

## Reactive oxygen species generation is likely a driver of copper-based nanomaterial toxicity

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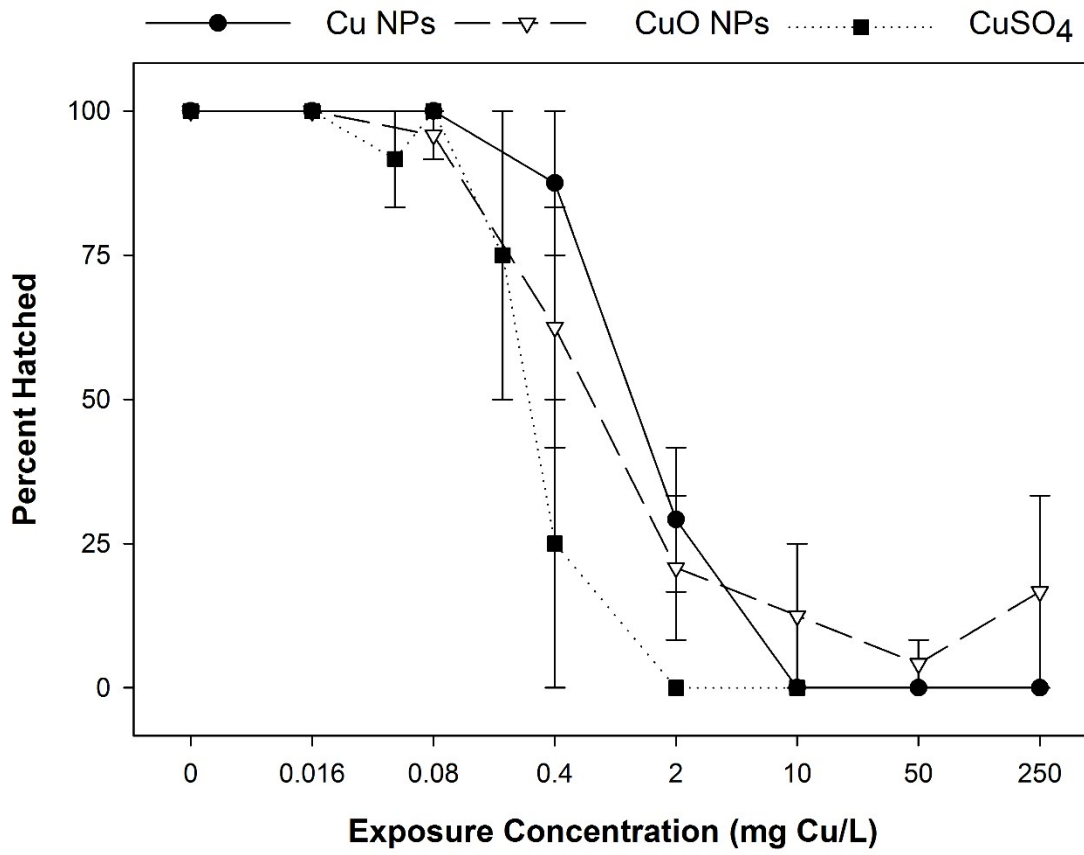
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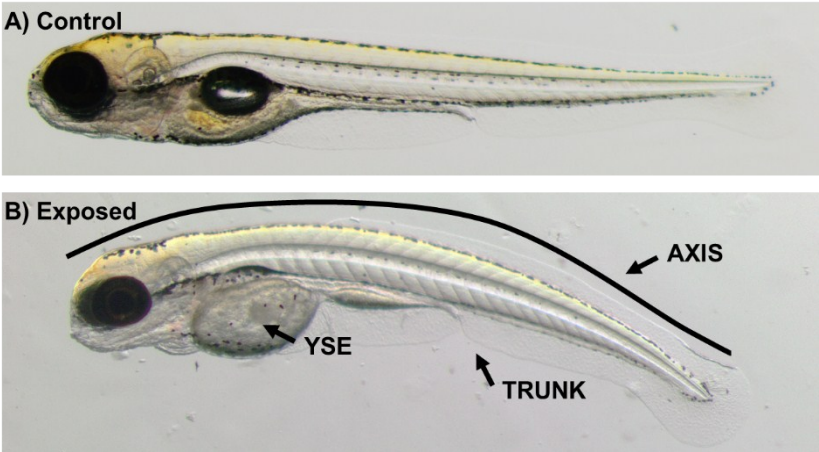
**Supporting Information:**

**7 pages, 2 tables, 3 figures**

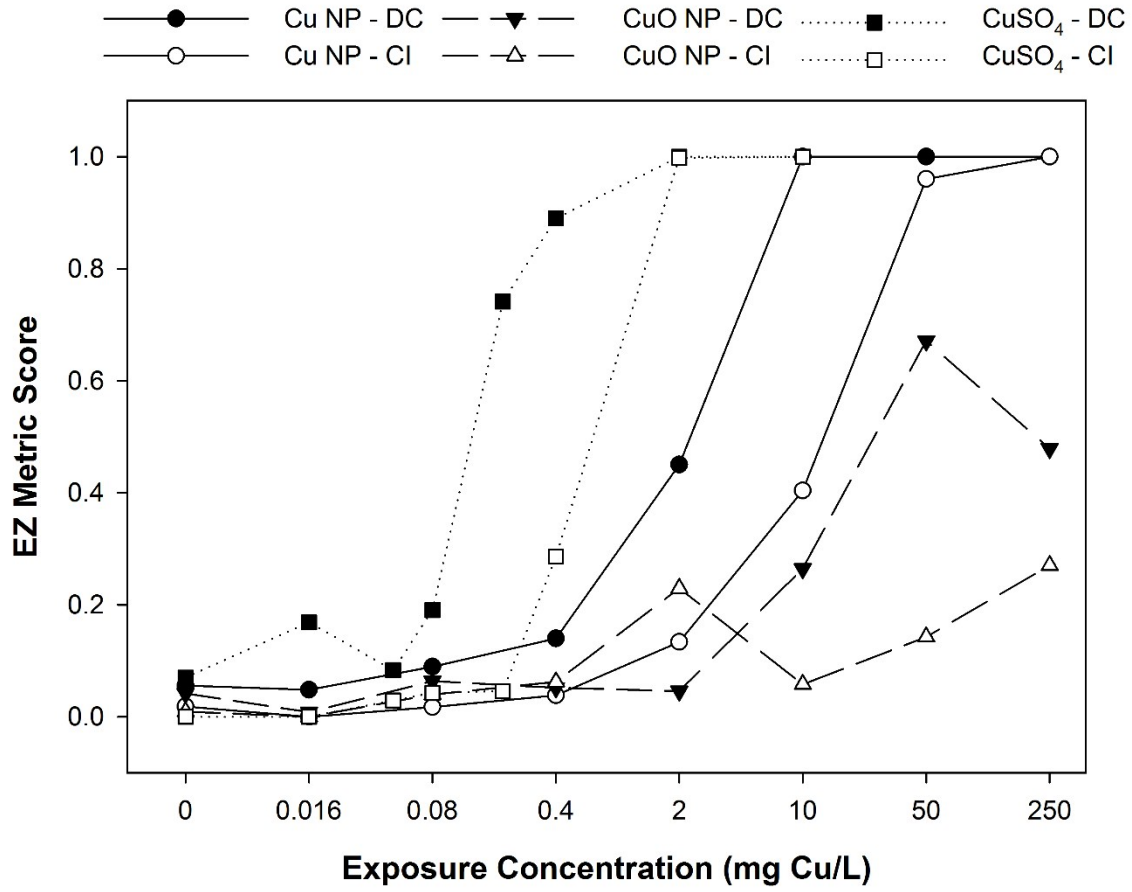
Figure S1. Hatching success of chorion intact embryonic zebrafish as assessed at 120 hpf.



**Figure S2.** Representative image of significant malformations observed in 120 hpf zebrafish.



**Figure S3.** Calculated EZ Metric values for each type of Cu exposure and both chorion statuses.



**Table S1.** Reported values for measuring zeta potential per Lowry *et al.* 2016.<sup>1</sup>

	<b>Cu NPs</b>	<b>CuO NPs</b>
Electrophoretic Mobility	-1.508 $\mu\text{mcm/Vs}$	-1.854 $\mu\text{mcm/Vs}$
<b>Particle Properties</b>		
Size Distribution	See Figure 1	See Figure 1
Composition	Cu core with 1.4 nm CuO shell	CuO
Surface Functionalization	None	None
Shape	Spherical	Spherical
Model used to compute zeta-potential	Smoluchowski	Smoluchowski
<b>Media Dependent Factors</b>		
pH	7.2	7.2
Specific conductance	0.482 mS/cm	0.477 mS/cm
Ionic Composition	2.8 mg/L Na <sup>+</sup> , 0.10 mg/L K <sup>+</sup> , 0.33 mg/L Mg <sup>2+</sup> , 0.11 mg/L Ca <sup>2+</sup> , 0.0020 mg/L Sr <sup>+</sup> , 4.9 mg/L Cl <sup>-</sup> , 0.70 mg/L SO <sub>4</sub> <sup>2-</sup> (values derived from Atkinson and Bingman 1997) <sup>2</sup>	
Ionic Strength	0.18 mM	0.18 mM
Macromolecules/NOM present	None	None
Temperature	26.7 C	26.7 C
Viscosity	0.8508	0.8508
Particle Concentration	10 mg Cu/L	10 mg Cu/L
<b>Measurement Parameters</b>		
Applied voltage	148 V	148 V
Number of measurements made and averaged	12	12
Total number of replicate measurements	3	3
Observed trends in replicate measurements	None	None

**Table S2.** Summary of hydrodynamic diameter and zeta potential values obtained over time in FW, represented graphically in Figure 1. Error represents standard error of two measurements.

	Hydrodynamic Diameter (nm)		Zeta Potential (mV)	
	Cu NP	CuO NP	Cu NP	CuO NP
<b>Day 0</b>	643 ± 342	1257 ± 282	-17.5 ± 0.2	-20 ± 2
<b>Day 1</b>	682 ± 163	1417 ± 121	-16 ± 4	-20 ± 3
<b>Day 2</b>	657 ± 147	1566 ± 352	-11 ± 3	-10 ± 2
<b>Day 3</b>	762 ± 161	1371 ± 570	-12 ± 4	-11 ± 2
<b>Day 4</b>	2080 ± 322	3578 ± 368	-9 ± 5	-8 ± 1
<b>Day 5</b>	752 ± 93	3035 ± 1984	-12 ± 2	-11 ± 2

## References

- 1 G. V. Lowry, R. J. Hill, S. Harper, A. F. Rawle, C. O. Hendren, F. Klaessig, U. Nobbmann, P. Sayre and J. Rumble, *Environmental Science: Nano*, 2016, **3**, 953–965.
- 2 M. J. Atkinson and C. Bingman, *Journal of Aquaculture and Aquatic Sciences*, 1997, **8**, 39–43.