Environron. Chem. **2014**, *11*, 247–256
doi:10.1071/EN13141_AC ©CSIRO 2014

Supplementary material

Isotopically modified silver nanoparticles to assess nanosilver bioavailability and toxicity at environmentally relevant exposures

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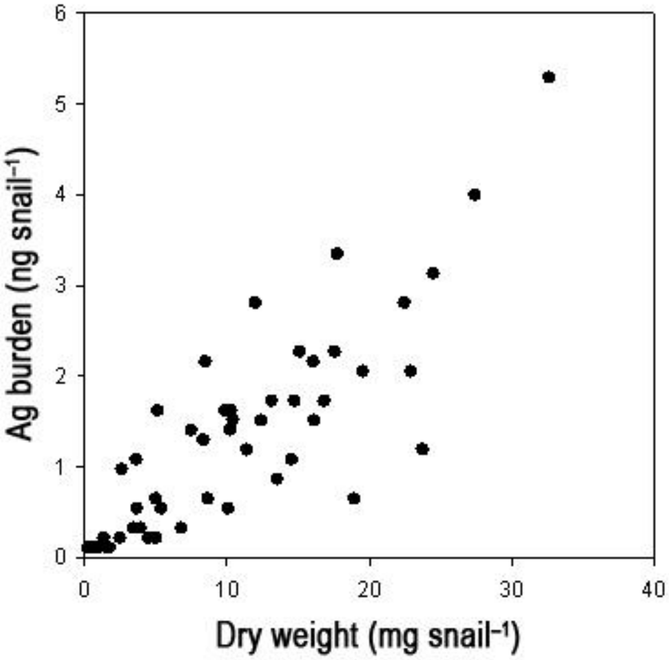

Fig. S1. Ag burden (ng snail^{-1}) in unexposed snails as a function of weight (dry weight), n = 52. The line represents the linear regression relationship (R^2 = 0.88, $P < 0.001$, slope 0.122 ± 0.013)

Snail body size influence on uptake rate constants

In addition to exposure characteristics (e.g. concentrations, speciation), body size (allometry) can influence metal bioaccumulation. For example, size-dependent relationships have been used to describe metal concentrations in a marine bivalve[1], selenium uptake by mussels[2] and lead elimination by freshwater gastropods[3]. Typically, metal uptake is faster for small individuals than for large individuals. Not surprisingly, the rate constant of Ag uptake from water (k_{uw}) appeared faster for smaller than for larger size snails (Fig. S2). This appears the case for both Ag NPs and Ag^+. The faster k_{uw}s determined in this study for labelled Ag NPs (open circles, Fig. S2) compared to that reported by Croteau et al.[4] for unlabelled citrate-capped Ag NPs (solid circle, Fig. S2) might thus reflect a snail size effect rather than a nanoparticle effect. Experimenting with similar size organisms is thus crucial when using *L. stagnalis* to characterise the bioavailability and toxicity of Ag nanoparticles. To minimize the confounding influence of size when experimenting with *L. stagnalis*, we recommend using snails whose size range from 8 to 15 mg DW.

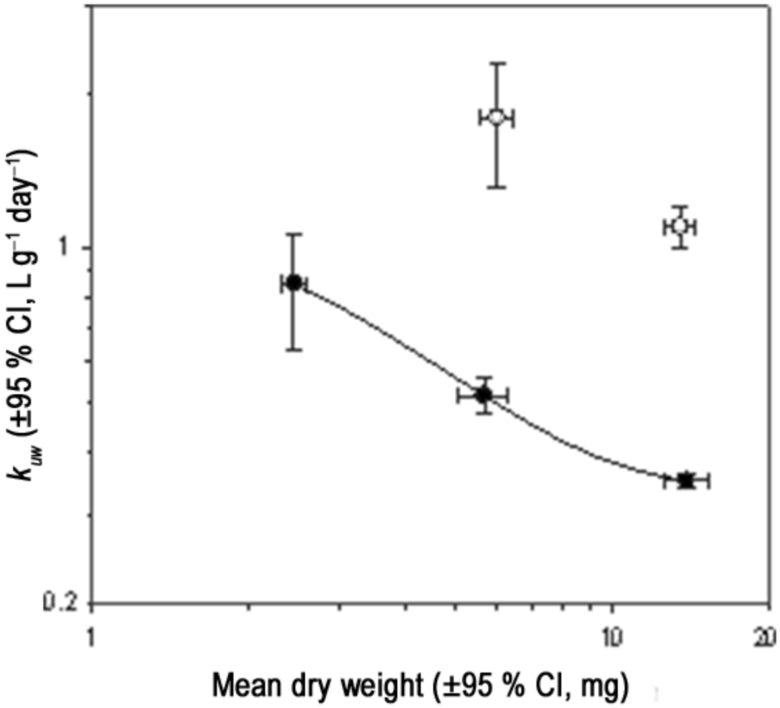

Fig. S2. Rate constant of Ag uptake (k_{uw},) as a function of weight (mg DW individual^{-1}) for snails exposed to dispersed Ag NPs coated with citrate in MHW (solid circles) and for Ag^+ added as $AgNO_3$. Data for the larger size snails are from Croteau et al.[4] and Ag^+ relationships

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Table S1. Concentrations of ^{109}Ag NPs and predicted amount of 109AgNPs added to algal mats after pouring 10 mL of serially diluted solutions of ^{109}Ag NPs

[^{109}AgNPs] (ug L^{-1})	Added ^{109}Ag (ng)
0.045	0.45
0.225	2.25
1.125	11.25
2.25	22.5
3.375	33.75

Table S2. Dietary Ag exposure concentrations (±s.d.), Ag assimilation efficiencies (AE) (±s.d.) and food ingestion rates (IR) (± s.d.) for snails exposed to diatoms mixed with increasing amounts of ^{109}Ag NPs

DL, detection limit

Dietary exposure (µg g^{-1})	AE (%)	IR (g g^{-1} day^{-1})
0.046 ± 0.020	<DL	<DL
0.16 ± 0.046	<DL	<DL
1.2 ± 0.26	61 ± 10	0.11 ± 0.03
1.9 ± 0.35	45 ± 15	0.11 ± 0.03
2.9 ± 0.39	39 ± 11	0.11 ± 0.06

Table S3. Comparison of exposure concentrations and lowest detectable Ag concentration in *L. stagnalis* soft tissues when a tracer is used (isotopically enriched ^{109}Ag) or not

	With ^{109}Ag	Without a tracer
Aqueous Ag concentration yielding to a significant uptake after 24-h exposure	6 ng L^{-1}	0.2 µg L^{-1}
Dietary Ag concentrations yielding to a significant uptake after 3–4-h exposure	0.07 µg g^{-1}	2 µg g^{-1}
Lowest detectable tissue concentration	1 ng g^{-1}	0.1 µg g^{-1}

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