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

Nico W. van den Brink¹, Anita Jemec Kokalj², Patricia Silva³, Elma Lahive⁴, Karin Norrfors⁵, Marta Baccaro¹, Zahra Khodaparast³, Susana Loureiro³, Damjana Drobne², Geert Cornelis⁵, Steve Lofts⁴, Richard D. Handy⁶, Claus Svendsen⁴, Dave Spurgeon⁴, Cornelis A.M. van Gestel⁷

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 al1. All analyses were performed with Genstat, 19th Edition (https://www.vsni.co.uk/software/genstat/).

![Graph showing measured and modelled concentrations of Ag in Daphnia magna](image)

Figure OI1. Measure and modelled concentrations of Ag in Daphnia magna (mg Ag/kg bodyweight (d.w.) based on Ribeiro et al. 20171. 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 see1. For statistical details see below.

Model no SF (equation 1 and 2) using data of1

Response variate: Daphnia concentrations

Nonlinear parameters: k₁, k₂

Summary of analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>variance ratio</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>3771.7</td>
<td>1885.87</td>
<td>97.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>18</td>
<td>346.8</td>
<td>19.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4118.5</td>
<td>205.93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage variance accounted for 61.2

Estimates of parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₁</td>
<td>0.1897</td>
<td>0.0253</td>
</tr>
<tr>
<td>k₂</td>
<td>0.03107</td>
<td>0.00654</td>
</tr>
</tbody>
</table>

Model with SF (equation 3a and 3b) using data of1

Response variate: Daphnia concentrations

Nonlinear parameters: k₁, k₂, SF

Summary of analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>variance ratio</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3</td>
<td>3874.4</td>
<td>1291.48</td>
<td>89.96</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>17</td>
<td>244.1</td>
<td>14.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4118.5</td>
<td>205.93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage variance accounted for 71.1

Estimates of parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₁</td>
<td>0.363</td>
<td>0.121</td>
</tr>
<tr>
<td>k₂</td>
<td>0.1240</td>
<td>0.0587</td>
</tr>
<tr>
<td>SF</td>
<td>0.0991</td>
<td>0.0327</td>
</tr>
</tbody>
</table>
**Case study i: Ag uptake from enchytraeids exposed to Ag$_2$S 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$_{CaCl_2}$ 5.7, 3.7% organic matter, CEC 8.96 cmol$_c$/kg) spiked with 20 nm Ag$_2$S 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 avoid 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$_2$O, 123.3 mg/L MgSO$_4$.7H$_2$O, 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 °C. 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

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>variance ratio</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>1217.2</td>
<td>608.593</td>
<td>129.05</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>30</td>
<td>141.5</td>
<td>4.716</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>1358.7</td>
<td>42.458</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage variance accounted for 41.7

Estimates of parameters

<table>
<thead>
<tr>
<th>Parameter estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>0.06277</td>
</tr>
<tr>
<td>$k_2$</td>
<td>0.04094</td>
</tr>
</tbody>
</table>

Scenario: Predict separate $k_1$

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

Summary of analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>variance ratio</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>1212.7</td>
<td>1212.7</td>
<td>257.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>31</td>
<td>146</td>
<td>4.709</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>1358.7</td>
<td>42.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage variance accounted for 41.8

Estimates of parameters

<table>
<thead>
<tr>
<th>Parameter estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Scenario: Predict separate $k_1$, 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

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>variance ratio</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>1212.5</td>
<td>1212.5</td>
<td>257.23</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>31</td>
<td>146</td>
<td>4.714</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>1358.7</td>
<td>42.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage variance accounted for 41.7

Estimates of parameters

<table>
<thead>
<tr>
<th>Parameter estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Scenario: Predict separate $k_2$

Nonlinear regression analysis
- Response variate: worm concentration
- Nonlinear parameters: $k_1$, $k_{2\text{fast}}$, $k_{2\text{slow}}$

Summary of analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>variance ratio</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3</td>
<td>1241.8</td>
<td>413.937</td>
<td>102.73</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>29</td>
<td>116.9</td>
<td>4.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>1358.7</td>
<td>42.458</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage variance accounted for 50.2

Estimates of parameters

<table>
<thead>
<tr>
<th>Parameter estimate</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>0.0852</td>
</tr>
<tr>
<td>$k_{2\text{fast}}$</td>
<td>0.0545</td>
</tr>
<tr>
<td>$k_{2\text{slow}}$</td>
<td>0.0183</td>
</tr>
</tbody>
</table>
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$_2$S 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

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

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$_2$S 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](https://via.placeholder.com/150)

Figure OI2. Fitting of the kinetic dissolution model (dashed straight line) to experimental dissolution data from Levard et al.$^4$ for Ag$_2$S 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 (Olivier 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

$$\ln\left(\frac{[Ag^+]_0}{[Ag^+]_0}\right) = -k_{ads}t$$

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.$^5$

<table>
<thead>
<tr>
<th>Property</th>
<th>Olivier soil</th>
<th>Baccaro et al.$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.80</td>
<td>5.2</td>
</tr>
<tr>
<td>TOC (%)</td>
<td>0.38</td>
<td>5.4</td>
</tr>
<tr>
<td>CaCO$_3$ (%)</td>
<td>&lt; LOD</td>
<td>0.2 %</td>
</tr>
<tr>
<td>CEC (cmol kg$^{-1}$)</td>
<td>8.6</td>
<td>22.94</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>5</td>
<td>81.7</td>
</tr>
</tbody>
</table>
Silt (%) | 89 | 11.7
Clay (%) | 6 | 6.6

Figure O13. Fitting of the kinetic adsorption model (dashed straight line) to experimental data from Zhu et al. for Ag⁺ modelling on the Olivier soil.

References

5. L. Zhan, MSc, Louisiana State University and Agricultural and Mechanical College, 2013.