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

Supplementary Material

The bioaccumulation testing strategy for manufactured nanomaterials: physico-chemical triggers and read across from earthworms in a meta-analysis.

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Supplemental Table S1. Linear equations for the curve fits of the physico-chemical parameters plotted against calculated bioaccumulation factors (BAFs), as shown in Figure 4 of the main manuscript.

Panel Letter/Metric	**Material and Equation**
Figure 4(A) Primary particle size	Copper: $y = 1.0369 + (– 0.0613)*x$
Figure 4(B) Hydrodynamic diameter	Copper: $y = 0.2428 + 0.0033*x$
Figure 4(C) Metal dissolution rate	Copper: $y = 0.3294 + 0.2793*x$
Figure 4(D) Particle settling rate	Copper: $y = 0.6022 + (– 2.0430)*x$
Figure 4(E) Hydrodynamic diameter	Cadmium: $y = 1.9520 + (– 0.0065)*x$ Tellurium: $y = (– 0.0433) + 0.0009*x$
Figure 4(F) Metal dissolution rate	Cadmium: $y = 1.0861 + 0.4100x$ Tellurium: $y = 0.0864 + (– 0.2648)*x$
Figure 4(G) Particle settling rate	Cadmium: $y = 1.7975 + (– 55.5760)*x$ Tellurium: $y = 0.0356 + (– 0.3757)*x$

The equations are for the curve fits shown in Figure 4 using a polynomial, linear equation $y = b + a*x$ where y is bioaccumulation factor and x is the respective metric value, with constants a and b shown (SigmaPlot 13). Unfortunately with only two Ag materials (Ag_2S NPs and Ag NPs, without coatings) there was not enough data to derive regression equations for Ag alone.

Supplemental Table S2: Selected ionic radii and charge densities for the metal ions.

Material	Metal ion	Coordination number	Ionic radius (Å)*	Charge density (ρ)**
Ag NPs	Ag^{+}	2	0.67	0.79
Ag_2S NPs	Ag^{+}	2	0.67	0.79
$AgNO_3$	Ag^{+} in $[Ag(H_2O)_4]^{+}$	4	1.00	0.24
CdTe Bulk and CdTe QDs	Cd^{2+}	4	0.78	1.01
	Te^{2-}	4	2.21	0.04
CuO NPs	Cu^{2+}	4	0.57	2.58
$CuSO_4$	Cu^{2+} in $[Cu(H_2O)_6]^{2+}$	6	0.73	1.23

* Ionic radius **(Å)** derived from Shannon[1].

** Charge density (ρ) calculated using Eq. 1;

$$\rho = \frac{q}{\frac{4}{3}\pi r^3} \quad (1)$$

where *q* is the ion charge and *r* denotes the Shannon ionic radius. The equation extracted from Huang *et al*.[2]

Supplemental Table S3. Multiple linear regression analysis using IBM SPSS Statistics 25.

(A) Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.932[a]	.868	.851	.2106274

a. Predictors: (Constant), SettlingRate, IonicRadius, HydrodynamicDiameter, DissolutionRate, ParticleSize, ChargeDensity

b. Dependent Variable: CalcBAF

(B) ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	13.451	6	2.242	50.534	.000[b]
	Residual	2.041	46	.044		
	Total	15.492	52			

a. Dependent Variable: CalcBAF

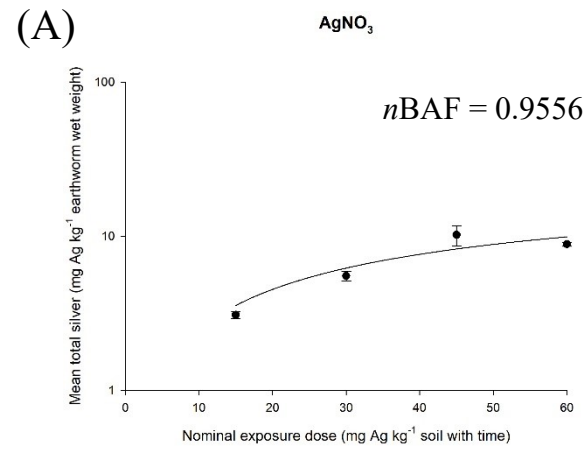
b. Predictors: (Constant), SettlingRate, IonicRadius, HydrodynamicDiameter, DissolutionRate, ParticleSize, ChargeDensity

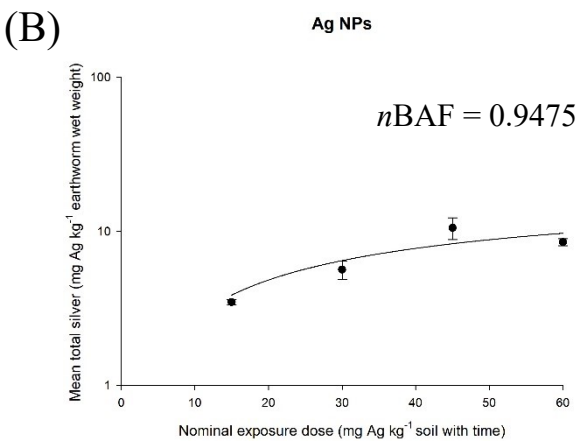
(C) Coefficients[a]

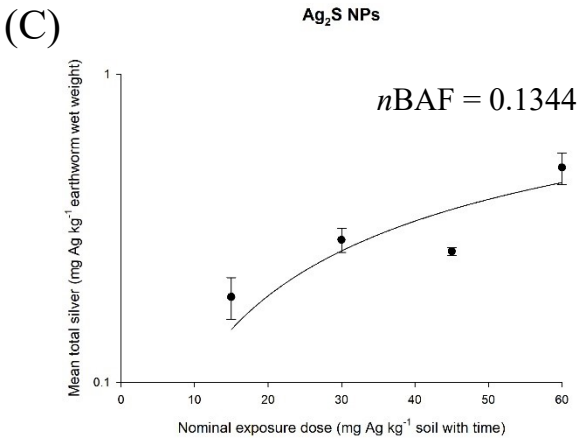
Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
1	(Constant)	2.306	.358		6.447	.000	1.586	3.026
	ChargeDensity	1.064	.559	2.110	1.904	.063	-.061	2.188
	IonicRadius	-.348	.360	-.429	-.966	.339	-1.073	.377
	ParticleSize	-.435	.139	-2.743	-3.126	.003	-.715	-.155
	HydrodynamicDiameter	-.002	.001	-.113	-1.234	.223	-.004	.001
	DissolutionRate	-1.090	.330	-.769	-3.305	.002	-1.754	-.426

SettlingRate	4.933	2.629	.470	1.876	.067	-.359	10.225

a. Dependent Variable: CalcBAF

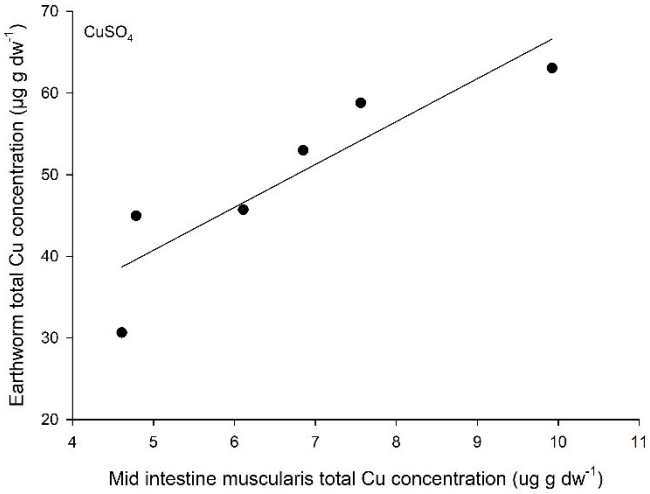




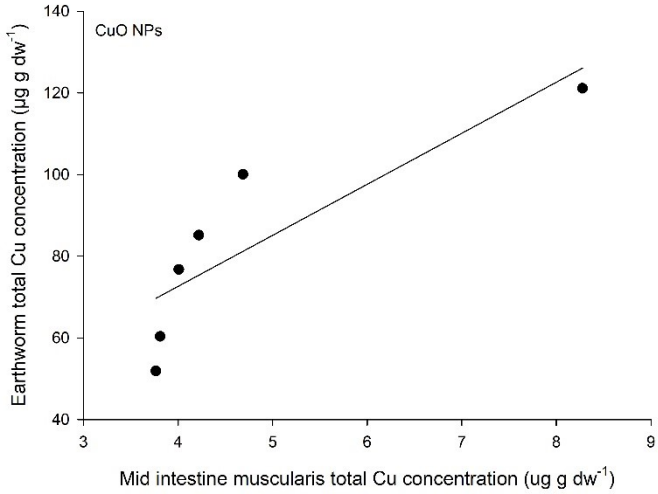


Supplemental Figure S1. Nominal chemical dose exposure in soil with silver materials (A) $AgNO_3$, (B) Ag NPs, (C) Ag_2S NPs, plotted against measured total metal concentration in the earthworm (*Eisenia fetida*) tissue (mean ± S.E.M, $n = 4$). Data for silver from Baccaro *et al*. [3]

(A)

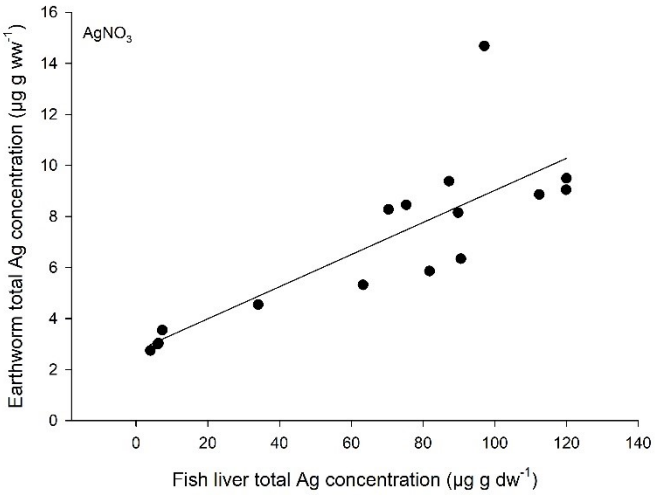


(B)

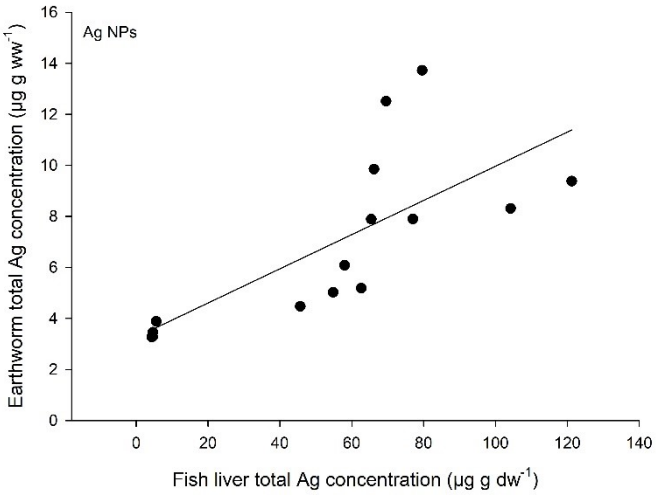


Supplemental Figure S2. Correlations between tier 2 (earthworms) and tier 3 (gut sacs) exposed to $CuSO_4$ or uncoated CuO NPs. The earthworms were exposed to 200 mg kg^{-1} of the Cu materials for 14 days, and the fish gut was exposed to 6.354 mg L^{-1} for 4 h. Data were ranked and then correlated. The r^2 values were 0.8006 and 0.7222 for the $CuSO_4$ and uncoated CuO NPs, respectively. The equations of the lines are (A) $y=5.2514x+14.489$ and (B) $y=12.498x+22.623$. The earthworm total Cu concentrations were taken from Tatsi *et al*.[4] and the fish data from Boyle *et al*.[5]

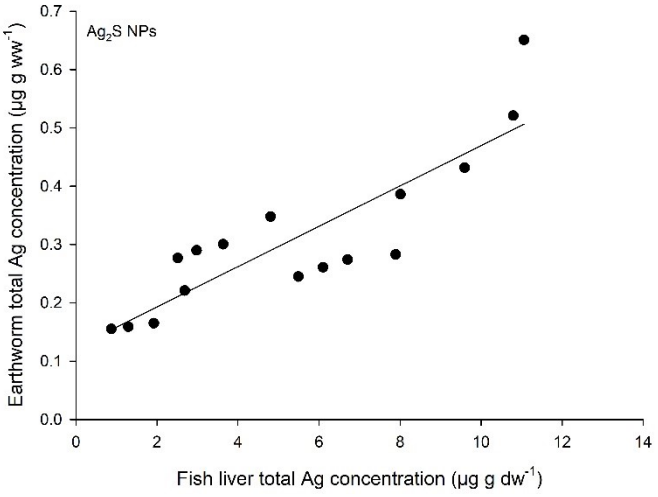
(A)


AgNO3
Earthworm total Ag concentration (µg g ww-1)
Fish liver total Ag concentration (µg g dw-1)

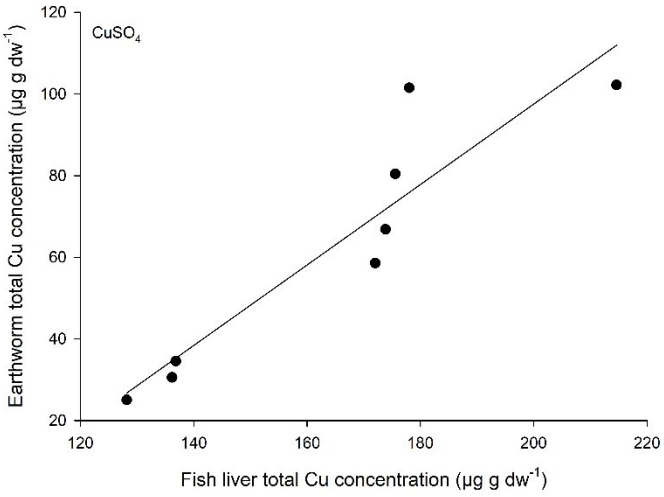
(B)


Ag NPs
Earthworm total Ag concentration (µg g ww-1)
Fish liver total Ag concentration (µg g dw-1)

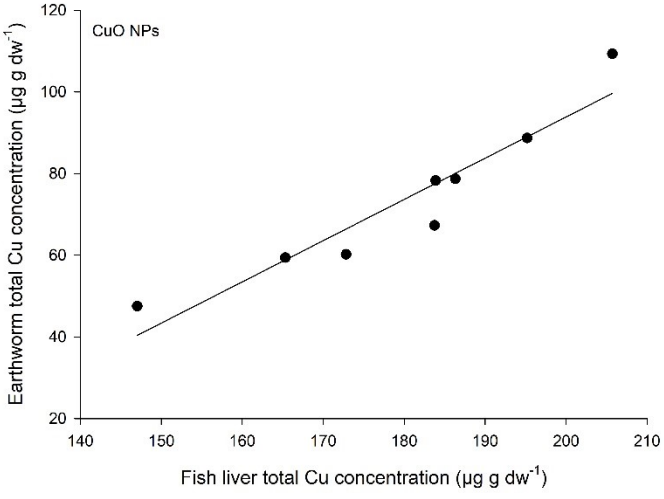
(C)


Ag2S NPs
Earthworm total Ag concentration (µg g ww-1)
Fish liver total Ag concentration (µg g dw-1)

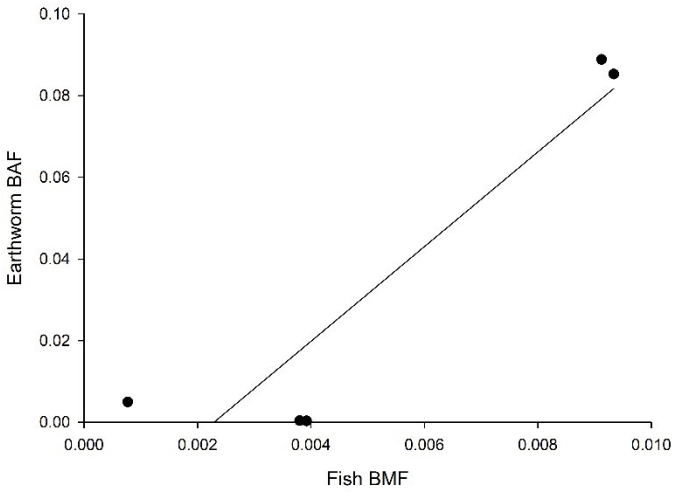
(D)


CuSO4
Earthworm total Cu concentration (µg g dw-1)
Fish liver total Cu concentration (µg g dw-1)

(E)


CuO NPs
Earthworm total Cu concentration (µg g dw-1)
Fish liver total Cu concentration (µg g dw-1)

(F)


Earthworm BAF
Fish BMF

Supplemental Figure S3. Correlations between tier 2 (earthworms) and tier 4 (fish liver) of the testing strategy using for (A) $AgNO_3$, (B) Ag NPs, (C) Ag_2S NPs, (D) $CuSO_4$ and (E) uncoated CuO NPs. For the Ag materials, the earthworms were exposed to soil containing nominally 100 mg Ag kg^{-1} for 28 days, and the fish were fed a diet containing nominally 100 mg Ag kg^{-1} for 28 days. Both species were sampled every week (1, 2, 3 and 4). The data from each species at each week was ranked and correlated together. The r^2 values were 0.6702, 0.5306 and 0.7502 for the $AgNO_3$, Ag NPs and Ag_2S NPs, respectively. The equations of the line are (A) y=6e-05x+2.7308, (B) y=7e-05x+3.2678 and (C) y=3e-05x+0.1238. The total Ag earthworm concentrations are from Baccaro *et al*.[3] and the fish liver concentrations are from Clark *et al*.[6] For the Cu materials, earthworms were exposed to soil containing nominally 200 mg kg^{-1} for 14 days (Tatsi *et al*.[4]), and the fish were fed a diet containing nominally 750 mg kg^{-1} for 14 days (Boyle *et al*.[6]). Both species were sampled at day 14 only. The r^2 values are 0.8536 and 0.8911 for the $CuSO_4$ and uncoated CuO NPs, respectively. The equation of the lines are (D) y=0.9852x-99.51 and (E) y=1.01x-108.14. Panel (F) is the relationship between the calculated earthworm BAFs and fish BMFs values. The r^2 value is 0.8561 and the correlation coefficient is 0.500. The equation of the line is y=11.597x-0.0266.

References:

1. R. Shannon, Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides, *Acta Crystallographica Section A*, 1976, **32**, 751-767.
2. S. Huang, P. Du, C. Min, Y. Liao, H. Sun and Y. Jiang, Poly(1-amino-5-chloroanthraquinone): Highly Selective and Ultrasensitive Fluorescent Chemosensor For Ferric Ion, *Journal of Fluorescence*, 2013, **23**, 621-627.
3. M. Baccaro, A. K. Undas, J. de Vriendt, J. van den Berg, R. Peters and N. W. van den Brink, Ageing, dissolution and biogenic formation of nanoparticles: how do these factors affect the uptake kinetics of silver nanoparticles in earthworms?, *Environmental Science: Nano*, 2018, **5**, 1107-1116.
4. K. Tatsi, B. J. Shaw, T. H. Hutchinson and R. D. Handy, Copper accumulation and toxicity in earthworms exposed to CuO nanomaterials: Effects of particle coating and soil ageing, *Ecotoxicology and Environmental Safety*, 2018, **166**, 462-473.
5. D. Boyle, N. J. Clark, T. L. Botha and R. D. Handy, Comparison of the dietary bioavailability of copper sulphate and copper oxide nanomaterials in *ex vivo* gut sacs of rainbow trout: effects of low pH and amino acids in the lumen, *Environmental Science: Nano*, 2020, **7**, 1967-1979.
6. N. J. Clark, D. Boyle and R. D. Handy, An assessment of the dietary bioavailability of silver nanomaterials in rainbow trout using an *ex vivo* gut sac technique, *Environmental Science: Nano*, 2019, **6**, 646-660.