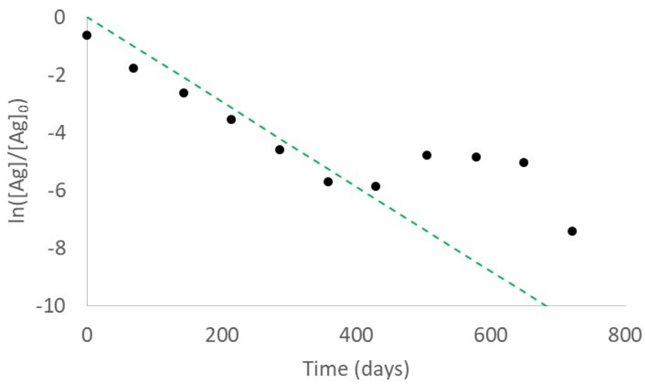

Figure OI3. Fitting of the kinetic adsorption model (dashed straight line) to experimental data from Zhu et al. for Ag^{+} modelling on the Olivier soil.

References

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