Supporting Information

Manuscript: Influence of hardness on the bioavailability of silver to a freshwater snail after waterborne exposure to silver nitrate and silver nanoparticles

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Table S1. Composition of different media used in exposures to AgNO₃ and AgNP. Media were prepared according to US EPA's "Methods for measuring the acute toxicity of effluents and receiving water to freshwater and marine organisms," 2002.

	Reagent added (mg/L)				Final Water Quality
	NaHCO ₃	CaSO ₄ •2H ₂ O	MgSO ₄	KCl	Hardness ¹
Soft	48	30	30	2	40-48
Moderately Hard	96	60	60	4	80-100
Hard	192	120	120	8	160-180
Very Hard	384	240	240	16	280-320

¹ Hardness expressed as mg CaCO₃/L.

Figure S1. The relationship of snail length and weight (n= 60).

The characteristics of PVP- capped AgNPs

Table S2. Hydrodynamic diameter of PVP AgNPs (by DLS and FI-FFF) and zeta potential. Stock diluted to 4 mg/l with DI and MOD water were analyzed.

Sample	By DLS		By FI-FFF	Zeta potential (mV)
	z-average (nm)	PdI *	Peak maximum (nm)	
PVP in DI	33 ± 1	0.409 ± 0.01	30.45 ± 2.19	-13.9 ± 0.9
PVP in MOD	34 ± 2	0.487 ± 0.08	30.43 ± 0.74	-9.3 ± 0.6
PVP in MOD (24h)	36 ± 1	0.403 ± 0.01	32.08 ± 0.74	-9.7 ± 0.9

*PdI= polydispersity index

Table S3. Core diameter of PVP particles in MOD measured by TEM:

PVP	Average Core diameter $\pm$ SD (nm)	Number of particles measured
0.43 μ M (4 mg/l)	11.3 ± 2.6	113
75 nM	11.1 ± 2.4	100
50 nM	11.4 ± 2.6	103
25 nM	11.9 ± 3.1	101

Figure S2. Absorption in the UV-VIS range of a diluted solution of PVP particles (2.2 ppm). Measurements taken after dilution and after 24 h.

For PVP in MOD water, there was a 5.75% loss of UV signal after 24h. For PVP in DI water, there was 1.38% loss of UV signal after 24h.

PVP Images:

Figure S3. PVP particles at 75 nM concentration in MOD water.

Figure S4. PVP particles at 50 nM concentration in MOD water.

Figure S5. PVP particles at 25 nM concentration in MOD water.

PVP TEM Summary

The 4 mg/l PVP particles in media showed very little aggregation when measured by TEM after 24 hours, which was consistent with FFF, DLS and UV-VIS results. In the case of much more dilute samples (75, 50 and 25nM) some aggregation (Figure S2 and S3) was observed, also growth of nanoparticles (as seen in Figure 1). Size of nano-aggregates varied, but in general the size of large nanoparticles and nano aggregates were between 50 and 100 nm. The average particle size did not change significantly; aggregates were not included to calculate this value.

The characteristics of PEG-capped AgNPs

Table S4. Hydrodynamic diameter of PEG AgNPs (by DLS and FI-FFF) and zeta potential. Stock diluted to 2.4 mg/L with DI and MOD water were analyzed.

Sample	By DLS		By FI-FFF	Zeta potential (mV)
	z-average (nm)	Pdl *	Peak maximum (nm)	
PEG in DI	39 ± 1	0.162 ± 0.01	46.85 ± 0.55	-14.6 ± 0.9
PEG in MOD	44 ± 1	0.239 ± 0.03	44.73 ± 1.87	-10 ± 1
PEG in MOD (24 h)	40 ± 1	0.375 ± 0.04	**	-11.2 ± 0.8

*Pdl= polydispersity index

** PEG in MOD water was not measurable after 24 h.

Table S5. Core diameter of PEG particles in MOD measured by TEM:

PEG	Average Core diameter $\pm$ SD (nm)	Number of particles measured
0.24 μ M (2.2 mg/l)	12.5 $\pm$ 3.6	105
75 nM	16.3 $\pm$ 6.8	100
50 nM	14.3 $\pm$ 3.9	105
25 nM	ND*	ND

*The average core diameter was not determined at 25nM because of aggregation of the sample.

Figure S6. Absorption in the UV-VIS range of diluted solution of PEG particles (2.2 ppm). Measurements were taken after dilution and after 24 hr.

For PEG in MOD water a 57.42% loss of UV signal was observed after 24 hours. For PEG in DI water there was a 6.55% loss of UV signal after 24 h.

PEG Images:

Figure S7. PEG particles at 2.2 mg/l concentration in MOD water.

A

B

Figure S8A and B. PEG particles at 75 nM concentration in MOD water.

A

B

Figure S9A and B. PEG particles at 50 and 25 nM concentration in MOD water.

PEG TEM Summary

There seems to be an influence on particle size (average) as well as aggregation (as seen in Figure 5B) in the case of dilute solution (PEG 75nM), which is not observed in the concentrated solution. Aggregation was observed in the 50 nM solution, and was extensive in the 25 nM solution.

Elimination Rate Constants

Growth rate constants (k_g) were estimated by fitting data to an exponential growth rate equation:

$$Wt_{\text{snail}} = Wt_{\text{snail}}^0 e^{k_g t} \quad (\text{Eq. S1})$$

where Wt_{snail} is the average dry weight of snail at a time point, Wt_{snail}^0 is the average dry weight of snail at the initial time of the depuration, and t is the time of the experiment (d).

The elimination constants, k_e (d^{-1}), were determined by fitting data with either a one (Eq. S2) or two-compartment (Eq. S3) exponential decay equation:

$$[M]_{\text{snail}} = [M]_{\text{snail}}^0 e^{-k t} \quad (\text{Eq. S2})$$

$$[M]_{\text{snail}} = \underbrace{[M]_{\text{snail}}^0 e^{-k_f t}}_{\text{Fast-exchanging compartment}} + \underbrace{[M]_{\text{snail}}^0 e^{-k_s t}}_{\text{Slow-exchanging compartment}} \quad (\text{Eq. S3})$$

where $[M]_{\text{snail}}$ is the silver concentration (nmol g^{-1}) at a time point, $[M]_{\text{snail}}^0$ is the silver concentration at the initial time of depuration, and t is the depuration time (d). The loss rate constant k is representative of the total loss of metal over the depuration time of the experiment. If growth of the organisms is negligible then k equals k_e . If growth is significant, then k_e equals $k - k_g$. For modeling, the rate constant from the slow-exchanging compartment (k_s) was used.

Membrane Transport Processes

Binding of metals to membrane sites was assumed to approximate Michaelis-Menten reaction kinetics as described:

$$\text{Influx}_{\text{organism}} = \frac{B_{\text{max}} \times [M]_{\text{exposure}}}{K_{\text{metal}} + [M]_{\text{exposure}}} \quad (\text{Eq. S4})$$

Where $\text{Influx}_{\text{organism}}$ ($\text{nmol g}^{-1} \text{d}^{-1}$) is the rate of accumulated metal, B_{max} (nmol g^{-1}) is representative of the number of transport sites, $[M]_{\text{exposure}}$ is the exposure metal concentrations, and K_{metal} (nmol l^{-1}) represents the specific metal affinity for the binding site. The parameters B_{max} and K_{metal} were determined by fitting accumulation data to Eq. S4.

Table S6. Speciation of Ag in different exposure media as determined by MINEQL+.

	pH	Percentage		
		Ag ⁺	AgCl [°]	AgSO ₄
DI	5.5	100		
Soft	6.5	93	7	
Moderately Hard	7.5	87	12	
Hard	8	77.7	20.6	1.4
Very Hard	8	64.8	32.7	1.9

Figure S10. Uptake rates ($\text{nmol g}^{-1}\text{d}^{-1} \pm 95\% \text{ C.I.}$) of Ag in *L. stagnalis* soft tissue were measured following 24 hr waterborne exposures to AgNO_3 in hard and soft water. Each symbol represents Ag concentrations (less background concentration) for 10 snails and 3 water samples ($\pm 95\% \text{ C.I.}$). Similar sized snails were used for the two experiments (7.5 and 8.1 mg for hard and soft, respectively).

A

B

Figure S11. Uptake of Ag ($\text{nmol g}^{-1} \text{d}^{-1} \pm 95\%$ C.I.) in snail tissue following exposure to PVP, PEG AgNP, and AgNO_3 versus measured total Ag (nM). Each symbol represents Ag concentrations (less background concentration) for 10 snails and 3 water samples ($\pm 95\%$ C.I.). Results are from different experiments conducted in soft (A) and hard (B) water.

Figure S12. Comparison of the influence of water quality on the influx of silver (nmol g⁻¹ d⁻¹ ±95% C.I.) in snail tissue from PEG (A) and PVP (B) coated nanoparticles. Each symbol represents Ag concentrations (less background concentration) for 10 snails and 3 water samples (±95% C.I.). Separate experiments were conducted for each water hardness and particle coating.

Figure S13. Snail's average dry weight (mg $\pm$ 95% C.I.) during depuration (n=10 for each data point) following 72 h exposure with (A) PVP AgNPs and (B) PEG AgNPs. The solid lines represent growth as predicted by equation S1.

Figure S14. Proportion of loss of Ag over time in *L. stagnalis* for experiments with (A) PVP AgNPs and (B) PEG AgNPs. Each data point represents the average loss from ten snails $\pm$ 95% CI. The dashed and solid lines represent loss from growth dilution (Eq. S1) and physiological loss (Eq. S3 PVP, Eq. S2 PEG), respectively.

Biological retention of PVP and PEG AgNPs

The snails' weight increased over the 14 d depuration phase in both PVP and PEG AgNP elimination experiments. An exponential growth curve was fit to the dry weight data of the snail tissue and the growth constant, $k_g \text{ d}^{-1}$, was determined to be 0.02 ± 0.007 for the experiment with PVP-NP (Figure S12A). A two compartment model was assumed for the experiment with PEG-NP, and the growth constants of $0.06 \pm 0.01 \text{ d}^{-1}$ and $0.03 \pm 0.004 \text{ d}^{-1}$ were determined for 0 to 4 days and 4-14 days, respectively (Figure S12B).

The silver in snail tissue was eliminated at different rates from organisms exposed to PVP AgNP compared to PEG AgNPs. The elimination rate constant (k_{ew}) was determined by fitting a two parameter (PVP data, Figure S13A) or one parameter (PEG data, Figure S13B) exponential decay equation and then correcting for the loss due to growth. The $k_{ew} \text{ (d}^{-1}\text{)}$ for PVP-NP was calculated as $0.04 \pm 0.01 \text{ (S.E.)}$. The elimination rate constant for PEG-NP in snail tissue was determined to be negligible as growth was greater than the loss of Ag.

Steady-state concentrations of Ag from PVP or PEG AgNPs

We used Eq. 3 as well as the rate constants of total uptake and loss determined in MOD water to calculate the steady-state concentrations of Ag from PVP or PEG AgNPs in *L. stagnalis* assuming an exposure concentration of 0.2 nM. This concentration was chosen to represent a realistic environmental concentration of AgNPs (Gottschalk et al., 2013). The steady-state Ag concentrations were 4 nmol/g and 100 nmol/g, respectively for PVP and PEG AgNPs. The 25-fold differences in Ag concentrations can be ascribed to the lack of efflux observed when Ag is accumulated after exposures to PEG AgNPs. Thus, elimination rates have a greater effect than uptake rate on the potential for bioaccumulation of Ag from PEG AgNPs.