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

Supplementary Material

The bioaccumulation testing strategy for nanomaterials: correlations with particle properties and a meta-analysis of *in vitro* fish alternatives to *in vivo* fish tests

Handy, R.D., Clark, N.J., Boyle, D., Vassallo, J., Green, C., Nasser, F., Botha, T.L., Wepener, V., van den Brink, N.W. and Svendsen, C.

Supplemental Table S1. Linear equations for the curve fits of the physico-chemical parameters plotted against calculated biomagnification factors (*n*BMFs) for the materials, as shown in Figure 3 of the main manuscript.

Panel Letter/Metric	**Fish body organ and Equation**
Figure 3(D) Primary particle size	Liver: $y = -0.0709 + 0.0199x$
	Mid intestine: $y = 0.0033 + 0.0013x$
	Hind intestine: $y = -0.0634 + 0.0136x$
	Kidney: $y = -0.0802 + 0.0048x$
Figure 3(E) Hydrodynamic diameter	Liver: $y = 1.1036 - 0.0060x$
	Mid intestine: $y = 0.0999 - 0.0006x$
	Hind intestine: $y = 0.8245 - 0.0052x$
	Kidney: $y = 0.1539 - 0.0008x$
Figure 3(F) Metal dissolution rate	Liver: $y = 0.0933 + 27.1619x$
	Mid intestine: $y = 0.0003 + 2.5198x$
	Hind intestine: $y = -0.0158 + 21.8548x$
	Kidney: $y = -0.0032 + 4.6501x$

The equations are for the curve fits shown in Figure 3 using a polynomial, linear equation $y = b + a*x$ where y is biomagnification factor and x is the respective metric value, with constants a and b shown (SigmaPlot 13).

Supplemental Table S2. Multiple linear regression analysis using IBM SPSS Statistics 25.

(A) Trout liver *n*BMF linear regression analysis

(i) Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.911[a]	.830	.811	.2387600	.830	43.838	2	18	.000

a. Predictors: (Constant), DissolutionRate, ParticleSize

(ii) ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.998	2	2.499	43.838	.000[b]
	Residual	1.026	18	.057		
	Total	6.024	20			

a. Dependent Variable: BMF_Liver

b. Predictors: (Constant), DissolutionRate, ParticleSize

(iii) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.639	.141		-4.537	.000	-.936	-.343					
	ParticleSize	.021	.003	.699	7.122	.000	.015	.027	.609	.859	.693	.983	1.018
	DissolutionRate	28.076	4.033	.683	6.962	.000	19.603	36.549	.591	.854	.677	.983	1.018

a. Dependent Variable: BMF_Liver

(B) Trout mid intestine *n*BMF linear regression analysis

(i) Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.822[a]	.676	.640	.0278175	.676	18.799	2	18	.000

a. Predictors: (Constant), DissolutionRate, ParticleSize

(ii) ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.029	2	.015	18.799	.000[b]
	Residual	.014	18	.001		
	Total	.043	20			

a. Dependent Variable: BMF_MidIntestine

b. Predictors: (Constant), DissolutionRate, ParticleSize

(iii) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.043	.016		-2.616	.017	-.077	-.008					
	ParticleSize	.001	.000	.560	4.142	.001	.001	.002	.471	.699	.556	.983	1.018
	DissolutionRate	2.362	.470	.680	5.026	.000	1.374	3.349	.606	.764	.674	.983	1.018

a. Dependent Variable: BMF_MidIntestine

(C) Trout hind intestine *n*BMF linear regression analysis

(i) Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.906[a]	.820	.799	.1610478	.820	38.802	2	17	.000

a. Predictors: (Constant), DissolutionRate, ParticleSize

(ii) ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.013	2	1.006	38.802	.000[b]
	Residual	.441	17	.026		
	Total	2.454	19			

a. Dependent Variable: BMF_HindIntestine

b. Predictors: (Constant), DissolutionRate, ParticleSize

(iii) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.407	.098		-4.148	.001	-.614	-.200					
	ParticleSize	.014	.002	.729	6.962	.000	.010	.019	.598	.860	.716	.964	1.037
	DissolutionRate	18.411	2.783	.693	6.616	.000	12.540	24.282	.555	.849	.680	.964	1.037

a. Dependent Variable: BMF_HindIntestine

(D) Trout kidney *n*BMF linear regression analysis

(i) Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.783[a]	.614	.571	.0886015	.614	14.298	2	18	.000

a. Predictors: (Constant), DissolutionRate, ParticleSize

(ii) ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.224	2	.112	14.298	.000[b]
	Residual	.141	18	.008		
	Total	.366	20			

a. Dependent Variable: BMF_Kidney

b. Predictors: (Constant), DissolutionRate, ParticleSize

(ii) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.181	.052		-3.464	.003	-.291	-.071					
	ParticleSize	.005	.001	.679	4.598	.000	.003	.007	.615	.735	.674	.983	1.018
	DissolutionRate	4.955	1.497	.489	3.311	.004	1.811	8.100	.400	.615	.485	.983	1.018

a. Dependent Variable: BMF_Kidney

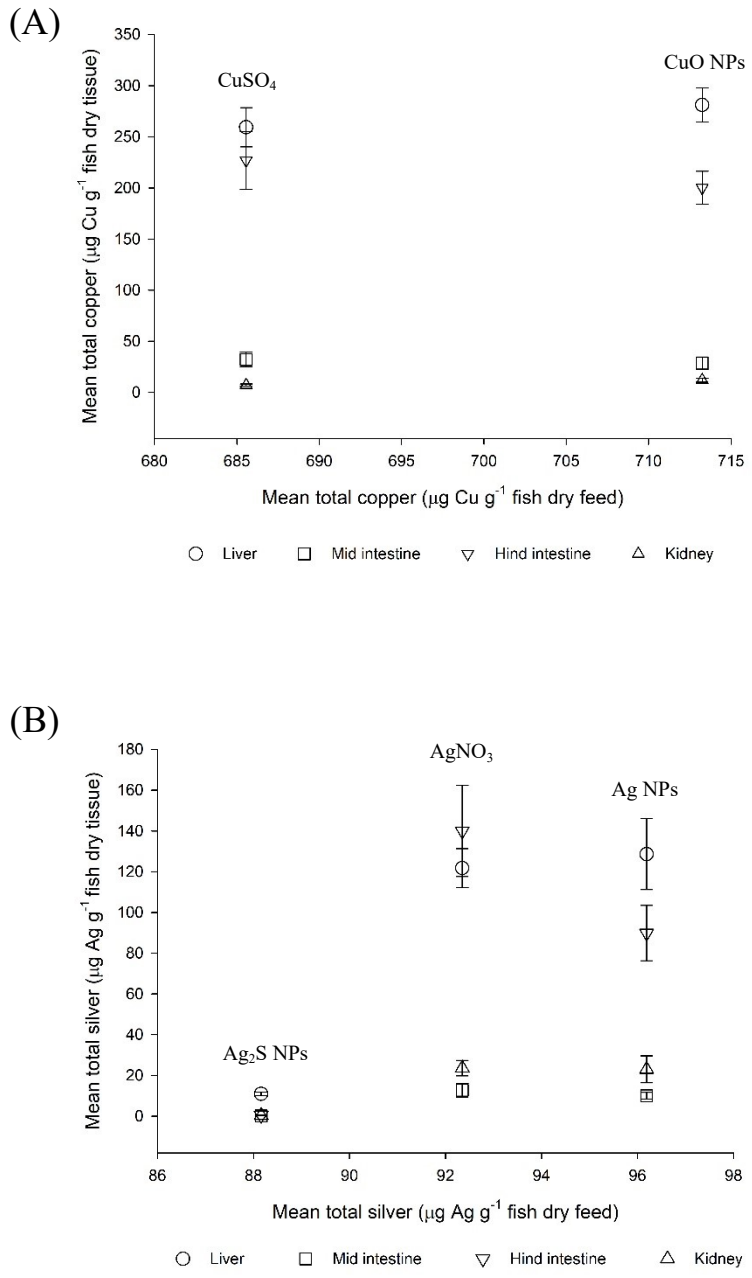
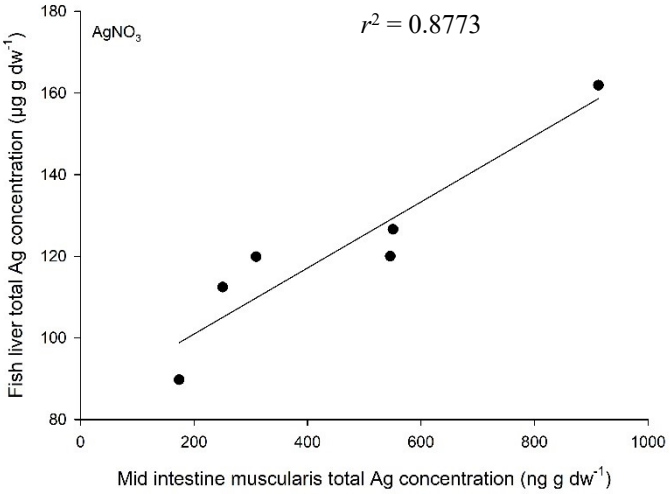
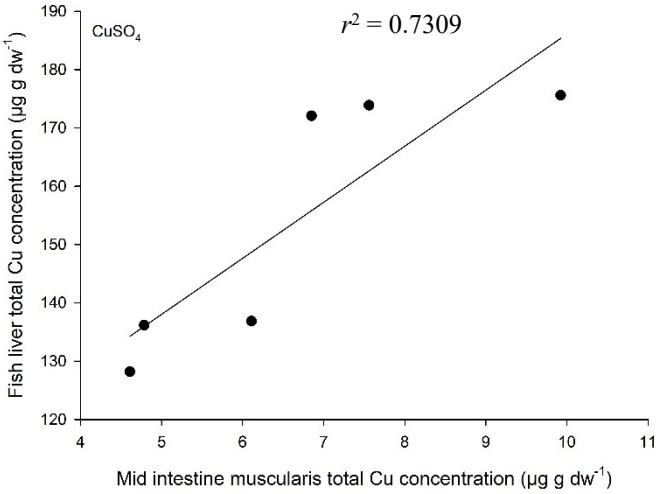


Figure S1. Measured total metal concentration in the fish feed plotted against the mean total metal concentration in rainbow trout (*Oncorhynchus mykiss*) tissue (mean ± S.E.M, n = 4 - 9) as liver, mid intestine, hind intestine and kidney, sampled following the last experimental uptake time point for (A) copper- and (B) silver-based test materials. Copper data sourced from Boyle *et al*.[1] Silver data sourced from Clark *et al*.[2]

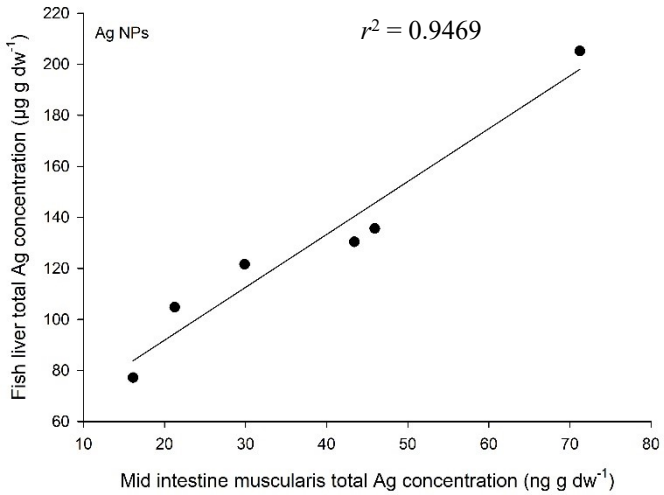
(A)


AgNO3
r2 = 0.8773
Fish liver total Ag concentration (µg g dw-1)
Mid intestine muscularis total Ag concentration (ng g dw-1)

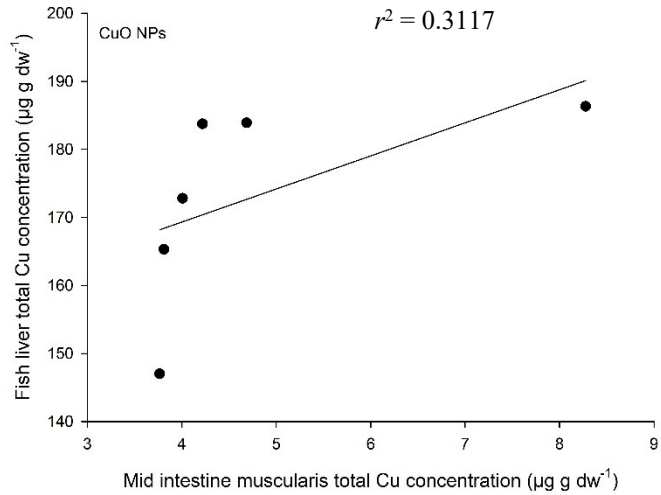
(D)


CuSO4
r2 = 0.7309
Fish liver total Cu concentration (µg g dw-1)
Mid intestine muscularis total Cu concentration (µg g dw-1)

(B)


Ag NPs
r2 = 0.9469
Fish liver total Ag concentration (µg g dw-1)
Mid intestine muscularis total Ag concentration (ng g dw-1)

(E)


CuO NPs
r2 = 0.3117
Fish liver total Cu concentration (µg g dw-1)
Mid intestine muscularis total Cu concentration (µg g dw-1)

(C)

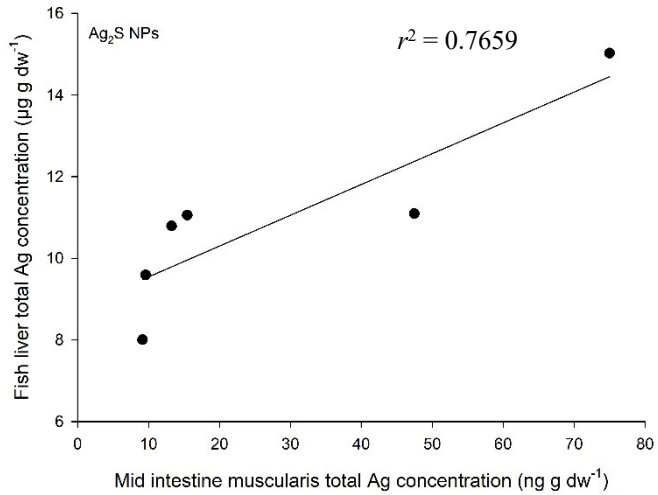

Ag2S NPs
r2 = 0.7659
Fish liver total Ag concentration (µg g dw-1)
Mid intestine muscularis total Ag concentration (ng g dw-1)

Figure S2. Correlations between tier 3 (*ex vivo* exposure, mid intestine muscularis) and tier 4 (*in vivo* exposure, liver concentrations) of the testing strategy. The materials used are silver (left panels) or copper (right panels) based. The data were ranked and then correlated. Copper data sourced from Boyle *et al*.[3] for gut sacs. Silver data sourced from Clark *et al*.[2, 4]

References

1. D. Boyle, N. J. Clark, B. P. Eynon and R. D. Handy, Dietary exposure to copper sulphate compared to a copper oxide nanomaterial in rainbow trout: bioaccumulation with minimal physiological effects, *Environmental Science: Nano*, 2021, **8**, 2297-2309.
2. N. J. Clark, D. Boyle, B. P. Eynon and R. D. Handy, Dietary exposure to silver nitrate compared to two forms of silver nanoparticles in rainbow trout: bioaccumulation potential with minimal physiological effects, *Environmental Science: Nano*, 2019, **6**, 1393–1405.
3. 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.
4. N. J. Clark, R. Clough, D. Boyle and R. D. Handy, Development of a suitable detection method for silver nanoparticles in fish tissue using single particle ICP-MS, *Environmental Science: Nano*, 2019, **6**, 3388-3400.