Toxicokinetics of pristine and aged silver nanoparticles in freshwater benthic organisms: the role of exposure route

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Introduction

- ✓ Nanomaterials (NMs) may undergo several physical-chemical changes during their life-cycle, and can reach biological receptors in unpredictable forms → **this can determine their biological fate through different uptake routes.**
- ✓ **Toxicokinetic studies** may help understanding and assessing NM bioavailability, ways of uptake/elimination and biological fate.
- ✓ In freshwater systems, **benthic organisms** can be exposed to NMs through **water and sediment** and therefore **different routes** of uptake NMs are expected.

Objective

Determine toxicokinetics of silver nanoparticles, in their pristine **(Ag-NPs; 3-8 nm, 50 nm and 60 nm)** and simulated aged form **(Ag_2S-NP),** through different exposure routes, in freshwater benthic organisms.

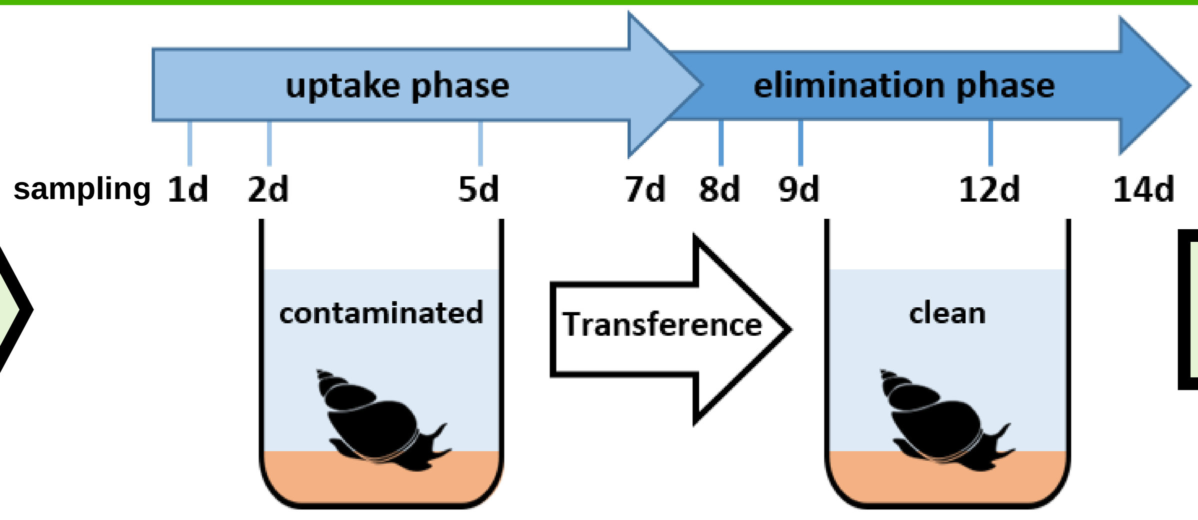
Material and Methods

Test chemicals

- ❖ **3-8 nm Ag-NPs (AMEPOX Enterprise)**
- ❖ **50 nm Ag-NPs (AppNano)**
- ❖ **60 nm Ag-NPs (AMEPOX Enterprise)**
- ❖ **20 nm Ag_2S-NPs (AppNano)**
- ❖ **Ag^+ → $AgNO_3$ (Sigma Aldrich)**

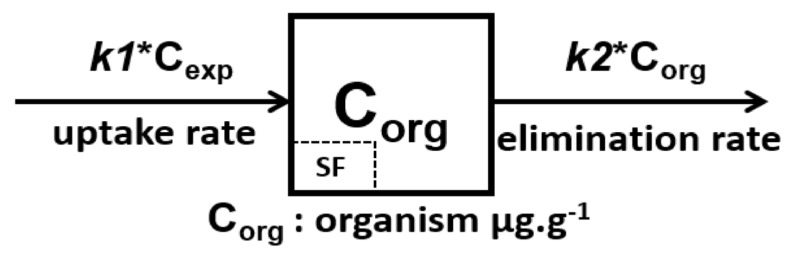
Physa acuta (pond snail)

Dugesia tigrina (planarian)



- ✓ Different routes of exposure:
  - ✓ **Water 10 µg Ag.L^{-1} (with and without sediment)**
  - ✓ **Sediment 10 mg Ag.kg^{-1}**
- ✓ No food
- ✓ Separation of snail soft body and shell
- ✓ Total Ag body concentrations: measured with Atomic absorption spectroscopy

One-compartment model



Uptake phase

$$C_{org} = C_{org\ t\ =\ 0} + SF * C_{exp} * k1 * t + (1 - SF) * C_{exp} * \left(\frac{k1}{k2}\right) * (1 - e^{-(k2*t)})$$

Elimination phase

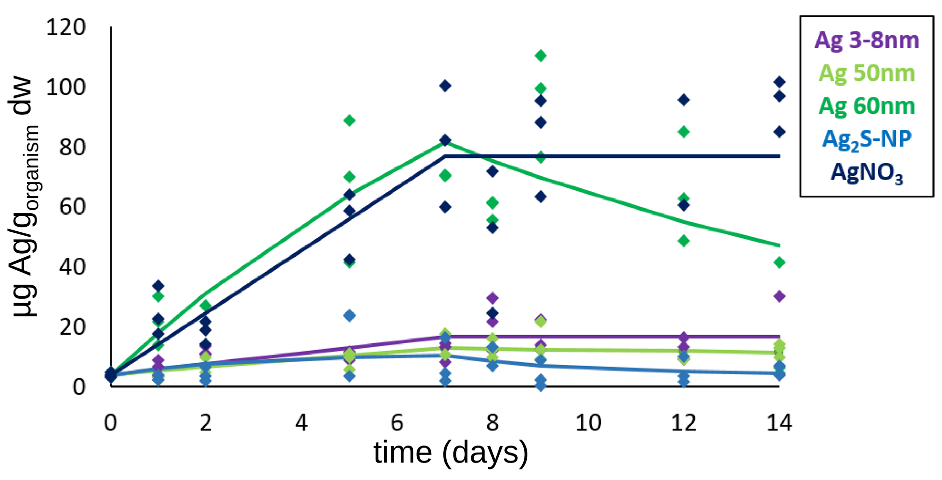
$$C_{org} = C_{org\ t\ =\ 0} + SF * C_{exp} * k1 * t_e + (1 - SF) * C_{exp} * \left(\frac{k1}{k2}\right) * (1 - e^{(-k2*te)}) * e^{-k2*(t - t_e)}$$

k1 - uptake rate constant (L.g$^{-1}_{organism}$.day^{-1} or $g_{sediment}$.g$^{-1}_{organism}$.day^{-1}); k2 - elimination rate constant (day^{-1}); SF - stored fraction (0 ≤ SF ≤1) (unit less); C_{exp} - concentration in the exposure medium (µg.L^{-1} or mg.kg^{-1}); $C_{org\ t=0}$ - concentration in the organism at time 0 (µg.g^{-1}); t - time (day); t_e - time of transference of organisms from contaminated to clean medium (day)

Van den Brink N.W., et al. (2019) Tools and rules for modelling of the uptake and bioaccumulation of nanomaterials in invertebrate organisms. Environmental Science: Nano. DOI: 10.1039/C8EN01122B

Results

Contaminated water (no sediment)

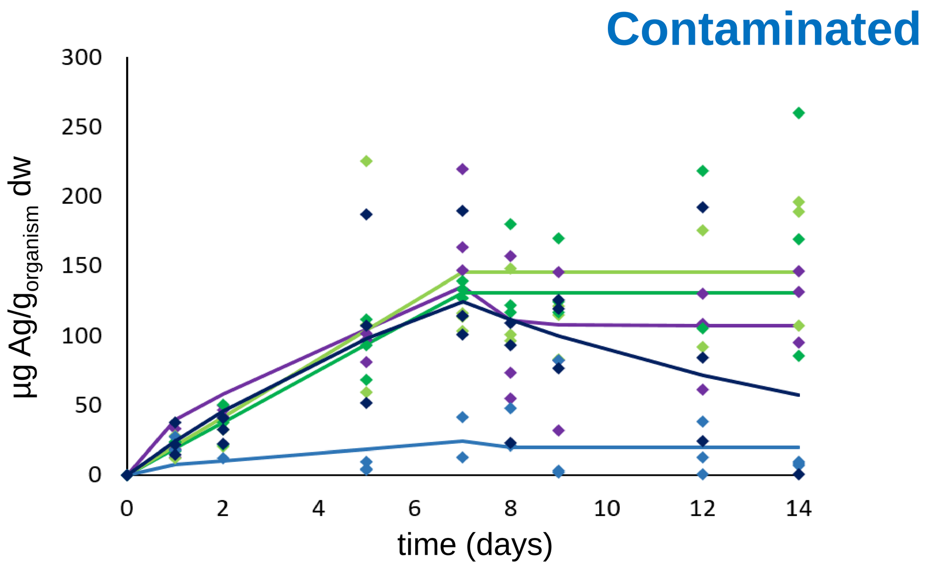


Tables 1 and 2. Kinetics parameters of Ag forms in *Physa acuta* soft body samples. Values in between brackets represent 95% confidence intervals.

Table 1

Ag form	*k1* (L.g$^{-1}_{organism}$.day^{-1})	*k2* (day^{-1})	SF
3-8 nm	0.18 (0.08-0.29)	0 (-)	1
50 nm	0.16 (-0.01-0.34)	0.02 (-2.81-2.86)	0
60 nm	1.43 (0.32-2.54)	0.08 (-0.66-0.82)	0
Ag_2S-NP	1.07 (-0.97-3.10)	0.38 (-0.86-1.61)	0
$AgNO_3$	1.1 (0.77-1.43)	0.001 (-)	1

Contaminated sediment



The three graphs show the uptake and elimination kinetics of Ag forms in *Physa acuta* soft body samples. Lines represent the fit of a one-compartment model to the data.

Table 2

Ag form	*k1* ($g_{sediment}$.g$^{-1}_{organism}$.day^{-1})	*k2* (day^{-1})	SF
3-8 nm	7.24 (-19.5-33.97)	2.01 (-8.63-12.64)	0.22
50 nm	2.34 (1.43-3.25)	0.001 (-)	1
60 nm	2.03 (1.38-2.69)	0.003 (-)	1
Ag_2S-NP	8.31 (-)	18.11 (-)	0.04
$AgNO_3$	2.98 (-0.18-6.13)	0.11 (-0.84-1.06)	0

Contaminated water (with sediment)

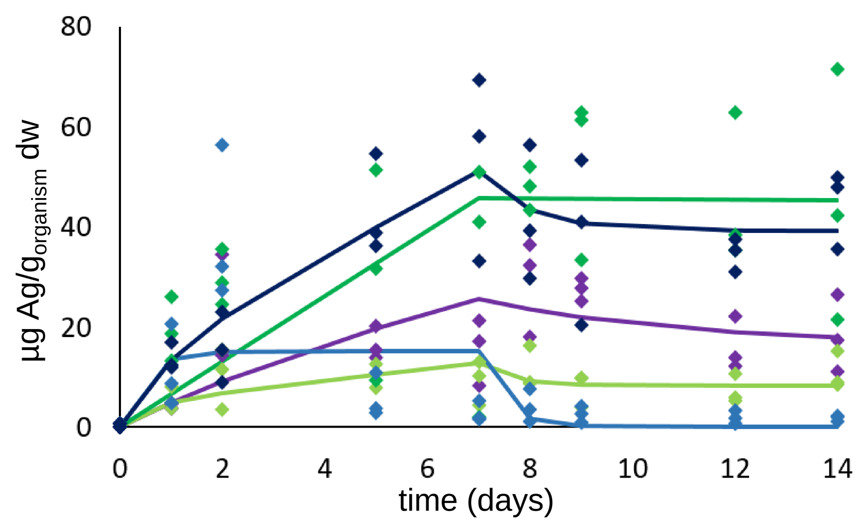


Table 3. Kinetics parameters of Ag forms in *Physa acuta* soft body samples. *kw* is uptake rate constant in water, *ks* is uptake rate constant in sediment, *ke* is the elimination rate constant. Values in between brackets represent 95% confidence intervals.

Ag form	*kw* (L.g$^{-1}_{organism}$.day^{-1})	*ks* ($g_{sediment}$.g$^{-1}_{organism}$.day^{-1})	*ke* (day^{-1})	SF
3-8 nm	0.31 (-)	0.16 (-)	0.46 (-0.68-1.6)	0.45
50 nm	0.4 (-)	0.42 (-)	1.92 (-5.18-9.02)	0.14
60 nm	0.36 (-)	0.2 (-)	0.33 (-)	0.42
Ag_2S-NP	2.29 (-)	0.37 (-)	2.52 (-4.77-9.8)	0
$AgNO_3$	1.26 (-)	0.42 (-)	1.43 (-0.89-3.76)	0.31

- ✓ Planarians showed **very little to no uptake**.
- ✓ In exposures to contaminated water and clean sediment, the Ag background concentration in sediment increased in time while Ag concentration in water decreased, due to Ag association with the sediment → kinetics were modelled considering **changes in Ag concentration in both media**, adapting the model described in **Van den Brink et al. (2019).**
- ✓ In this model, a **stored fraction (SF)** is included in equations for both the uptake and elimination phase, assuming that Ag **storage is a biological process also occurring during the uptake phase**.

Discussion and Conclusions

- ✓ When considering water and sediment Ag concentrations in the model, **uptake rate constants (k1) were in general higher for water uptake**.
  - ➢ Ag concentrations in water and sediment (sediment background concentration increasing in time) reached very similar values → **water exposure more relevant in uptake** than sediment.
- ✓ Higher k1 values in sediment exposures, probably due to higher exposure concentrations.
- ✓ Similar uptake patterns were observed for different Ag forms in snails from water exposures, uptake being higher for Ag 60 nm and $AgNO_3$, possibly related to their higher dissolution. In the sediment exposure, all Ag forms presented similar uptake rates, except for Ag_2S-NP → **exposure route may influence uptake.**
- ✓ In general, higher uptake rate constants for snails exposed to Ag_2S-NPs, $AgNO_3$ and Ag-NPs 60 nm, and lower for Ag-NPs 3-8 nm and 50 nm, suggesting that **NP form is also determining uptake.**
- ✓ Planarians revealed very little to no uptake when exposed through contaminated water. These organisms secrete mucus that can act as a defense mechanism against the entrance of contaminants. Exposures to higher concentrations should be performed.
- ✓ Results suggest that **different exposure routes may lead to different uptake rates** and that the NP form is also determining Ag uptake.

Acknowledgments

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