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

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

David Boyle[1], Nathaniel, J. Clark[1], Tarryn L. Botha[2] & Richard D. Handy[1,3]*

[1]School of Biological and Marine Sciences, University of Plymouth, Plymouth PL4 8AA, UK

[2]Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa

[3]Visiting Professor, Department of Nutrition, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq

*Corresponding author: Richard D. Handy

School of Biological and Marine Sciences, University of Plymouth, Plymouth PL4 8AA, UK

Tel: +44 1752 584630

r.handy@plymouth.ac.uk

Supplementary Information

Supplementary Table 1. Confirming exposure of gut sacs to Cu at a concentration of 6.354 mg L^{-1} of Cu as CuO ENMs or $CuSO_4$. Total mass (ng) of Cu found in the luminal saline (rinse 1) and EDTA wash (rinse 2).

Treatment	Sample type	Stomach	Anterior intestine	Mid intestine	Posterior intestine
Control	Luminal saline	33 ± 8	694 ± 393	24 ± 9	292 ± 174
	EDTA wash	22 ± 9	119 ± 42	9 ± 3	49 ± 25
$CuSO_4$	Luminal saline	4068 ± 1583	4117 ± 730	838 ± 169	2151 ± 768
	EDTA wash	1229 ± 470	457 ± 69	161 ± 25	408 ± 151
CuO ENMs	Luminal saline	6052 ± 2024	6121 ± 1798	698 ± 221	2460 ± 449
	EDTA wash	430 ± 205	535 ± 273	154 ± 61	573 ± 116

Data are means ± SEM of *n* = 5/6 samples. Controls were loaded with saline, only (Cu < 0.1 μg L^{-1}).

Supplementary Table 2. Confirming exposure of gut sacs to Cu at a concentration of 6.354 mg L^{-1} of Cu as CuO ENMs or $CuSO_4$ and in the presence or absence of 5 mM L-histidine. Total mass (ng) of Cu found in the luminal saline (rinse 1) and EDTA wash (rinse 2).

	Sample type	Control	$CuSO_4$	CuO ENMs	$CuSO_4$ and histidine	CuO ENMs and histidine
Mid-intestine	Luminal saline	11 ± 7	816 ± 127	509 ± 72	1050 ± 155	498 ± 103
	EDTA wash	0 ± 0	160 ± 59	45 ± 21	145 ± 16	162 ± 59
Posterior-intestine	Luminal saline	230 ± 54	3353 ± 444	3680 ± 499	1530 ± 278	1327 ± 229
	EDTA wash	23 ± 9	362 ± 91	532 ± 169	214 ± 68	168 ± 37

Data are means ± SEM of *n* = 5/6 samples. Controls were loaded with saline, only (Cu < 0.1 μg L^{-1}).

Supplementary Table 3. Confirming exposure of gut sacs to Cu at a concentration of 6.354 mg L^{-1} of Cu as CuO ENMs or $CuSO_4$ and in the presence or absence of 5 mM L-cysteine. Total mass (ng) of Cu found in the luminal saline (rinse 1) and EDTA wash (rinse 2).

	Sample type	Control	$CuSO_4$	CuO ENMs	$CuSO_4$ and cysteine	CuO ENMs and cysteine
Mid-intestine	Luminal saline	56 ± 39	2317 ± 683	1183 ± 204	2288 ± 532	1916 ± 508
	EDTA wash	13 ± 4	195 ± 52	175 ± 20	189 ± 42	229 ± 111
Posterior-intestine	Luminal saline	315 ± 260	4537 ± 1017	3124 ± 769	1853 ± 183	2387 ± 599
	EDTA wash	145 ± 138	629 ± 224	405 ± 54	393 ± 101	273 ± 62

Data are means ± SEM of n = 3-5 samples. Controls were loaded with saline, only (Cu < 0.1 µg L^{-1}).

Supplementary Table 4. Percentage of total Cu ($CuSO_4$ or CuO ENMs) dosed in gut sacs and in the presence or absence of 5 mM L-histidine that was recovered from the luminal saline (rinse 1) and EDTA wash (rinse 2) after 4 h.

	Sample type	$CuSO_4$	CuO ENMs	$CuSO_4$ and histidine	CuO ENMs and histidine
Mid- intestine	Luminal saline	27.3 ± 3.6^{abcde}	18.8 ± 2.9^{abcde}	37.9 ± 4.0^{cde}	17.1 ± 3.0^{abcde}
	EDTA wash	5.7 ± 2.0^{abcd}	1.8 ± 0.8^{a}	5.5 ± 0.7^{abcd}	5.5 ± 1.6^{abcd}
Posterior-intestine	Luminal saline	54.1 ± 8.0^{de}	64.4 ± 5.9^{e}	29.1 ± 5.6^{bcde}	23.4 ± 4.2^{abcde}
	EDTA wash	6.0 ± 1.5^{abcd}	8.8 ± 2.1^{abcde}	4.2 ± 1.3^{abc}	3.2 ± 0.8^{ab}

Data are means ± SEM of n = 5/6 samples. Measured Cu concentrations in rinses are shown in Supplementary Table 2; background Cu concentrations in controls were subtracted prior to calculations. Values with different lower case letters are significantly different (Kruskal-Wallis Test, $p < 0.001$).

Supplementary Table 5. Percentage of total Cu ($CuSO_4$ or CuO ENMs) dosed in gut sacs and in the presence or absence of 5 mM L-cysteine that was recovered from the luminal saline (rinse 1) and EDTA wash (rinse 2) after 4 h.

	Sample type	$CuSO_4$	CuO ENMs	$CuSO_4$ and cysteine	CuO ENMs and cysteine
Mid- intestine	Luminal saline	42.8 ± 11.1^{ab}	37.7 ± 18.1^{ab}	49.3 ± 13.3^{ab}	44.6 ± 12.7^{ab}
	EDTA wash	4.1 ± 0.6^{ab}	5.2 ± 2.4^{ab}	3.7 ± 0.8^{ab}	5.3 ± 2.8^{ab}
Posterior-intestine	Luminal saline	62.8 ± 9.8^{a}	43.0 ± 5.8^{ab}	23.7 ± 2.2^{ab}	37.0 ± 9.6^{ab}
	EDTA wash	6.5 ± 1.7^{ab}	4.6 ± 1.3^{ab}	3.9 ± 1.5^{b}	2.5 ± 1.1^{b}

Data are means ± SEM of n = 3-5 samples. Measured Cu concentrations in rinses are shown in Supplementary Table 3; background Cu concentrations in controls were subtracted prior to calculations. Values with different lower case letters are significantly different (Kruskal-Wallis Test, $p < 0.001$).

Supplementary Table 6. Fluid flux and total Cu accumulation into the serosal compartment of gut sacs.

	Control	$CuSO_4$	CuO ENMs	$CuSO_4$ and histidine	CuO ENMs and histidine
Total Cu concentration in serosal saline ($\mu g\ mL^{-1}\ g^{-1}$)					
Mid-intestine	0.37 ± 0.07^{ab}	0.43 ± 0.14^{ab}	0.78 ± 0.14^{a}	0.02 ± 0.01^{b}	0.23 ± 0.09^{ab}
Posterior-intestine	0.30 ± 0.13^{ab}	0.60 ± 0.26^{ab}	0.36 ± 0.11^{ab}	0.55 ± 0.14^{ab}	0.37 ± 0.11^{ab}
Fluid flux ($\mu L\ g^{-1}\ h^{-1}$)					
Mid-intestine	40.1 ± 16.3^{abcd}	65.7 ± 14.7^{bcd}	61.9 ± 7.0^{bcd}	72.2 ± 10.9^{cd}	161.6 ± 92.5^{d}
Posterior-intestine	-29.1 ± 7.9^{ab}	-20.9 ± 4.6^{abcd}	-12.3 ± 5.3^{abcd}	-33.7 ± 14.6^{abc}	-142.4 ± 80.3^{a}

Data are means ± SEM of n = 5/6 samples. Different lowercase letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.05$).

Supplementary Table 7. Fluid flux and rates of total Cu accumulation into the serosal compartment of gut sacs.

	Control	$CuSO_4$	CuO ENMs	$CuSO_4$ and cysteine	CuO ENMs and cysteine
Total Cu concentration in serosal saline ($\mu g\ mL^{-1}\ g^{-1}$)					
Mid-intestine	0.35 ± 0.19^{a}	0.67 ± 0.67^{a}	0.28 ± 0.05^{a}	0.60 ± 0.15^{a}	0.62 ± 0.18^{a}
Posterior-intestine	0.79 ± 0.44^{a}	1.03 ± 0.23^{a}	0.44 ± 0.12^{a}	1.03 ± 0.30^{a}	0.78 ± 0.15^{a}
Fluid flux ($\mu L\ g^{-1}\ h^{-1}$)					
Mid-intestine	-4.0 ± 9.9^{ab}	9.7 ± 11.1^{ab}	13.9 ± 13.5^{ab}	13.8 ± 4.8^{ab}	83.5 ± 94.7^{a}
Posterior-intestine	1.2 ± 9.6^{ab}	-10.8 ± 4.9^{b}	-3.6 ± 4.7^{ab}	-3.9 ± 3.8^{ab}	-8.5 ± 8.2^{b}

Data are means ± SEM of n = 3-5 samples. Different lowercase letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.05$).

Supplementary Table 8. Partitioning of Cu throughout gut sacs exposed to $CuSO_4$ or CuO ENMs and in the presence and absence of 5 mM L-histidine expressed as a percentage of the Cu dose at the start of the 4 h incubation.

		$CuSO_4$	CuO ENMs	$CuSO_4$ and histidine	CuO ENMs and histidine
Mid-intestine	Mucosa	10.0 ± 2.9^{bcd}	14.2 ± 3.8^{cd}	14.4 ± 2.8^{d}	6.2 ± 1.5^{abcd}
	Muscularis	2.4 ± 0.6^{abcd}	0.7 ± 0.4^{abcd}	5.1 ± 1.2^{abcd}	0.6 ± 0.3^{abcd}
	Serosal saline	0.1 ± 0.1^{abc}	0.3 ± 0.1^{abcd}	0 ± 0^{a}	0 ± 0^{a}
Posterior-intestine	Mucosa	11.0 ± 3.3^{bcd}	11.1 ± 3.0^{bcd}	17.8 ± 5.9^{d}	7.4 ± 1.9^{bcd}
	Muscularis	3.5 ± 0.2^{abcd}	0.8 ± 0.2^{abcd}	6.5 ± 2.6^{abcd}	0.6 ± 0.2^{abcd}
	Serosal saline	0.3 ± 0.2^{abcd}	0.1 ± 0.1^{ab}	0.4 ± 0.3^{abcd}	0.1 ± 0.1^{ab}

Data are means ± SEM of n = 5/6 samples. Background Cu concentrations in controls are shown in Figure 3 and were subtracted prior to calculations. Values with different lower case letters are significantly different (Kruskal-Wallis Test, $p < 0.001$).

Supplementary Table 9. Partitioning of Cu throughout gut sacs exposed to $CuSO_4$ or CuO ENMs and in the presence and absence of 5 mM L-cysteine expressed as a percentage of the Cu dose at the start of the 4 h incubation.

		$CuSO_4$	CuO ENMs	$CuSO_4$ and cysteine	CuO ENMs and cysteine
Mid-intestine	Mucosa	3.2 ± 0.6^{abcd}	2.1 ± 1.8^{abcd}	3.5 ± 1.3^{abcd}	4.1 ± 1.4^{abcd}
	Muscularis	1.2 ± 0.4^{abcd}	1.5 ± 1.0^{abcd}	3.4 ± 0.8^{abcd}	3.5 ± 0.3^{abcd}
	Serosal saline	0.3 ± 0.1^{abc}	0.1 ± 0.1^{ab}	0.2 ± 0.1^{ab}	0.3 ± 0.1^{abc}
Posterior-intestine	Mucosa	20.8 ± 1.2^{d}	$13.1 \pm 5.2b^{cd}$	16.1 ± 2.6^{cd}	14.9 ± 1.2^{cd}
	Muscularis	6.7 ± 0.7^{abcd}	5.6 ± 1.7^{abcd}	8.6 ± 2.0^{abcd}	5.3 ± 1.2^{abcd}
	Serosal saline	0.2 ± 0.2^{ab}	0.1 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.1 ± 0.1^{ab}

Data are means ± SEM of n = 3-5 samples. Background Cu concentrations in controls are shown in Figure 3 and were subtracted prior to calculations. Values with different lower case letters are significantly different (Kruskal-Wallis Test, $p < 0.001$).

Supplementary Table 10. Net uptake rate (nmol cm^{-2} h^{-1}) for Cu (as $CuSO_4$) in trout gut sacs.

	Stomach	Anterior intestine	Mid intestine	Posterior intestine
Mucosa	0.274 ± 0.094	0.134 ± 0.060	0.354 ± 0.092	0.446 ± 0.128
Muscularis	0.097 ± 0.052	0.120 ± 0.074	0.088 ± 0.024	0.238 ± 0.080

Data are means ± SEM of n = 6 samples.